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RESEARCH ARTICLE

EFFECTS OF DEPRIVATION OF VITAMIN A ON THE INTESTINE OF PARATELPHUSA SPINIGERA WOOD MASON

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ABSTRACT

This study shows the effect of vitamin A deprivation in the epithelial tissues of the intestine of *Paratelphusa spinigera*, a fresh water crab. Histological studies were conducted both in control or normal and vitamin A –deficient crabs. The administration of vitamin A –deficient diet was continued for 90-95 days and chronological studies were done at 30-35 days, 60-65 days and 90-95 days and the histopathological changes were analyzed. Gradual degeneration of mucous glands leading to metaplasia and keratinization of columnar epithelial cells to squamous type, atrophy of villi with congestion of blood vessels in the serosal layer were observed in acute deficiency of vitamin A. These results also suggest that a decrease in the level of vitamin A may have a detrimental effect on the intestinal epithelium of the crab.

INTRODUCTION

Vitamin A is very essential in maintaining the normal morphology and function of epithelial cells in many organs (Roberts and Sporn; De Luca. 1984). Various authors like (De Luca. 1926) described the relationship between vitamin A, protein and normal division with differentiation of epithelial cells. Vitamin A, protein and Fe deficient animals are characterized by an almost immediate reduction of growth and a progressive keratinizing metaplasia of epithelium. Several workers (Mori, (1904), Goldschmid, (1915) reported the cornification of the conjunctiva in rats and similar changes in the lining of the pharynx and trachea and alimentary canals due to vitamin A-deficiency. The normal epithelium of alimentary tract is converted into metaplastically stratified squamous and keratinized epithelium in rat due to vitamin A-deficiency and this keratinisation allows these tissues to undergo further infections resulting in capricious appetite, lachrymation, progressive weakness and death (Wolbach and Howe, (1925), Seifried, (1930), Howell *et al*. (1941), Eddy and Dalldrof, (1938), Sherman, (1961), Aydelotte, (1963), Anderson, (1966), KonStantinov, (1969), Tvedten *et al*., (1973), Cloarec *et al*., (1979), Dutta, (2012), Alex, (2012). This deficiency also strongly disturbs the regulation of mitosis (Alov, (1957), Parnell and Burton, (1962).

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Vitamin A-deficiency was found to increase the proliferation rate of mucous cells in the duodenum of rat significantly (Reifen *et al*. (1998)). Morphologic alterations occurred at 50% and 70% of the small intestine length in vitamin A-depleted lambs and consistent ultrastructural alterations were vesicular microvillar degeneration and disruption of the endothelium (Holland *et al*., 1993). These results suggest that vitamin A -depleted diet have a detrimental effect on the small intestinal epithelium. But vitamin A repletion appears to minimize the detrimental effects (Holland *et al*., 1993). According to Reifen *et al*., 1998, retinoids have potency in differentiation and their association with formation of epithelial mataplasia. After intestinal absorption carotene is converted into vitamin A in the small intestine (Thompson *et al*., 1949, Mc Gillivary, 1951). Carotene is first converted to retinal and then reduced to retinol and finally become completely esterified and transported through lymph. The biosynthesis of vitamin A from carotene, is under influence of cell free homogenate fractions of rat-intestinal mucosa (Goodman, 1967). According to Pande and Krishnamurthy, 1959, the damage of intestinal mucosa due to vitamin A-deficiency probably affects the absorption of vitamin A and also conversion of carotene into vitamin A. Krieg and Loliger, 1963 reported that due to total absence of vitamin A supply, there is complete blockage of kupffer cells, destruction of parenchyma, inhibition of bile secretion and finally fail to absorb vitamin A from the small intestine.

Aydelotte, 1963 reported that increase of mucous cell-size in the tracheal epithelium is due to vitamin A-deficiency. According to Luets'kii and Baran, 1974, the membrane permeability of the small intestinal mucosa is increased due to vitamin A-deficiency. They show that enzyme's activity is sharply decreased by large doses of vitamin A yet indicating the stimulation activity of Fe, Mg^{2+}, Na^{+}, K^{+} ATP-ase and Mg^{2+} ATP-ase. Further vitamin A, protein deficiencies etc. sharply inhibit the transportation of ATP-ase in the cell membranes of the small intestinal mucosa. Wolbach, 1954 reported that the morphology of the small intestine is not significantly affected by vitamin A-deficiency. A decreased growth rate in rat is one of the earliest, and at the present time, the most sensitive index of vitamin A-deficiency. Reifen *et al*., 1998 found a lower number of goblet cells in the colon and the duodenum of vitamin A-deficient rats. Villous atrophy of the intestine and flattening of mucous gland with gradual thickening are some of the effects of vitamin A-deficiency (Fousa *et al*., 1975, Coop and Angus, 1975, Holland *et al*., 1993). Corey and Hayes, 1972 and De Luca *et al*., 1972, reported that in the intestinal mucosa the number of goblet cells is significantly reduced and various biochemical functions are altered (De Luca *et al*., 1972 Zile *et al*., 1977, Coop and Angus, 1975, Holland *et al*., 1993). According to Leut's kii *et al*., 1974, retinyl palmitate-hydrolase decreased markedly during vitamin A-deficiency in the intestinal mucosa of the small intestine.

