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HISTOLOGICAL EXAMINATION OF TISSUES OF EXPERIMENTAL FISH

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Nutrition and pathology are related disciplines. Gounelle (1961) observed that "Every disease has a nutritional aspect. Pathology is concerned with specific disease process and resultant tissue changes in the individual what ever its systemic position. The study of disease process involves at a greater extent the histological examination of various organs and tissues. The histological examination of tissue in a nutritional experiment provide an opportunity to observe the resultant changes occurring at cellular level. Many times a particular treatment may not produce any physiological or reproductive effect immediately; however histological examination may reveal considerable changes. It is also important before advocating any feed or a combination of feed for field use we have to ascertain whether the said combination contain any type of toxic factor. This can be settled only by conducting an experiment in which histopathological examination forms the major component.

General histological picture in fishes

A knowledge of general histology is essential for interpreting changes due to experimental manipulation. The basic histologic pattern is similar in all vertebrates and a knowledge of mammalian histology is helpful. However it

should be noted that fishes are not primitive vertebrates and they have undergone considerable adaptation to suit their aquatic environment. Even among fishes there is considerable variation between various species. It is not possible to discuss each individual separately. We will confine to a general discussion on histology.

Integumentory

The skin of fish has got following layers (1) cuticle (2) epidermis (3) basement membrane (4) dermis (5) hypodermis. Cuticle is a muco poly saccharide layer secreted by epidermis and may contain sloughed cells, immunoglobulines, lysozyme and fatty acids. Epidermis is a startified squamous epithelial layer formed from Malpighian cells. Many of the cells are mucoid (goblet cells). In addition club cells (Shreckstoffzellen) which produce alarm substances, granule cells and cysts are found in epidermis. Dermis consist of stratum spongiosum containing chromatophores (melano phores Lipophores erythrophores), mast cells, scale buds and the stratum compactum which provides structural strength.

Hypodermis is a lose tissue containing fat cells and is highly vascular.

Structure of Bone: Bone tissue is a cellular.

Muscles are organised as distinct zones in many species. There are two or three types.

(i) Muscularis lateralis superficialis (red muscles) rich vascular supply.

(ii) Muscularis lateralis profoundus (white) poor vascular supply.

(iii) Pink muscles - contain both type of fibres. In red muscles nerve ending are engrappe (middle), in white muscle they are en platte or terminal.

Respiratory system

- Gills are made up of four holobranchs and each holobranch is divided into two hemibranch. Each hemibranch has a row of filament projecting like the teeth of a comb. They are the primary lamellae. The surface of primary lamellae has numerous semilunar projections called secondary lamellae. The gillarch is osseous structure from which radiate the bony support of primary lamellae. The arches contain afferent arteries and efferent arteries. The gillarch and lamellae are covered by epidermal tissue. At primary lamellae the epidermis thicker and contains numerous mucus cells. Below the epidermal layer lymphoid cells eosinophilic granule cell and phagocytic cells are seen in loose connective tissue.

Secondary lamellae consist of an envelope of epithelial cells one layer thick separated by contractile pillar cells which are arranged in rows 9-10 microns apart. The pillar cells spread on basement membranes in the form flanges and coalesce with the neighbouring pillar cells forming the lining of blood sinuses which connect afferent and efferent vessels. The pillar cells can regulate blood flow and blood pressure through gills.

Circulatory system

Heart: Muscle fibres are approximately 6 micron in diameter and are similar to mammalian one with intercalated discs. In atrium muscles are arranged in the form trabeculae with a lining of endothelium which is phagocytic. Sinus venosus is mainly collagenous connective tissue.

Ventricle had two layers of muscles. Outerlayer is compact and inner layer spongy and in the form of trabeculae. Outer layer receives nutrition from coronary vessel where as inner spongy layer takes it directly from luminal blood. Bulbus arteriosus is formed of elastic tissue. Pedicardium is similar to other vertebrates.

