Society of Toxicologic Pathology – India (STP-I)

STP-I News Letter
Editorial team

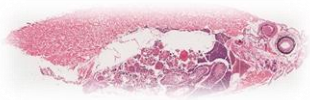
Dr. Praful Patel
Dr. M.R. Balaji
Dr. P.Kalaiselvan
Dr. Sowmya Bharat

Society of Toxicologic Pathology – India (STP-I)

"TOX-PATH" NEWSLETTER AUGUST 2020

The Zebrafish has become a prominent vertebrate model for disease and has already contributed to several examples of successful phenotype-based drug discovery. For the zebrafish to become useful in drug development more broadly, key hurdles must be overcome, including a more comprehensive elucidation of the similarities and differences between human and zebrafish biology. Recent studies have begun to establish the capabilities and limitations of zebrafish for disease modelling, drug screening, target identification, pharmacology and toxicology. As understanding increases and as the technologies for manipulating zebrafish improve, it is hoped that the zebrafish will have a key role in accelerating the emergence of precision medicine. This STP-I News Letter on "Zebrafish is the new mouse" will help fellow pathologist to better understand basic structural anatomy of Zebrafish.

August, 2020



Zebrafish is the new mouse!

Dr. Yougesh Murkunde, M.V.Sc.,PhD., DIBTP
CEFTE

NIH has ranked Zebrafish as the third most important experimental organism after man[1]. Does this surprise you? Let's have a quick review of the rapidly increasing popularity gained by the zebrafish use and its relevance to our toxpath community.

Frankly, Zebrafish needs no introduction anymore! You must be knowing that it is smallest, inexpensive, in-vivo vertebrate model which is easy to maintain, reproduce in large amount, manipulate genetically and develops rapidly. In 2013, the full genome sequence of zebrafish was published revealing approx. 70 % of the genes as Human homologue. And, 82% of human genes associated with disease have a zebrafish homologue[2]. CNS, digestive tract, pancreas, liver, CVS, kidney, musculature, and innate immune system; and many critical pathways that are required to grow these organs are highly conserved with humans! Thus, any type of disease that causes changes in these body parts in humans could theoretically be modeled in zebrafish. This high genetic and

organ system homology to humans provides multifactorial advantages of zebrafish over the classical vertebrate model for early screening of drug molecules[3].

Drug discovery today has evolved into many dimensions and scientists are looking I to many of the animal alternative platforms that included cell cultures, stem cells, 3D tissue models, organs on chips, in silico, as well as humanized chimeric mouse models and non-mammalian animal testing i.e. C. elegans and zebrafish[4,5]

Nevertheless, regulatory requirements are rigorous; thus, acceptance of alternative toxicology assays by government agencies rely on well-established predictivity of toxicity[6]. There comes zebrafish model in picture making a bridge between in vitro assays and mammalian in vivo studies. Recent studies have demonstrated the potential of zebrafish to serve as a tractable alternative to mice due to the congruence of cellular mechanisms, gene similarity, and comparable tissue biology. There is a long history of zebrafish being studied for genetics, cell biology, embryology, and environmental toxicology. Most toxicological studies using zebrafish have focused on environmental contaminants, but an increasing number are emerging in the field of pharmaceutical toxicology[7]. Now, one can still underestimate their relevance but nearly ten compounds from zebrafish screens are about to enter the clinic; and this could be enough to prove the ability to move fundamental discoveries from zebrafish to humans[8]. Let's see few anatomical and pathologically relevant information from published research.

Digestive system

The zebrafish digestive system contains liver, pancreas, gall bladder, and a segmented intestine.