Bang *et al*., 1975 reported on the vitamin A-activity that keratotic and squamousal change with stunted growth was because of malnutrition rather than this deficiency or complete lack of vitamin A in their diet. Batia *et al*., 2010 and Abdullav *et al*., 1979 found that in cattle, nutritional protein, Fe, Zn and vitamin A deficiencies cause keratinisation and increase of epithelial cell-membranes of oesophagus to becoming multilayered and thickening of mucosa of the stomach with intestinal inflammation. Odonoghue, 1955 observed keratinization of epithelial cells with an increased susceptibility to infection in beef cattle due to vitamin A-deficiency. The rate of differentiation of sensitive goblet cells from oligomucous cells and other precursor cells seems to be blocked due to vitamin A-deficiency (Zile *et al*., 1977, Rojanapo *et al*., 1980) and vitamin A-deficient diet shows conversion of mucous and ciliated cells in the trachea, stomach, intestine etc into keratinizing metaplastic cells instead of differentiating into the normal mucous and ciliated cells.

According to Hunter and Hermann, 1961, there is reduction of human epidermal 'keratin cell' counts when vitamin A is orally administered and after discontinuation of therapy produces variable pattern of keratin cells. Uni Z *et al*., 1998 mentioned that severe vitamin A-deficiency altered the small intestine of chickens at both the biochemical and the morphological levels and it caused the loss of mucosal protein , reduced villus height and crypt depth and diminished activities of disaccharides, transpeptidase and alkaline phosphate. Vitamin A-deficiency interferes with the normal growth rate in chickens because it influences functionally of the small intestine by altering proliferation and maturation of cells in the small intestinal mucosa (Uni Z *et al*., 2000). According to Natarajan *et al*., 2009, retinoids are important mediators of cellular differentiation and proliferation in various epithelia of the body including the small intestine and altered retinoid metabolism in the intestine of cirrhotic rats might have an influence on changes in intestinal epithelial cell differentiation seen in liver cirrhosis. Retinoic acid supplementation of rats with low vitamin A status affects vitamin A metabolism at both the whole body level and in specific organs like liver, kidney, lung and small intestine as mentioned by Christofer *et al*., 2007.

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 & 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 60-89 gm, 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 ± 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 accordingly to Dutta, 2012.

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 ---------- 15.0 ml

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
KI ---------- 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, 2012.

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 everyday 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

In vertebrates the biogenesis of vitamin A is mainly occurred in the intestine (Goswami and Barua, 1971). Crab intestine also follows similar phenomenon of biogenesis of retinol, retinoic acid and dehydroretinol like vertebrate intestine. Besides the site of biosynthesis, intestine is also a good source of vitamin A in both fresh water and marine crabs. In order to study the effects of vitamin A –deficiency in epithelial tissues, the present experiments were conducted by taking 30 normal or controlled experimental crabs which were dissected at regular intervals of 30-35, 60-65, 90-95 days and histological slides of the intestinal epithelium of *Paratelphusa spinigera* were prepared with conventional histological techniques.

Histology of intestinal epithelium was carried out by taking sample of normal group of crabs. The intestinal tissues were first cut into small pieces and preserved in 10% formaldehyde solution and washed in tap water which were further dehydrated, stained with paraffin at 65 ^{0}C (Dutta, 2012). The different functional and histological structures together with the vitamin A –deficient were examined and comparative physiological effects in the affected intestinal epithelium were analyzed.

Intestinal epithelium of normal *Paratelphusa spinigera*

The cross section of the routine histological structures showed serosal layer, muscularis, epithelial cells, villi, blood vessels and infiltrating haemocytes (Plate1). Serosa forms the outermost layer of the intestinal wall. It is the visceral peritoneum with underlying loose connecting tissue. The muscular coat consists of an outer longitudinal and a thinner circular layer of muscles with blood vessels lying between them. The intestinal mucosa consists of villi which are lined by high columnar epithelial cells containing mucous secreting cells (Plate Ia). Similar histological structures were observed throughout the experimental period which was repeated after 30-35 days, 60-65 days and 90-95 days of administration of control diet.

