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EEFECTS OF VITAMIN A DEFICEINCY ON THE CYTOMORPHOLOGY OF HEPATOPANCREAS OF *PARATELPHUSA SPINIGERA* WOODMASON

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ABSTRACT
The present study was carried out for the first time to analyze the morphological and functional state of hepatopancreas in *P. spinigera* with different dietary vitamin A level. The study evaluated the growth and changes in the cytomorphology of hepatopancreatic cells. The functional histology was carried out at the (1) Initial period, after (2) 30-35 days, (3) 60-65 days and (4) 90-95 days of administration with control diet. Starting from the early period of deficiency, degeneration of nucleus and haemocytic cells and reduced lumen diameter of the tubules were observed. It has been found that after 90-95 days, the hepatopancreatic epithelium is highly affected and there are several histopathological lesions leading to metaplasia and keratinization of normal columnar epithelium to squamous nature with necrosis of mucous glands.

INTRODUCTION

The fresh water crab, *Paratelphusa spinigera* is an ecologically successful species and is available in the mud soil of the banks of wetlands of Assam. These are the most favourable habitats where they can construct burrows around 60 cms long. The humid air trapped inside the burrows gives the crab enough moisture to survive.

The crustacean hepatopancreas is a bilaterally bilobed organ located on either side of the midgut in the main body cavity, *i.e.* Cephalothorax, directly under the carapace. It consists of numerous branching tubules closely set and firmly held by connective tissue. The canals of these tubules unite together forming larger canals which ultimately fuse forming two large hepatopancreatic ducts that open into the pyloric stomach, one on each side.

The hepatopancreas of the decapod crustaceans is the vital organ analogous to the liver of vertebrates which is responsible for many biological functions. In crustacean, the hepatopancreas is the primary organ responsible for absorption and storage of ingested materials (Loizzi, 1971; Storch and Welsh, 1977; Vogt *et al.,* 1889; Johnston *et al.,* 1998). According to some authors, fat soluble vitamins A, D, E and K are essential in most animals for good health and especially in growth, development, maintenance and reproduction (He *et al.,* 1992). Vitamins interact with other nutrients in processes such as metabolism, digestion and developing blood cells and some vitamins are said to slow down the aging process and also strengthen the immune system. Many studies have evaluated the dietary essentiality of vitamin A for penaeids (He *et al.,* 1992; Liang and Ji, 1998; Reddy *et al.,* 1999a, 1999b). Several studies have revealed that avitaminosis A strongly disturbs mitosis in various vertebrates (Zile, 2001). Earlier workers reported that vitamin A congeners are necessary for the maintenance of proper epithelial cell differentiation thereby reducing the rate of keratinization to reach normal condition (Mock and Main, 1979, Chen *et al.,* 1981). According to Poston (1986) vitamin A is essential for normal structure and function of eyes and gills in fish. In case of crustacean, eye is the main organ where vitamin A is concentrated which suggest that the role of this vitamin in crustaceans may be more limited than in vertebrates (Conklin, 1997). Kalita (2008) gave superficial description about the cytomorphology of the blood and epithelial tissues of fresh water crab. In crustaceans, the hepatopancreas is an important for the absorption and storage of nutrients and can synthesize digestive enzymes for food digestion and the stored nutrients are transported to the muscles, gonads and other tissues during the growth and reproductive stages (Yao *et al.,* 2008, Jiang *et al.,* 2009). Almbro *et al.,* (2011) found that the two dietary compounds vitamin E and Beta carotene have interactive antioxidant properties which promote sperm competitiveness in male cricket, *Teleogryllusoceanicus*. Wang *et al.* (2014) recorded an important role of the hepatopancreas

for nutrition metabolism and ovarian maturation in the Portunus trituberculatus. The shrinkage of the hepatic cells can result in cirrhosis - the contracting of the blood vessels thereby greatly impeding the portal flow through the liver (Nikalje *et al.,* 2012). The histopathological studies not only give an early indication of pollution hazard, but also provide useful data on nature and degree of damage to cells and tissues (Shaikh *et al.,* 2010).