Intestine

The zebrafish is agastric but an enlarged anterior intestine known as the intestinal bulb shows patterns of motility like the mammalian stomach[9,10]. Cross-sections reveals a mucosa, muscularis externa, and serosa layer. The intestinal mucosal contains many of same epithelial cell lineages found in mammals including absorptive columnar-shaped enterocytes, goblet cells, and enteroendocrine cells. Enterocytes have a basolateral nucleus, apical microvilli, and an intestinal brush border. An underlying lamina propria contains blood capillaries, lymphatic vessels, muscle fibres, and mesenchymal cells. The mucosal layer is covered by circular and longitudinal smooth muscle tiers of the muscularis externa[9,10]. However, in the zebrafish intestine the submucosa layer, crypts, Paneth cells, and Brunner's glands are absent[9,10]. Intestinal ridges resemble the spatially separate villi in the mouse or human small intestine. Based on the height of villar ridges, intestine can be divided into three segments. In the last section, ridges are absent and shorter and broader in the segment prior to that[10].

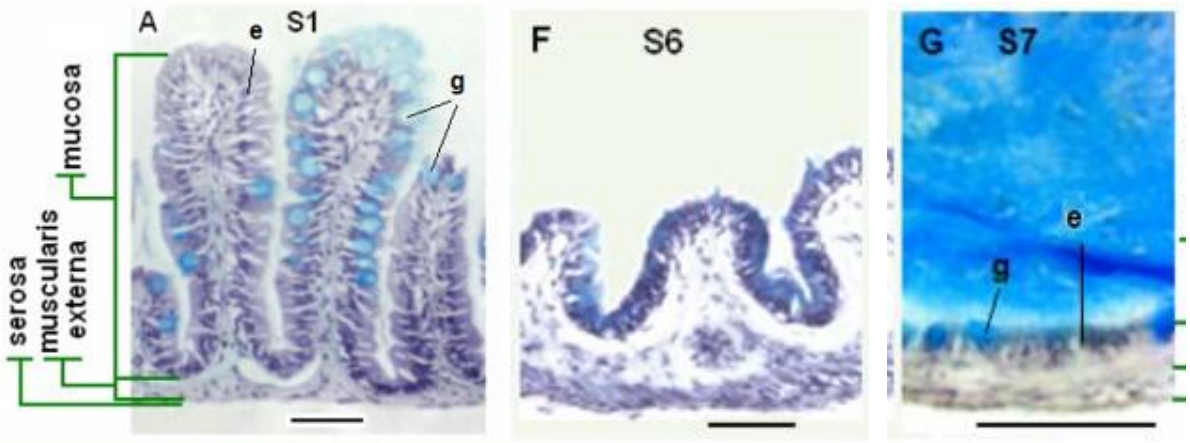


Fig. 1. Three type of segments, based on the height of villar ridges. Goblet cells (stained blue) are interspersed among the absorptive cells. Examples of enterocytes (e) and goblet cells (g) Scale bars: 50 μ m. (Source: Z. Wang, et al.2010)

Liver

Hepatic cellular composition, function, signaling, and response to injury as well as the cellular processes that mediate liver diseases are similar in zebrafish and humans[11].Zebrafish have been used to model neonatal cholestasis, cholangiopathies, such as polycystic liver disease, alcoholic liver disease, and non-alcoholic fatty liver disease[12]. The zebrafish liver encompasses three lobes that lie along the intestinal tract. The zebrafish liver differs from the mammalian liver in that the hepatocytes are not organized in cords or lobules; the portal triads are not apparent and Kuppfer cells are absent. The female hepatocytes are very basophilic as a result of the production of vitellogenin[13].

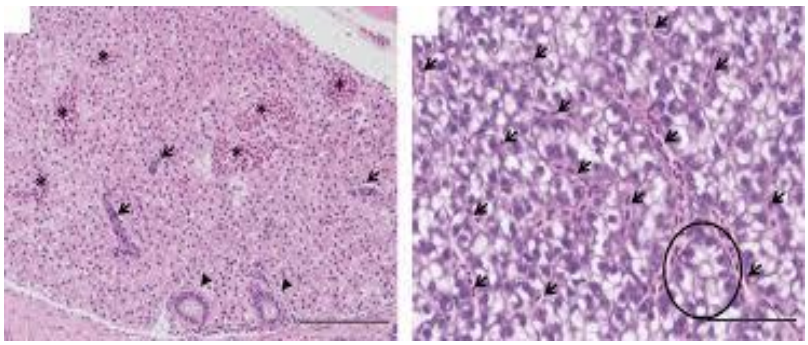


Fig. 2 Note the difference in staining of male and female zebrafish liver (Source: Vliegenthart et al[14]) (1) male zebrafish liver (H & E ×200). biliary ducts (arrows), bile ductules (arrowheads) and blood vessels (*). (2) female zebrafish liver (H&E ×400). sinusoidal spaces (arrows) and tubular arrangement of hepatocytes (encircled)