Intestinal epithelium after administration of vitamin A – deficient diet

For the histopathological study of the intestinal epithelium 30 vitamin A – deficient *Paratelphusa spinigera* were sacrificed. The growth of the crabs was hampered due to vitamin A – deficient diet supplementation and the intestinal epithelium was found to be severely affected. The extensive information received from various researchers as mentioned earlier helped in the study of the histopathology of the intestinal epithelium (Dutta, 1986). Chronological studies were made in different time intervals (i) 30- 35days, (ii) 60-65 days, (iii) 90-95 days after the repletion of vitamin A –deficient diet.

Table 1. Measurements (μ) of mucous glands and epithelial nuclei in the intestine of P. spinigera in normal/control crabs after administration of vitamin A -deficient diet and after supplementation of vitamin A congeners

P. spinigera and Nature of Administration	Mucous (μ) Gland			Epithelial (μ) Nucleus		
				Columnar		Squamous
	Length (Range, Mean±SD)	Breadth (Range, Mean±SD)	Diameter (Range, Mean±SD)	Length (Range, Mean±SD)	Breadth (Range, Mean±SD)	Size (Range, Mean±SD)
Normal/Control	5.4-8.4, 7.1±1.231	3.2-4.9, 3.9±0.947		2.2-4.4, 3.8±0.710	1.3-2.1, 1.7±0.626	
Vitamin A deficient (30-35) days	4.2-7.1, 6.2±0.882	3.0-4.1, 3.1±0.812		Metaplasia		1.0-3.0, 1.8±0.626
Vitamin A deficient (60-65) days	2.7-5.1, 3.4±0.547	2.0-3.0, 1.9±0.447		Metaplasia		1.5-3.2, 1.9±0.547
Vitamin A deficient (90-95) days	6.0-8.5, 7.1±1.330			Keratinized		
After supplementation Of Retinoic Acid	5.3-8.2, 7.2±1.112	3.2-4.8, 4.0±0.535		3.1-4.2, 3.9±0.479	1.3-2.0, 1.6±0.431	
After supplementation Of Retinol	5.2-8.0, 6.1±1.131	3.1-4.6, 3.7±0.509		2.1-4.1, 3.7±0.880	1.2-2.0, 1.5±0.556	
After supplementation Of Dehydroretinol	3.2-5.9, 4.1±0.821	2.1-3.3, 3.0±0.485		1.9-3.9 , 3.1±0.714	0.9-1.0, 0.8±0.574	

*SD : Standard Deviation,
*Blank Cells : Not Applicable

Histopathology of intestinal epithelium of after administration of vitamin A – deficient diet

After 30-35 days of administration of vitamin A –deficient diet

Mucous glands were degenerated after 30-35 days of vitamin A –deficiency diet supplementation. But a few mucous cells were found to be enlarged due to accumulation of mucin (plate 2). Atrophy of villi took place with the appearance of metaplasia of columnar epithelial cells to squamous type and congestion of blood vessels in the serosal layer was observed. A few villi were seen showing flatting at the tip.

After 60-65 days of administration of vitamin A –deficient diet

There was more congestion of blood vessels in the serosal layer than the first month of vitamin A-deficiency. The columnar epithelium of the villi was gradually converted into stratified squamous epithelium. The mucous secreting cells became gradually degenerated resulting in the necrosis of the mucous membrane though very few attained enlargement (Plate 3).

After 90-95 days of administration of vitamin A –deficient diet

Degenerative changes were continued in the epithelial cells of the mucous membrane of the intestine. The columnar epithelia of the villi which were converted into squamous epithelia had become keratinized due to severe deficiency of vitamin A. Most of the villi showed marked degree of congestion and a few villi produced coagulating necrotic changes. Mucous secreting cells were few in number and except few enlarged types all others were found in the degenerated stages which were indicative of necrosis of mucous glands. Vacuolation in the mucous membrane and congestion of blood vessels were observed in acute deficiency of vitamin A.