The work on the biopotency of vitamin A on the blood and epithelial tissues of invertebrate is very scanty. So, the present study was carried out to find the effect of vitamin A- deficient diet on the epithelial tissues of the fresh water crab (P. spinigera)

MATERIALS AND METHODS

About 90 mature specimens of freshwater crab *Paratelphusa spinigera* were collected from the different paddy fields and the Pagladia river of Baksa and Nalbari district of Assam. Both the sexes were collected for investigation. The specimens were first placed in a prediluted bath containing 0.1% $KMno_4$ solution for a few minutes as a prevention of dermal infection. The sizes of the crabs were almost of uniform range and weighing between 60-89gm, carapace length and breadth were 5-6 cm and 5-9 cm respectively. Animals were transported very carefully to four aquaria, avoiding rough handling and injuries. The aquaria were under continuous aeration and pH was 6.7 $\pm$ 0.6.

Vitamin A-deficient diet

The vitamin A -deficient diet was prepared by taking the ingredients as reported by earlier workers (Jone and Foster, 1942, Bulher and Halver, 1961, Jones *et al.,* 1971). However the proportion of ingredients was used according to Dutta (1986).

Methyl cellulose	35.0 g
Gelatin	5.0 g
Caesin	50.0 g
Starch	50.0 g
Glucose	15.0 g
Sucrose	35.0 g
*Salt mixture	10.0 g
**Vitamin mixture	
Water	300.0 mL
Ground nut oil	15.0mL

Composition of salt mixture

NaCl	10.0 g
KCl	12.0 g
$CaCO_3$	22.0 g
$MgSO_4$	22.0 g
$CuSO_4$	trace
Kl	trace
$FePO_4$	trace
$MnSO_4$	trace
$Ca_3(PO_4)_2$	15.0 g

Vitamin mixture

Amount in milligrams supplied by 100.0 g diet. Thianine HCl, 0.5; Riboflavin, 0.5; Pyridoxine, 0.5; Ca pentothenate, 3.0; Inositol, 3.0; Nicotine acid, 3.0; Folic acid, 0.1; Biotin, 0.2; Ascorbic acid, 10.0; Crystalline vitamin D_3, 0.2; and α-tocopherol acetate, 22.0.

Preparation of Diet

The vitamin A-deficient diet was prepared by taking the above mentioned ingredients following the procedure of Dutta (1986).

Control Diet

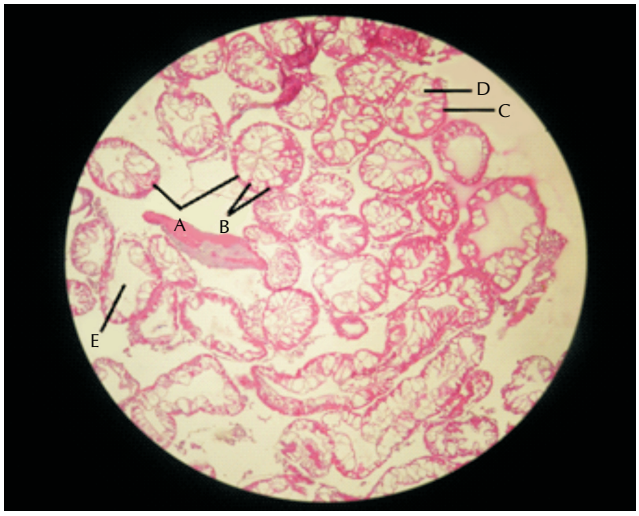
In order to examine the effect of vitamin A -deficient diet, a control diet was prepared by taking the vitamin A -deficient diet (as prepared and described above) and retinyl acetate (2.0 mg/crab). The final preparation of the diet was completed at the time of administration of food by taking the vitamin A -deficient diet and simultaneously adding retinyl acetate as required along with 2 drops of groundnut oil. Both the control and the vitamin A deficient diets were administered (10.0g/ 100.0g body weight of the crab) to the batches of crabs concerned. In the morning time the experimental aquaria were cleaned twice in a week and filled with fresh tap water. The diet was administered in the form of small pallets (0.5g approx).

For histopathological studies of the hepatopancreas, 30 controlled or normal experimental crabs were dissected at regular intervals of 30-35, 60-65 and 90-95 days and histological slides were prepared with conventional histological techniques. Then the different functional as well as histological structures were examined and recorded along with the changes in the gross histological structure.