Pancreas

The zebrafish pancreas shares a basic structure and cellular makeup with the mammalian pancreas[15]. The pancreas is scattered along the right lateral aspect of the intestinal tract. One large islet and 3-6 smaller islets occupy the main pancreas. The tail of the pancreas is embedded with single beta cells or clusters of small islets[16]. An exocrine compartment produces digestive enzymes in zymogen granules, and an endocrine compartment (the islets), critical for blood sugar homeostasis[15]. The exocrine lobules are comprised of acinar glands and large ducts lined by cuboidal cells with a very dark, basophilic cytoplasm. The pancreatic islet consists of a central core of insulin-producing β-cells, surrounded by, glucagon-secreting β-cells, somatostatin producing β-cells, and ghrelin-producing ε-cells[13,15].

Fig.3 Pancreas. (a) Endocrine pancreas (Brockman body); (b) exocrine pancreas; (Source: Menke et al[15])

Heart

In the zebrafish, the heart is situated ventral to the esophagus. Deoxygenated venous blood enters the sinus venosus which is lined with a thin wall of collagenous connective tissue. The blood subsequently passes through the sino-atrial valve into the atrium. The atrium has a thin, muscular wall, and thin trabeculae form a loose meshwork in the lumen. The contraction of the atrium and dilation of the ventricle forces the blood into the ventricle via the atrioventricular valve. The ventricle has a much thicker wall than the atrium. There is a compact outer layer of muscle and a spongy inner layer with numerous trabeculae. The blood is pumped into the onion-shaped bulbus arteriosus via the ventricular-bulbar valve. The bulbus arteriosus has a thick wall consisting of fibro-elastic tissue and some smooth muscle fibers. From the heart, the ventral aorta distributes blood to the gills via the afferent branchial arteries [13,17,18].

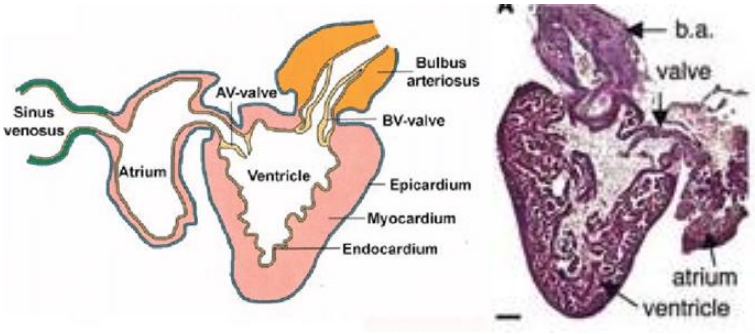


Fig 4. Heart: A- Schematic representation of the adult zebrafish heart, B. -H&E stained whole heart. (Source: A- doi:10.5339/gcsp.2013.4[19]; B-Poss, Kenneth D., et al[20])

Kidney

The zebrafish kidney lies in a retroperitoneal location, just ventral of the vertebral column. It has a distinct head and trunk regions. Similar to the mammalian kidney, it has nephrons with a glomerulus, proximal tubules, distal tubules, and collecting ducts.
However, the distal tubules are difficult to distinguish from the proximal tubules with routine H&E staining. The renal interstitium contains hematopoietic cells [13,21,22].