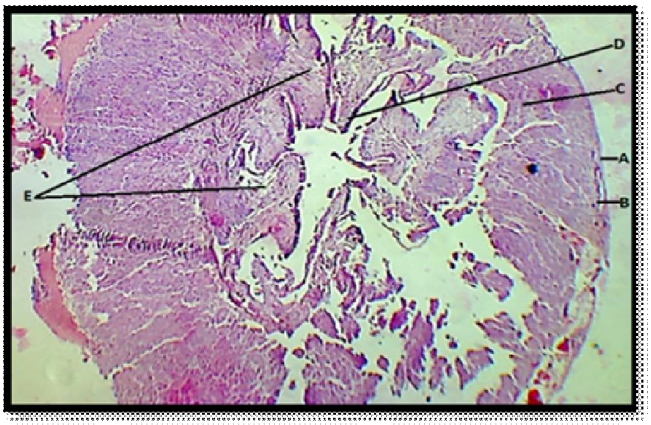

Fig. 1. Intestine of normal *P.spinigera*, (Giemsa stain, HE X 280)

A - Serosal layer
B - Epidermis
C – Muscularis
D - Villi
E - Mucous Glands

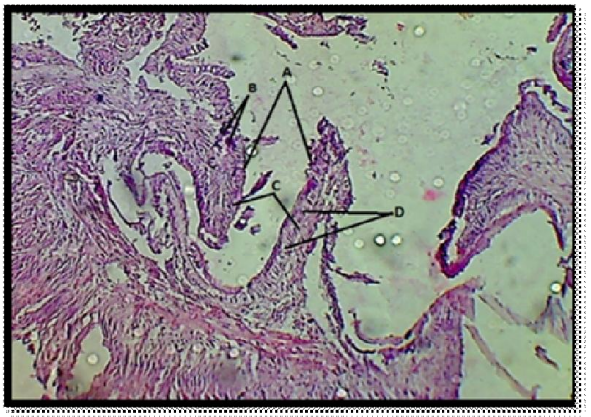

Fig. 1.a. Intestine of normal *P.spinigera* (Magnified Villi), (Giemsa stain, HE X 320)

A - Villi
B – Blood Vessels
C – Columnar Cells
D – Mucous Glands

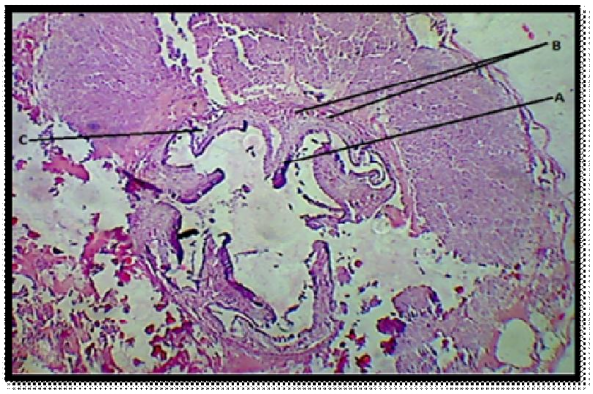

Fig. 2. Intestine of (30-35 days) Vitamin A-deficient *P.spinigera* (Giemsa stain, HE X 280)

A – Gradual Atrophy of Villi
B – Aggregation of Blood Vessels
C – Enlarged Mucous Glands

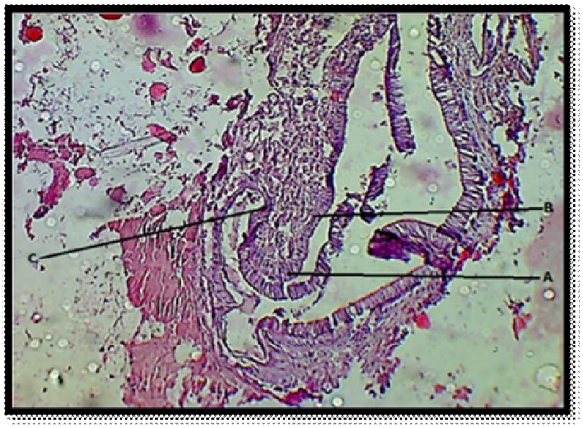

Fig. 2.a. Intestine of (30-35 days) Vitamin A-deficient *P.spinigera*, (Magnified) (Giemsa stain, HE X 320)

A – Villi
B – Gradual conversion of Columnar to Squamous type in the Villi Epithelium
C – Blood Vessel

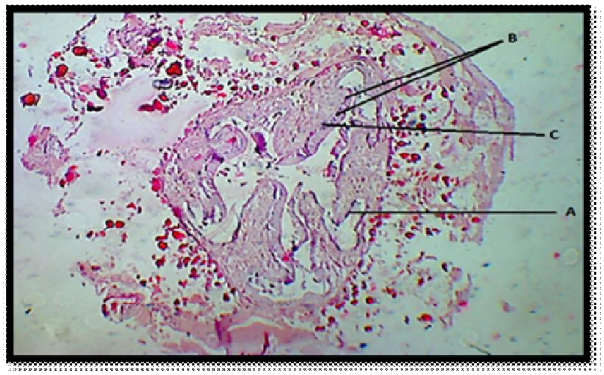

Fig. 3. Intestine of (60-65 days) Vitamin A-deficient *P.spinigera*, (Giemsa stain, HE X 280)