RESULTS

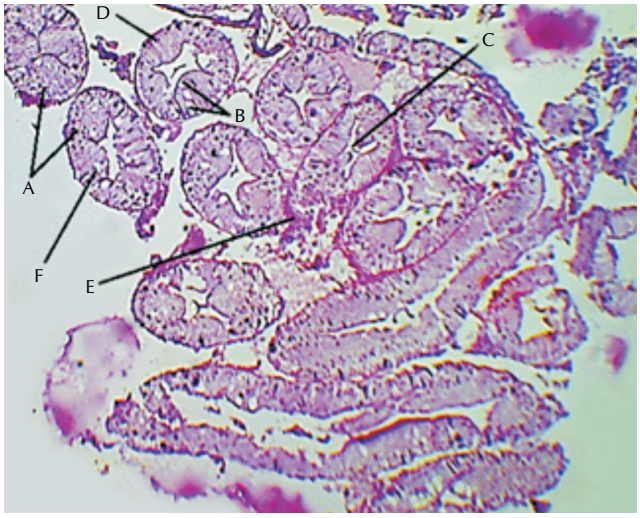
Functional histology of the controlled or normal crab

The hepatopancreas of the normal crab is a large, yellowish-brown compact organ (Fig. 1) consisting of a mass of blind tubules with scares intertubular space. Each hepatopancreatic tubule is lined by a simple epithelium that consists of four basic cell types: E-cell (embryonic), B-cell (blister like), R-cell (resorptive) and F-cell (fibrillar) according to the scheme of Jacob (1928). In control crabs, the hepatopancreatic tubules appeared densely packed and the intertubular spaces were reduced with a thin layer of connective tissues. The E-cells or 'embryonic cells' were small cells with a vacuolar apical complex and were found at the blind end of the tubules. These were the basal cells that lie close to the basement membrane on the outer side of the tall epithelial cells. In decapods crustaceans these cells are believed to be involved in mitotic activity (Gibson and Barkar and others 1979) for the production of other cell types. They were cuboidal and un-differentiated cells with round and prominent nuclei. These cells ranged from 8.24μ to 8.78μ in length and 3.44μ to 4.01μ in breadth respectively. The average length and breadth of the cells were measured 8.51μ ($\pm$0.208) and 3.64μ ($\pm$0.259). The R-cells or 'Resorptive cells' were the most numerous cell types which were generally elongated and granulated columnar cells. These cells were characterized by centrally or basally located nucleus



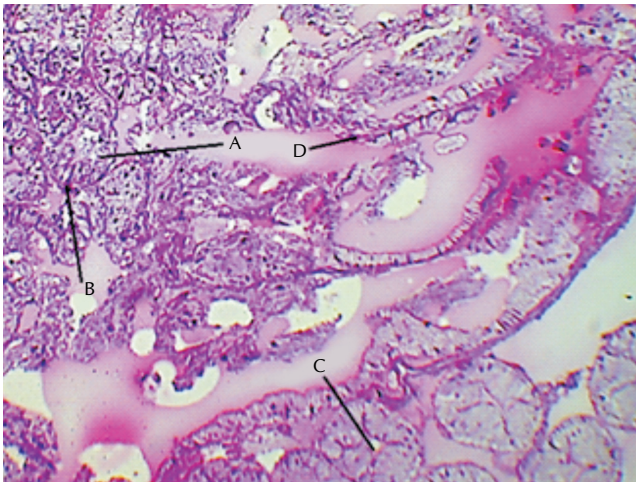
A: E-Cell (Embryonic Cell); B: F-Cells (Fibrillar Cell); C: R-Cell (Resorptive Cell); D: B-Cell (Blister like Cell); E: Lumen

Figure 1: Hepatopancreas of normal *P.spinigera* (Giemsa stain, HE x 280)