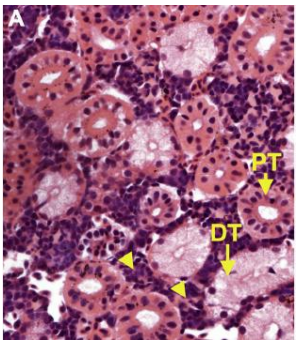


Fig 5. Kidney section includes proximal tubule (PT) (dark pink) and distal tubule (DT) (light pink) cross-sections along with a dense interstitial stroma (arrowheads) with intensely-purple stained nuclei that includes hematopoietic cells and is also the proposed location of renal progenitors.
(Source: https://doi.org/10.1016/j.trsl.2013.10.003 [23]**)**

EYE

The zebrafish eye is similar to the eye of all other vertebrates[21]. It consists of three layers: (1) the tunica fibrosa, which encompasses the cornea and the sclera; (2) the tunica vasculosa, which encompasses the choroid, the choroid rete, and the iris; and (3) the retina. The relatively flat cornea

consists of nonpigmented, stratified squamous nonkeratinizing epithelial cells, attached to a thick basement membrane considered to be analogous to the Bowman's membrane in mammals. Several layers can be distinguished in the retina: (1) the retinal pigment epithelium, in which photoreceptor cells (rods and cones) embed their outer segments; (2) the external nuclear layer, which contains the nuclei of the rods and cones; and (3) the bipolar cells that connect the rods and cones to (4) the ganglion cells, which send their axons through optic fibers that fuse into the optic nerve, which is connected to the brain [13,21].

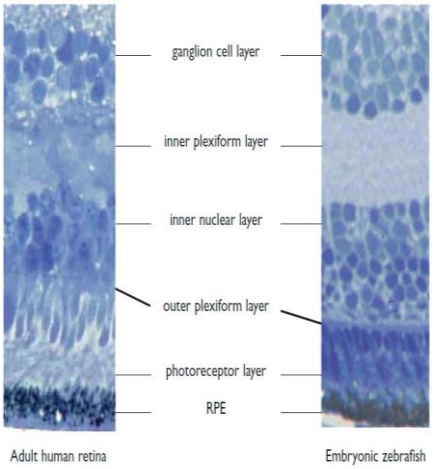


Fig:6 comparison of human and embryonic retinal layer (Source: P. Goldsmith and R. Solari [8])

Brain

The zebrafish brain is very similar in its basic components to the brain of higher animals and can be divided into five regions: the telencephalon, the diencephalons, the mesencephalon, the metencephalon, and the myelencephalon. The telencephalon is responsible for olfaction and for aspects of memory, reproductive behavior, feeding behavior, and color vision [13,24].

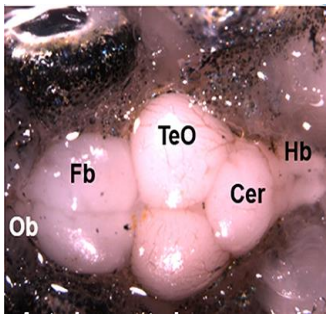


Fig 7, Brain: gross anatomy - Ob, olfactory bulbs; Fb, forebrain; TeO, optic tectum; Cer, cerebellum; Hb, hindbrain (Soure: Lindsey, B. et al.25)

SPLEEN

The zebrafish lacks lymph nodes. Together with the kidney, the spleen forms the major filtering organ for the removal of foreign agents and defective blood cells. Macroscopically, the spleen is a dark red organ, located in the peritoneal cavity, adjacent to one of the liver lobes. The splenic parenchyma consists mainly of erythrocytes and thrombocytes (red pulp) and periarterial sheaths of macrophages and reticular cells, supported by reticulin fibers, formed at the termination of splenic arterioles (ellipsoids) [13].

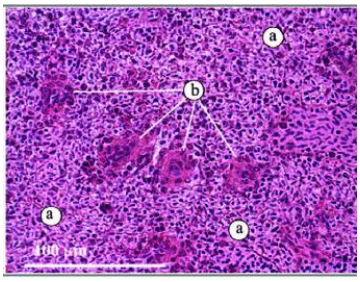


Fig 8. Spleen.
a) Red pulp, b) Ellipsoid. (Source: Menke et al [13])

THYMUS

The thymus is a bilateral organ consists of a uniform mass of lymphoid cells surrounded by a thin capsule of connective tissue and is linked to the pharyngeal epithelium along with the dorsomedial aspect of the brachial cavity and function as a maturation site for lymphocytes. Thin trabeculae extend into the thymic parenchyma, which supports the thymocytes and macrophages.