A – Atrophy of Villi with degeneration of Mucous Gland
B – Aggregation of Blood Vessels
C – Metaplasia in the Columnar Cell

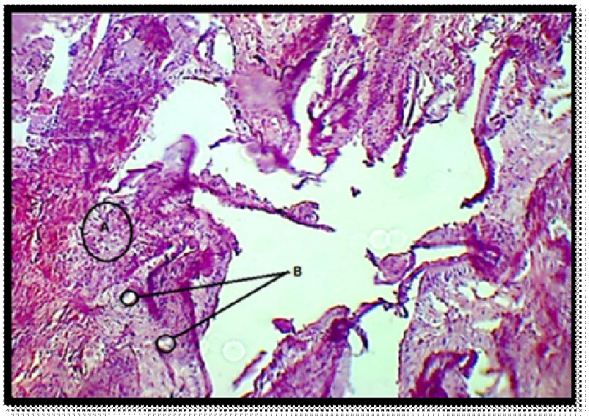

Fig. 3.a. Intestine of (60-65 days) Vitamin A-deficient *P.spinigera*, (Magnified), (Giemsa stain, HE X 320)

A – Metaplasia of Columnar Epithelium to Squamous type due to deprivation of Vitamin A
B – Abnormally enlarged Mucous Gland due to accumulation of large quantity of mucin

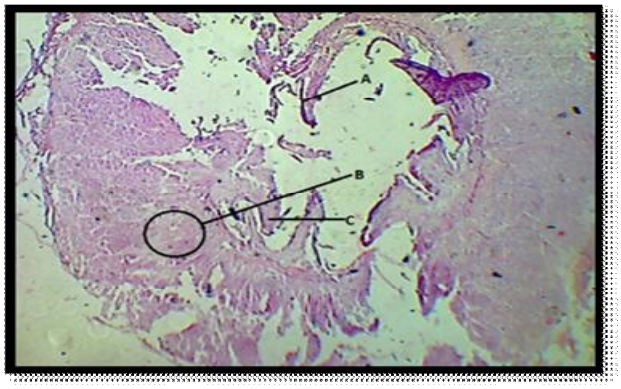

Fig. 4. Intestine of (90-95 days) Vitamin A-deficient *P.spinigera*, (Giemsa stain, HE X 280)

A – Atrophied Villi
B – Keratinized Epithelium
C – Necrotic Mucous gland

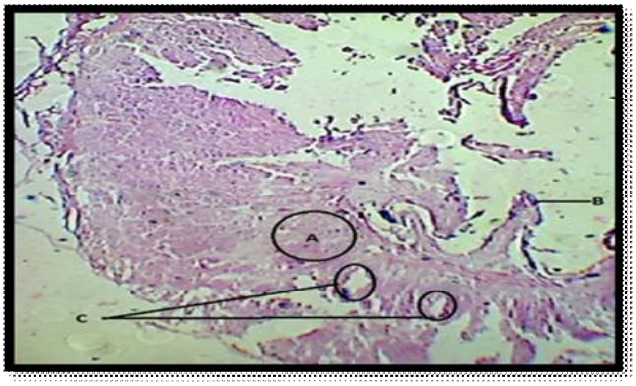

Fig. 4.a. Intestine of (90-95 days) Vitamin A-deficient *P.spinigera*, (Magnified), (Giemsa stain, HE X 328)

A – Keratinized Epithelium due to deprivation of Vitamin A
B – Atrophied Villi with degeneration of Mucous Gland
C – Abnormally enlarged Mucous Gland due to accumulation of large quantity of mucin

DISCUSSION

Several authors have described their findings regarding the structural and functional integrity of the intestinal epithelium with reference to vitamin A – deficiency and vitamin A – supplementation. Sherman, 1961, Zile *et al*., 1977 and Ganguly *et al*., 1980 observed the lesions in the normal epithelial structure caused due to vitamin A –deficiency. Alov, 1957, Parnell, 1964, De Luca *et al*., 1972, Pletsityi, 1978 and Darip *et al*., 1979 noticed that there was immediate reduction of growth and a progressive keratinizing metaplasia of epithelium (Mori, 1904, Goldschmidt, 1915). Again several authors like Wolbach and Howe, 1925, Seifried, 1930, Howell *et al*., 1941, Eddy and Dalldrof, 1938, Sherman, 1961, Aydelotte, 1963, Anderson, 1966, Konstantinov, 1969, Tvedten *et al*., 1973, Cloarec *et al*., 1979, Dutta, 2012, Alex, 2012 found that keratinization allows the tissues to undergo further infections resulting in weakness and death. The present investigations are in full support with the earlier workers. It has been found that due to vitamin A –deficiency progressive weakness occurred among the deficient diet administered crabs resulting in death of few crabs. Atrophy of villi in the small intestine was mostly observed due to vitamin A – deficiency and infection.