Arteries and veins: The basic pattern is same as in mammals.

Haemopoetic tissue: Haemopoetic tissue is located in stroma of spleen, interstitum of kidney and periportal areas of liver.

Renal haemopoetic tissue

Anterior kidney is exclusively haemopoetic and the support matrix of posterior kidney also contribute to a greater extent. It consist of a stroma of reticulo endothelial cells with numerous blood sinuses and blast cells.

Spleen

It is a lymphoid organ. Splenic capsule is fibrous without any trabeculae. The main elements in spleen are ellipsoids, spleenic pulp and melanomacrophage centres. The ellipsoids are thick walled filter capillaries derived from spleenic arterioles. Each consist of thick walled basement membrane bound tube in which artery is usually placed ecentrically sheathed with phagocytes and erythrocytes. Spleenic pulp consist of phagocytic tissue supported by argyrophilic fibres and with numerous blood sinuses.

Melano macrophage centres

Found in kidney, liver and spleen. These are foci containing numerous pigmented cells/phagocytes containing cercid, haemosiderin and melanin.

Thymus

Located at dorsal commissure of operculam as paired organ. It is an aggregate of small lymphocyte covered with fibrous capsule and stroma formed by fine argyrophilic cells and fibres. Epithelial cords are seen occasionally.

Reticulo endothelial system (RES)

RES in fish consist of promonocyte of haemopoetic tissues, monocytes of blood and lymph, macrophage of connective tissue, kidney and endothelial cells (Phogocyte) of atrial lining of heart. Melanomacrophage centres are also part of this system. There are no lymphnodes.

Excretory kidney

Kidney in fishes is a complex organ having haemopoetic, reticulo endothelial, endocrine and excretory functions. Anterior kidney is haemopoetic and posterior portion only had the nuphrons. Nephron structure varies between marine and freshwater species. In fresh water forms nephron comprises vascular glomerulus, ciliated neck, two proximal segments, one with brush border other without brush border, a narrow ciliated intermediate segment and a distal segment which joins collecting duct system. In marine forms nephron consist of glomerulus neck segments, two or three proximal segment occasionally inter-mediate segment found between 1 and 2 proximal segment and the collecting system. In euryhaline species nephron is similar to marine except it may have a distal segment.

Digestive system

1) Mouth is lined with stratified squamous mucoid epithelium on a thick basement membrane and condensed dermis attached to bony structure. Mouth and lips contains the taste buds also.

Oesophagus

Epithelial lining is stratified and rich in mucus cells. Muscularis is straited.

Stomach

Mucosa is lined with mucoid columnar epithelium. Mucosa is thrown into folds and pits. Submucosa contains eosinophilic granule cells. Muscularis comprise of several layers smooth muscle fibres.

Pybric caeca

Histological features are similar to intestine.

Intestine has a simple mucoid columnar epithelium, overlaying a submucosa containing large number of Eosinophilic granule cells and limited by a dense muscularis mucosa and fibroelastic layer. Rodlet cells are frequently seen (oval cells).

Liver: Histology of fish liver differs from mammalian in that the hepatocytes are arranged not in typical cords or lobules. Sinusoids are irregularly distributed and their number is much less compared to mammals. Sinusoids are lined by endothelial cells. Kuppfer cell are not present. The endothelial cells are fenestrated. Number of fat storage cells (cells of Iato) are seen in-space of disse. Billary system orginate as intracellular bile canaliculi which by anastomosis form the bile duct. The gall bladder is lined by transitional epithelium which contain rodlet cells.

Pancreas: It varies in its location. It may be found among the fat cells in mesentery of pyloric caecum; Sub capsular investment of spleen or around the hepatic portal vein. The acinar structure of exocrine pancreatic tissue is very

similar to that of the mammals. Pancreatic duct usually joins the common bile duct.