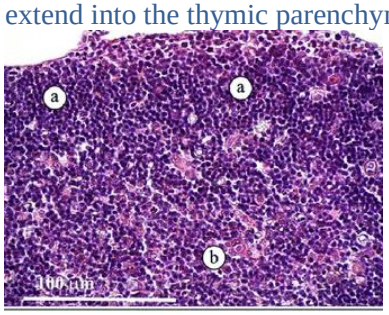


Fig 9. Thymus. (a) Cortex; (b) medulla. (Source: Menke et al [13])

Muscle

Structurally, zebrafish skeletal muscle is very similar to human muscle, and share the same muscle disease genes with humans. skeletal muscle is mainly composed of myocytes that develop from myoblasts. Myocytes are multinuclear, post-mitotic cells. individual muscle fibers (cells) are long and cylindrical cells, with multiple nuclei that are located on the perimeter of the fibers. Myoblasts differentiate, align, and fuse to make longer, multinucleated tubes called myotubes [13,26].

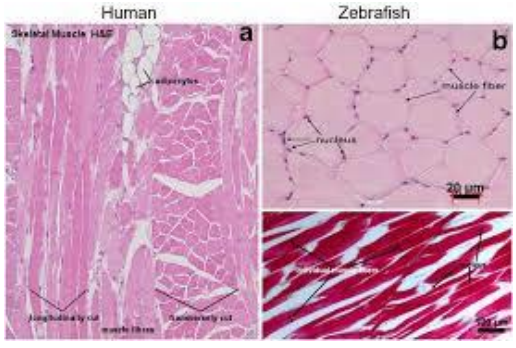


Fig 10
(a) H&E stained of human skeletal muscle fibers.
(b) H&E stained of muscle fibers in zebrafish (cross-section) and (c) Longitudinal.
(Source: Maiwulanjiang M [26])

Overall, toxicologic pathologists may appreciate the comparative histological features just like we did for rodents. Drug discovery researchers are driving high in utilizing zebrafish for drug development. Incidentally, much of the research uses larval stage and pathological processes are being evaluated by researchers using high end biomarkers. However, be it larvae or adult, world would need toxicologist pathologist's opinion for definitive information. Just like, phone camera has displaced 'point & shoot camera' off-the-shelf, Zebrafish may take over significant part of rodent research in near future. No doubt Pathologists would have to play significant role!

References :