This has been reported by previous workers like Fausa *et al*., 1975 and Coop and Angus, 1975. Holland *et al*., 1993 also reported microvillar degeneration and disruption of the capillary endothelium in the vitamin A – depleted lambs resulting in a detrimental effect on the small intestinal epithelium. Further he mentioned that repletion of vitamin A appeared to minimize the detrimental effects. The present work also shows a marked degree of congestion, coagulation of necrotic changes with vacuolation in the villi in acute deficiency of vitamin A. Pande and Kriishamurthy, 1959, Wong and Buck, 1971, Rojanapo *et al*., 1980, Dutta, 2012 recorded disappearance of mucin with reduced number of mucous glands due to vitamin A –deficiency. Aydelotte, 1963 also observed that with more acute vitamin A –deficiency the secretory glandular cells of tracheal epithelium that became enlarged at an early stage of deficiency were shed from the surface as the epithelium became keratinized.

Reifen *et al*., 1998 also found a lower number of goblet cells in the colon and in the duodenum of the vitamin A – deficient rats. Owing to avitaminosis, A Dutta, 2012 recorded complete necrosis of the mucous glands along with a few enlarged types in the intestinal villi. An almost similar conclusion has been drawn in the present investigation, where disappearance of mucous glands with a few enlarged types with indistinct and delicate membranes was noticed during the later stages of deficiency. Due to this deficiency, there was interference in the normal functioning of the mucous secreting glands and the mucin was not normally excreted resulting in gradual accumulation. As a result some of the mucous glands became atrophied, while a few abnormally enlarged due to gradual accumulation of mucin.

Several previous workers like Scott *et al*., 1961, have described the effects of vitamin A on mucous epithelium i.e. .normal development of mucous cells in white leghorn chick; protection of mucous membrane with epithelium from squamous cell- carcinoma in hamster (Chu and Richard, 1965); synthesis of mucopolysaccharides in the trunk epidermis of embryonic mouse (Hardy, 1967); morphologicai and functional integrity of the mucous glands (Darip *et al*., 1979 and Rojanapo *et al*., 1980); sequential alteration in the mucous metaplasia in the epidermis of the embryo of the chick (Tanak, 1982); alteration in proliferation and maturation of cells in the small intestinal mucosa (Uni z *et al*., 1998). All these observations are fully supported by the present work. Moreover Chirstofer *et al*., 2007 reported that retinoic acid supplementation of rats with low vitamin A –status affects vitamin A metabolism in some specific organs including small intestine. Lack of vitamin A resulted in the conversion of normal columnar or cuboidal epithelium into stratified squamous keratinized type (Wolbach and Howe, 1925, Seifried, 1930, Mc Collough and Dalldroff [56], Howell *et al*., 1941, Eddy and Dalldroff [8], Wolbach 1954; Sherman, 1961, Parnell and Burton, 1938, Aydelotte, 1963, Anderson, 1966, Konstantinov, 1969, Wong and Buck, 1971, Tvedten *et al*., 1973, Bang *et al*., 1975, Abdullaev *et al*., 1979, Dutta, 1971, Uni *et al*., 1998). A similar conclusion has been drawn in the present investigation where starting of metaplasia after 30-35 days, conversion of columnar to squamous type with necrosis of mucous membrane after 60-65 days and complete conversion of columnar to stratified squamous with keratinization after 90-95 days of vitamin A –deficiency has been observed.

Alov, 1957, Parnell and Burton, 1962 and Zile *et al*., 1977 have described that mitosis is strongly disturbed by avitaminosis A. The duration of the cell cycle of the jejunal crypt cells was found to be lengthened by vitamin A – deficiency and this increase in generation time was due mainly to a lengthening of the divided crypt cells of the vitamin A –deficient rats. This was strikingly different from that of vitamin A supplemented rats, suggesting an impaired migration of cells out of the crypts, which shows that vitamin A plays a significant role in the regulation of cell division in the small intestine. Several earlier workers further reported that this congener is necessary for the maintenance of proper epithel9ial cell differentiation, thereby reducing the rate of keratinization and allowing this stratified squamous type to reach a normal condition, i. e. Columnar or cuboidal variety (Hunter and Herman, 1961, Parnell, 1964, Dutta, 2012). The present findings are also in conformity with the earlier workers. The analysis indicates that vitamin A is essential for not only vertebrates but also for invertebrates to maintain the normal functional integrity of the intestinal epithelium and its deficiency has a deleterious effect on it.