Reproductive system

Testis: Comprised of series of tubules or blind sacs, the seminiferous tubules which are lined with spermatogenic epithelium which also has tall pyriform sertolicells. Leydig cells are (Polygonal) seen in between tubules interstitial.

Ovary: Structure varies from species to species.

Nervous system: It has two components (NS) (central nervous system) and PNS (Peripheral nervous system).

CNS: Brain and spinal cord are invested by single menigeal layer, the menix primitiva enclosing cerebro spinal fluid produced by choroid plexuses. CNS tissue is divided into grey and white matter. Other histological elements are same except Mauthenerian group of cells - they are two large neurons found in the medulla. The parts of brain are Telencephalon, Diencephalon, the mesencephalon and medulla oblangata. Cells constituting nervous system are neurons and supporting cells, the neuroglia (astrocytes Oligo dendroglia and microglia).

Special sense organs

Eye - basic structure is similar to that of mammals with species variation.

Labyrinth - It consist of semicircular and ottolith organs.

Lateral line system: Paired lateral line canal and in some head canals also. The mechano receptors are situated basally forming the neuromost which comprise pyriform cells with hair like structures.

Olfactory and gustutory senses

Olfactory tissue consist of focal groups of receptor cells, surrounded by mucoid and ciliated columnar epithelium. Sub epithelial loose connective tissue contains large number of eosinophilic granule cells. Axon of olfactory bulbs collect from bases of receptor cells.

Gustatory organs or taste buds are situated on outer surface of lips, head, barbels, fins, gillrackers, gill arches and mouth. Buds are formed by elongated cells forming a sphere the receptor the basal and supporting cells.

Endocrine system

Pituitary - situated in cella tursica of skull. Consist of neurohyophysis and adenohypophysis. Neurohypophysis consist of a stalk of axons whose neuro secretory neurons are situated in hypothalmic nuclei.

Adenohypophysis divided antomically into pars inter media and pars distalis composed of basophils, acidophils and chromophobes.

Thyroid gland - Consist of various follicles, lined with cuboidal cells distributed diffusely throughout connective tissue of pharyngeal area, around eyes, ventral aorta, hepatic veins, adrenal haemopoetic tissue.

Adrenals - Cortical tissue is located in anterior kidney as strands of lightly staining cuboidal eosinophilic cells. Medullary tissue or chromaffin tissue is found accompanying the sympathetic ganglia, in between anterior kidney and spine or even in anterior kidney.

Ultimobranchial gland - Serum calcium regulating gland. Appear as cords of polygonal cells lying ventral to oesophagus within the septum separating sinus venosus from the abdomen.

Corpuscles of stannius - Paired whitish cluster of tissue consisting large clear endocrine cells secreting into centre of the cluster are located retroperitonially on the surface of kidney.

Islet of Langerhans

Scattered throughout the pancreas are small islets which are poorly staining structures comprising of small fusiform alpha, Beta and Delta cells. In some teleost there is a major islet called Brockman body.

Urophysis - It is a whitish ventral expansion of spinal cord at the caudal end. It is composed of neurosecretory axons extending from cord.

Pseudobranch and Chroid body

Red gill like structure attached to internal surface of operculum derived from first gill arch. It consist of parallel blood capillaries supported by cartilage rods and have direct connection with choroid of eye.

Choroid rete:- consist arrays of capillaries alternating with rows of slender fibroblast like cells.

HISTOLOGICAL LESIONS DUE TO NUTRITIONAL FACTORS

Starvation: Marked reduction in sacroplasmal content of myofibrils with vacuolation and central migration of nuclei. In digestive tract reduction in goblet cells in muscosa. In submuscosa increase in fibro blast and collagen.

Pancreatic acini become shrunken. Increase in the size of melano-macrophage centres.

Proteins: Information on proteins is very little. Growth reduction and various abnormalities of bone may result from deficiency.

Carbohydrates: Excessive carbohydrates may lead to liver cell degeneration and excessive deposition of glycogene in liver. Blood sugar level increase was noticed in trout with degranulation and hypertrophy of beta cells of islet of langerhans.