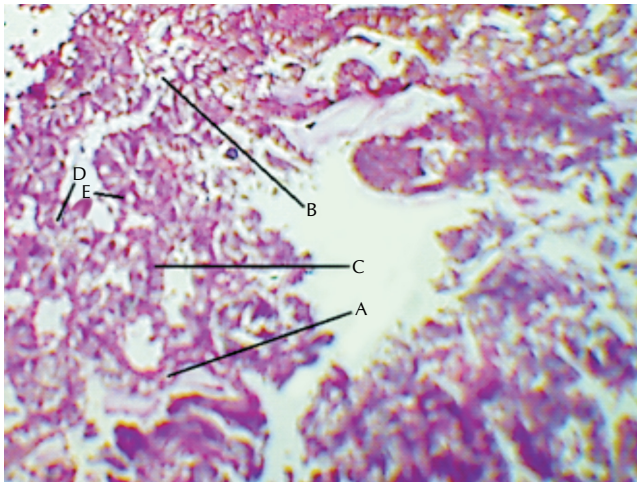
A: R-Cells converting into squamous types; B: Aggregation of Vacuoles; C: Reduced Lumen; D: Nucleus of R-Cell moving to the peripheral region; E: F-Cell becoming irregular shaped; F: B-Cell (Blister like Cell)

Figure 2: Hepatopancreas of (30-35 days) Vitamin A-deficient *P.spinigera* (Giemsa stain, HE x 280)



A: R-Cell converting into stratified squamous type; B: F-Cell (irregular shaped); C: Reduced Lumen; D: Disappearing E-Cell

Figure 3: Hepatopancreas of (60-65 days) Vitamin A-deficient *P.spinigera* (Giemsa stain, HE x280)



A: Vacuole in R-Cell; B: Vacuoles in Haemocytic Cells; C: Completely keratinized Columnar Epithelium; D: Enlarged Mucous Gland; E: Damaged Cell with unclear cellular contours.

Figure 4: Hepatopancreas of (90-95 days) Vitamin A-deficient *P.spinigera* (Giemsa stain, HE x 320)

and large number of storage vesicles in their cytoplasm. R-cells occurred throughout most of the medial and proximal tubular zones according to Al-Mohanna and Nott (1989). The length and breadth of the cells ranged from 22.48μ to 35.36μ and 21.05μ to 25.42μ with a mean value of 31.09μ ($\pm$3.419) and 22.2μ ($\pm$3.102) respectively. The corresponding diameter of the spherical nuclei ranged from 1.38μ to 2.38μ (1.85 $\pm$0.537). Among these large number of mucous gland cells were observed. The glandular cells were nothing but the mucous cells .Some of them was found oval to spherical in shape containing large amount of mucin for digestion of protein, fats and carbohydrates. The average length and breadth of the oval shaped cells were calculated 31.11μ ($\pm$2.17) and 26.50μ ($\pm$7.481) where the cells ranged from 28.94μ to 33.28μ in length and 21.2μ to 31.79μ in breadth respectively. The diameter of the spherical cells varied from 20.12ì to 28.21μ (22.14 $\pm$2.05). The B- cells or 'Blister-like cells' are fat globulated hepatic cells with a smaller nucleus and a large vacuole encompassed by a thin layer of cytoplasm. The nucleus was located in the basal region of the cell and the epical region contains vacuoles. B-cells were more abundant at the medial and distal zones of the hepatopancreatic tubule as described by the several earlier workers. These cells were more or less spherical which ranged from 22μ to 28μ (24.32 $\pm$2.088) in diameter. The nuclear diameter ranged from 1.8μ to 2.0μ (1.2$\pm$ 0.812). F-cells or 'fibrillar cells' are less frequent

and were scattered throughout the tubular tissues. These were the ferment cells which were dome like with a centrally or basically located nucleus. These cells were found located among R-cells and B-cells at the medial zone of the tubule. The average length and breadth of these cells were calculated as 6.88μ ($\pm$0.675) and 4.03μ ($\pm$0.82) where the ranged of the length and breadth of these cells were measured from 6.32μ to 7.21μ and 4.01μ to 4.12μ respectively. Subsequently the spherical nuclei measured from 2.5μ to 3.1μ (2.7 $\pm$0.114).

Effects on hepatopancreas after 30-35 days of administration of vitamin A - deficient diet

The following histological changes were observed under the light microscope in the hepatopancreas after 30-35 days of vitamin A deficiency.

The Columnar cells became stratified to squamous type. In the basal cells nuclei were found irregular shaped. Numerous vacuoles were seen with reduced cytoplasm. In the R-cells nuclei were seen moving to peripheral region from central region. Aggregation of variously seized vacuoles towards the base of the tubule together with reduced basal cells was observed. Mucous glands were found to be degenerated.