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REFERENCES

Abdullaev, D. V., Absaliamov, E. F. and Rish, M. A. 1979. "Parakeratosis of cattle," *Veterinariia*, (5), p.55-56.

Alex, J., Bermudez, Mahmoud El-Begearmi, Kirk C. Klasing, Steven Leeson, 2012. "Feeding Methods in Poultry,".

Alov, L. A. 1957 ."Eksptl. *Biol.I.Med.,"* 43(2), 206.

Anderson, W. A. D. 1966. "Pathology," 5th Edition, p.45.

Aydelotte, M. B. 1963. "Vitamin A Deficiency in chickens," *British Journal Nutrition*, 17, 205.

Bang, F. B., Bang, B. G. and Foard, M. A. 1975. *Am.J.Pathology,* 78(3), p.417.

Batya, B., Davidovici, Yalçin Tüzün, Ronni Wolf, 2010. "The role of vitamin A in differentiation and skin carcinogenesis," *Journal of Dermatological Science,* V, Vol. 25, I4, p.525-530.

Bulher, D. R. and Halver, J. E 1961. "Nutrition of salmonoid fishes. IX. Carbohydrate requirements of chinoook salmon," *J.Nutr.*, 74, p.307-318.

Chopra, D.P. 1983. "Cell Tissue Kinet," 16(2), p.155.

Chu, E. W. and Richard, A. M. 1965. *Cancer. Res.,* 25(6pt.1), p.884.

Cifelli, C. J., Green, J. B. and Green, M. H. 2007. "Use of model-based compartmental analysis to study vitamin A kinetics and metabolism," *American Journal of Clinical Nutrition*, 68(1), p.90-95.

Cloarec, L., Durou, M.R., Legras, B. and Esvant, J.Y. 1979. "Relation between ceruloplasmin and vitamin A in Sprague-Dawley rats," *Ann Nutr Aliment*, 33(3):385-93.

Coop, R.L., Angus, K.W., Coop, R. L. 1975. "The effect of continuous doses of Trichostrongylus colubriformis larvae on the intestinal mucosa of sheep and on liver vitamin A concentration," *Parasitology,* 70(1), p.1-9.

Corey, J. E. and Hayes, K. C., *J.Nutr*., 102, p.1585, 1972

Darip, M.D., Sirisinha, S. and Adrain, J. L. 1979. *Proc.Soc.Exptl, Biol.Med.,* 161(4), p.600.

De Luca, L., Maestri, N., Bonanni, F. and Nelson, D. 1926 "*Cancer",* 30.

DeLuca, L., Maestri, N., Bonanni, F. and Nelson, D. 1972. "Cancer," 30, p.1326.

Dutta, N. K. 2012. "Clinical, Hemato-histopathology and Methodology", p.115-130.

Eddy, W. H. and Dalldorf, G. 1938. "The avitaminoses the chemical, clinical andpathological easpects of the vitamin deficiency diseases," 338 pp.

Fausa, O., Larsen, T.E., Husby, G. and Thune, P. 1975. "Gastrointestinal investigations in dermatitis herpetiformis," *Acta Derm Venereol.,*55(3), p.203-206.

Ganguly, J., Rao, M.R.A., Murthy, S. K. and Sarada, K. 1980. "*Vitamins and hormons*,", 38, p.1.

Goldschmidt, M. 1915. "Experimenteller Beitrag zur Aetiologie der Keratomalacie," *Arch. f. Ophth*., 90:354–366.

Goodman, D. S. 1967. Prog. Biochem. *Pharmacol*.3, p.487.

Hardy, M. H. 1967. "Responses in embryonic mouse skin to excess vitamin A in organotypic cultures from the trunk, upper lip and lower jaw," Exp Cell Res, 46, p.367–384.

Holland, R. E., Pfeiffer, C.J., Bruns, N.J. and Webb, K. E. 1993. "Morphologic alterations in small intestinal epithelium of lambs fed vitamin A-depleted diet," *Dig Dis Sci.,* 38(2):p.333-343.

Howell, C. E., Hart, G. H. and Ittner, N. R. 1941. *Am. J. Vet. Res*., 2:60.

Hunter, R. and Hermann, P. 1961. *Journal of Investigative Dermatology,* 37(6), p.459.

Jones, J. H. and Foster, C. 1942. *Journal of Nutrition*, 24, p.245.