Lipids: Excessive fat may lead to fatty liver syndrome. Essential fatty acid deficiency may lead to depigmentation, fin erosion cardiac myopathy and fatty infiliteration of hepatic cells; ceroid in liver and thickening of cell membranes of fatty tissue. Rancid fat or fish fed with trash feed may develop lipoid liver condition in which extensive lipid infiliteration of hepatocytes with distortion of hepatic muralia. Haemopoetic tissue undergo degeneration with high levels of pale staining pigment in melano macrophage centres. Extra haemopoesis in portal triad and epicardium.

Vitamins

Fat soluble vitamins

Vitamin A: Hypo vitaminosis A in fish will result in Kerato malacia, blindness, and haemorrhages at the base of fins. Hyper vitaminosis result in wide spread epithelial squamous metaphasia and osteopathic conditions.

Calciferol (Vit. D).

This is an area which has not been explored properly.

Tocopherol (Vit. E)

Deficiency in the diet result in degeneration and necrosis of striated muscle fibres, steatitis lipoid degeneration and hepato renal syndrome. (Fatty degeneration of liver and nephrosis withnephro calcinosis).

Vitamin K

Deficiency result in prolonged clotting time. There will be extensive capillary haemorrhages in muscles and viscara coupled with anaemia.

Water soluble vitamins

Thiamin (Vitamin B

The histological changes due to thiamin deficiency can be seen in brain as haemorrhages and degeneration of neurons of periventricular nuclei.

Riboflavin

Deficiency of this vitamin result in vascularisation of cornea of eye leading to cataract. Haemorrhages in eyes and opercula have also been reported.

Pyrodoxine

No detailed study on histological lesion has not been done however symptoms of deficiency suggest nervous system involvement.

Biotin

Deficiency result in cuticular thickening.

Folic acid

Deficiency leads to suppression of haemopoeisis in kidney and spleen. Absence of blast cells are the main feature.

Cynocobalamin (Vitamin B_{12})

Not much information is available.

Choline

Deficiency result in fatty infiliteration of liver and haemorrhages.

Ascorbic acid

Deficiency of Vitamin C results in poor wound healing, failure of granulation tissue to fibrose and abberant development of cartilages and bone.

Minerals

Very little is known about mineral deficiency in fishes except goitre caused by iodine deficiency. The hepato renal syndrome and renal calcinosis are suspected to be due to mineral imbalance.

Hepatorenal syndrome and renal calcinosis

The liver showed peribiliary cirrhosis and in kidney biliary hyperplasia, extensive tubular necrosis or fibrosis. In a number of species hyaline droplet deposition occurs within the cells of proximal convoluted tubules. Extensive cast formation and urolithiasis may result in later stages after considerable tubular necrosis and fibrosis.

Nephrocalcinosis or urolithiasis is characterised by deposition of calcium or magnesium salts within renal tubules.

Toxic components

Aflatoxin at the level 1 PPB can induce neoplastic changes in liver other metabolites inducing neoplasia are dimethyl nutrosamine and carbontetra chloride.

Mercury, cadmium and other heavy metals may cause degeneration and necrosis in proximal convoluted tabules of

Antibiotics and chemothesapeutics

Continuous therapy may induce toxic changes in tissues such as depression of haeomopoesis and especially with sulphonamides tubular necrosis and cast formation.

Binders

Chemically substituted cellulose binders in artificial feeds may cause hepato renal syndrome.

Gossypol

An ingradient of cotton seed which accumulate in liver and kidney causing severe liver degeneration and glomerulonephritis in kidney.

Diabetes mellities - May be produced in fishes due to protein/carbohydrate ratio change or feeding silkworm pupae. In these cases hypertrophy of islets of langerhans with beta cell degranulation and mesengeal scapillary wall thickening in glomeruli of kidney are the main histological features.

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