Effects on hepatopancreas after 60-65 days of administration of vitamin A - deficient diet

After 60-65 days, more degenerative changes were noticed in the epithelial lining of the mucous membrane. The length and breadth and the diameter of the mucous glands were reduced and columnar cells became more stratified to squamous type with gradual degeneration of nucleus. Most of the vacuoles were more degenerated. The ferment cells showed irregular in shape, but still few cells were noticed having centrally located spherical nucleus, though in most cases nuclei were degenerated. The basal cells showed the gradual disappearance. The haemocytic cells were also disappearing with irregular cells with less synthesis of haemocytes. The lumen diameter also became reduced during this period.

Effects on hepatopancreas after 90-95 days of administration of vitamin A - deficient diet

After 90-95 days, there was complete loss of synthesis of haemocytic cells though the complete clumping nature of the haemocytes with irregular membrane was noticed. Vacuolation of the haemocytic cells appeared with loss of complete synthetic character. The vacuoles were much larger in case of R-cells in comparison to the controlled group. Mucous gland cells became degenerated. The columnar cells that converted into stratified epithelium had become keratinized. In this period the mucous gland cells became few in number, very few attained enlargement in their size and finally others were observed in the degenerated stages resulting in the shrinkage of the cells.

DISCUSSION

In the controlled crab, the hepatopancreatic tubules appeared densely packed and the intertubular spaces were reduced with a thin layer of connective tissue in between that may have blood vessels and fixed phagocytes (Johnson, 1980). B-cells and R-cells appeared in all tubules and the luminae were star-shaped (Fig. 1), which are the normal conditions for many decapod species (Johnson, 1980; Bell and Lightner, 1988; Factor, 1995; Cuartas and Petriella 2002). The functional histology of the hepatopancreas which consists of four type of cells such as E-cells, B-cells, R-cells and F-cells were affected after administration vitamin A - deficient diet and the mode of effect was observed in different degree of deficiency. It has been found that vitamin A deficiency showed degeneration of mucous glands with few enlarged cells, metaplasia of columnar to squamous type, aggregation of variously seized vacuoles, reduced basal cells during the first month of deficiency. Subsequent deficiency after the second month resulted metaplasia of columnar to squamous type with necrosis of mucous membrane and more degeneration of mucous glands. After the third month of deficiency there was complete conversion of columnar and cuboidal into stratified squamous epithelium with keratinization, complete degeneration of mucous gland with a very few enlarged cells. Several researchers have described their findings regarding the structural and functional integrity of the hepatopancreatic epithelium with reference to vitamin A deficiency. Most of the present findings are in full agreement with the earlier workers.

According to Davis and Burnett (1964) and Hopkin and Nott (1979, 1980), the hepatopancreatic tubule is divided into three zones: distal region containing E-cells, mid region containing R-cells and F-cells and proximal region containing B-cells, R-cells and F cells. According to them R-cells are the most abundant cell type in the hepatopancreas of the decapods and the present studies also support the above findings. But during the later period of vitamin A deficiency diet supplementation it has been found that some cells showed unclear nuclear and cellular contours and different cell types could not be recognized, which was similar to the observation laid by Analia V., Farnandej Gimenej *et al.* (2008).

Moreover the present findings are in full agreement with the findings of Loijji (1971) concerning the hepatopancreas of the cray fish (*Orconets virilis* and *Procambarus clarkii*), Lyon & Simkiss (1984) concerning the hepatopancreas of the cray fish (Austropotamobius pallipes) and Trinadaha Babu *et al.* (1989) concerning the hepatopancreas of the crab (*Portunus sanguinolentus*).

Some workers observed that the vitamin A deficiency diet has a deleterious effect on growth (He *et al.,* 1992) resulting cellular metaplasia, disorganized tissues shrinkage of cells etc in case of shrimp *Pleoticus muelleri*. In the present study also variation in the histological structure shrinkage of the cells with reduced lumen diameter was evident which characteristics were considered as the results of mal nourishment according to Rodriguer-Souza *et al.* (1996). Moreover it may be concluded that the lack of nutrient results in atrophy of hepatopancreas as described by Chandrakala Patil *et al.* (2007). Therefore the findings of these studies indicate that vitamin A deficient diet results in histopathological changes in the hepatopancreas and vitamin A is mostly essential to maintain the morphology and function of *Peratelphusa spinigera*.

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