Jones, J. H. Elizabeth, B., Bullard, B. and Rodriguer, A. 1971. *Trans.Am.Fish.Assoc*., 4, p.676.

Konstantinov, A. 1969. "The vitamin A status of chicks with rickets," Izv. Inst. srav. Pat. Zhovotnite. Sofia, 13: 281-287,

Kreig, R. and Loliger, H. C. 1963. Arch. Geflugelk, 27, p.483

Leuts'kii, K. M. and Baran, M. M. 1974. Ukr.Biokhim.Zh, 46(1), 105; 46(2), p.197.

Mcgillivray, W. A. 1951. "The conversion of carotene to vitamin A in sheep and cattle," *Aust J Sci Res B.*, 4(3), p.370-376.

Mori, M. 1904. "Über den sog. Hikan Xerosis conjunctivae infantum ev. Keratomalaci," Jahrb. Kinderheilk. 59:175-195.

Natarajan, S. K., Amirtharaj, G. J., Ramachandran, A., Pulimood, A. B. and Balasubramanian, K. A. 2009. "Retinoid metabolism in the small intestine during development of liver cirrhosis," *J. Gastroenterol Hepatol,* 24(5), p.821-829.

O'Donoghue, J. G. 1955. "Vitamin A –deficiency in beef cattle," *Canadian Journal of comparative medicine and veterinary science*, 19(12), p.367-372.

Pande, P. G. and Krishnamurthy, D. 1959 "Poultry Science," 38(1), p.13.

Parnell, J. P. 1964. Bull, New York Academed, 40(1), p.81.

Parnell, J. P. and Burton, S. S. 1962. Publ.Am.Assoc. *Advance Science,* 70, p.113.

Pletsityi, K. D. 1978. "Immunity and Vitamin A," Vopr. Onkol.Lrningr., 24(10), p.85.

Reifen, R., Nyska, A., Koperstein, L. and Zusman, I. 1998. "Intestinal and hepatic cell kinetics and mucous changes in vitamin A -deficient rats, " Int J Mol Med.,1(3), p.579-582.

Roberts, A. B. and Sporn, M. B. 1984. "Cellular biology and biochemistry of retinoids," The Retinoids, vol.2, pp.209–286.

Rojanapo W., Lamb, A. J. and Olson, J. A. 1980. *Journal of Nutrition*, 110(1), p.178.

Scott, M. L., Hill, E. W., Norris, L. C. and Henser, G. F. 1961. "Reinvestigation of the vitamin A and D requirements of laying and breeding hens and their progeny," *Poultry Science* 40, p.1245-1247.

Seifried, O. 1930. "Studies on a-avitaminosis in chickens : ii. lesions of the upper alimentary tract and their relation to some infectious diseases," *J. Exp. Med.*, 52(4):533–538.

Sherman, B. S. 1961. "The Effect of Vitamin A on Epithelial Mitosis In Vitro and In Vivo," *J. Invest. Dermatol*, 37: 469.

Tanaka, S. 1982. "Changes in morphology and cell proliferation during vitamin A-induced mucous metaplasia of developing chick scale cultured in vitro," *J. Exp. Zool.*, 10, 223(2), p.139-154.

Thompson, S. Y., Ganguly, J., Mawson, E. H. and Kon, S. K. 1949. Int. Dairy Congr. XII. Stockholm, p.238.

Tvedten, H. W., Whitehair, C. K. and Langham, R. F. 1973. *J. Am. Vet. Med. Assoc.*, 163(6):605-612.

Uni, Z., Zaiger, G., Gal-Garber, O., Pines, M., Rozenboim, I. and Reifen, R. 2000. "Vitamin A deficiency interferes with proliferation and maturation of cells in the chicken small intestine," *Br. Poult Sci.,* 41(4), p.410-415.

Uni, Z., Zaiger, G., Reifen, R. 1998. "Vitamin A deficiency induces morphometric changes and decreased functionality in chicken small intestine*," Br. J. Nutr.,* 80(4), p.401-407.

Wolbach, S. B. 1954. "Effects of vitamin A deficiency and hypervitaminosis A in animals. In The vitamins," Sebrell, p.106, vol. I.

Wolbach, S. B. and Howe. P. R. 1925. "Tissue changes following deprivation of fat-soluble A vitamin*," J Exp Med*., 42(6): 753–777.

Wong, Y. C. and Buck, R. C. 1971. "An electron microscopic study of metaplasia of the rat tracheal epithelium in vitamin A deficiency," Lab Invest, 24(1), p.55–66.

Zile, M., Elizabeth, B. C. and DeLuca, H. F. *J.Nutr.*,107, p.552, 1977

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