1. https://www.fda.gov/consumers/consumer-updates/zebrafish-make-splash-fda-research
2. K. Howe et al., "The zebrafish reference genome sequence and its relationship to the human genome," *Nature*, 496:498-503, 2013.
3. J.R. Goldsmith and C. Jobin. "Think Small: Zebrafish as a Model System of Human Pathology', Animal Models of Human Pathology 2012. https://doi.org/10.1155/2012/817341
4. J. Kramer et al., "The application of discovery toxicology and pathology towards the design of safer pharmaceutical lead candidates". Nat Rev Drug Discov **6,** 636–649, 2007 https://doi.org/10.1038/nrd2378
5. S. Cassar, et al., "Use of Zebrafish in Drug Discovery Toxicology. Chemical research in toxicology, 33(1), 95–118, 2020. https://doi.org/10.1021/acs.chemrestox.9b00335
6. I.A. Freires et al., "Alternative Animal and Non-Animal Models for Drug Discovery and Development: Bonus or Burden?" Pharm. Res 34 (4), 681–686. 2017
7. G.R. Garcia et al., "Advancements in zebrafish applications for 21st century toxicology". Pharmacol. Ther 161, 11–21,2016
8. P. Goldsmith and R. Solari, "The role of ZEBRAFISH in drug discovery"2019. https://www.ddw-online.com/drug-discovery/p148422-the-role-of-zebrafish-in-drug-discovery.html#
9. Wallace K.N. et al., "Intestinal growth and differentiation in zebrafish. Mech Dev;122: 157–73, 2005.
10. Wang Z., et al. "Morphological and molecular evidence for functional organization along the rostrocaudal axis of the adult zebrafish intestine." *BMC genomics* vol. 11 392, , doi:10.1186/1471-2164-11-392,2010
11. Goessling W. and Sadler KC, "Zebrafish: an important tool for liver disease research". *Gastroenterology*.;149(6):1361-1377,2015. doi:10.1053/j.gastro.2015.08.034
12. Pham, Duc-Hung et al. "Using zebrafish to model liver diseases-Where do we stand?." *Current pathobiology reports* vol. 5,2: 207-221,2017. doi:10.1007/s40139-017-0141-y
13. MENKE A.L. et al. "Normal Anatomy and Histology of the Adult Zebrafish" Toxicologic Pathology, 39: 759-775, 2011
14. A. D. B. Vliegenthart et al. "Zebrafish as model organisms for studying drug-induced liver injury", Br J Clin Pharmacol , 78:6 , 1217–1227,2014.
15. Kinkel MD, Prince VE. On the diabetic menu: zebrafish as a model for pancreas development and function. Bioessays. 2009;31(2):139-152. doi:10.1002/bies.200800123
16. https://zfin.org/action/ontology/term-detail/ZFA:0000140
17. Poon KL, and Brand T. "The zebrafish model system in cardiovascular research: A tiny fish with mighty prospects" Glob Cardiol Sci Pract. (1):9-28, 2013. doi:10.5339/gcsp.2013.4
18. Hu N, et al" Structure and function of the developing zebrafish heart".Anat Rec. Oct 1; 260(2):148-57,2000.
19. Poon, Kar Lai, and Thomas Brand. "The zebrafish model system in cardiovascular research: A tiny fish with mighty prospects." *Global cardiology science & practice* vol. 2013,1 9-28. 2013, doi:10.5339/gcsp.2013.4
20. Poss, Kenneth D., et al. "Heart regeneration in zebrafish. (Reports)." *Science*, vol. 298, no. 5601, 2002, p. 2188+. *Gale Academic OneFile*, https://link.gale.com/apps/doc/A95914067/AONE?

21. Roberts, R. J., and Ellis, A. E. (2001). The anatomy and physiology of teleosts, In Fish Pathology (R. J. Roberts, ed.), 3rd ed., pp. 12–54. Philadelphia, USA: W. B. Saunders.
22. Reimschuessel, R., and Ferguson, H. W. (2006). Kidney. In Systemic Pathology of Fish (H. W. Ferguson, ed.), 2nd ed., pp. 91–118. London, UK: Scotian Press
23. McCampbell KK and Wingert RA," New tides: using zebrafish to study renal regeneration" Translational Research, 163: 2, Pages 109-122,2014.
24. Wullimann, M. F., et al, "Neuroanatomy of the Zebrafish Brain: A Topological Atlas". Basel, Switzerland: Birkhauser, 1996
25. Lindsey, B. et al. "A Whole Brain Staining, Embedding, and Clearing Pipeline for Adult Zebrafish to Visualize Cell Proliferation and Morphology in 3-Dimensions. Frontiers in Neuroscience 2018. 11. 10.3389/fnins.2017.00750.
26. Berger J, & Currie P. D., "Zebrafish models flex their muscles to shed light on muscular dystrophies". Disease models & mechanisms, 5(6), 726–732,2012. https://doi.org/10.1242/dmm.010082
27. Mamuti Maiwulanjiang, "Histological Characterization of the Exercise-Dependent Alterations in the Musculoskeletal System of Zebrafish" Dissertation, UNIVERSITÄTSKLINIKUM HAMBURG-EPPENDORF, 2018

EIGHTH CONFERENCE AND CONTINUED EDUCATION PROGRAM ON TOXICOLOGIC PATHOLOGY OF SENSORY ORGANS, MEDICAL DEVICE AND DIGITAL PATHOLOGY

Conference is postponed indefinitely until further notice. The Coronavirus (COVID-19) global pandemic continues to impact all of our lives and must be viewed by all of us as a serious and evolving situation. The STPI will continue to assess the situation and will keep the members posted on the new date as and when it is scheduled. Please return to the STPI website or contact us for the most recent status of STP sponsored events.

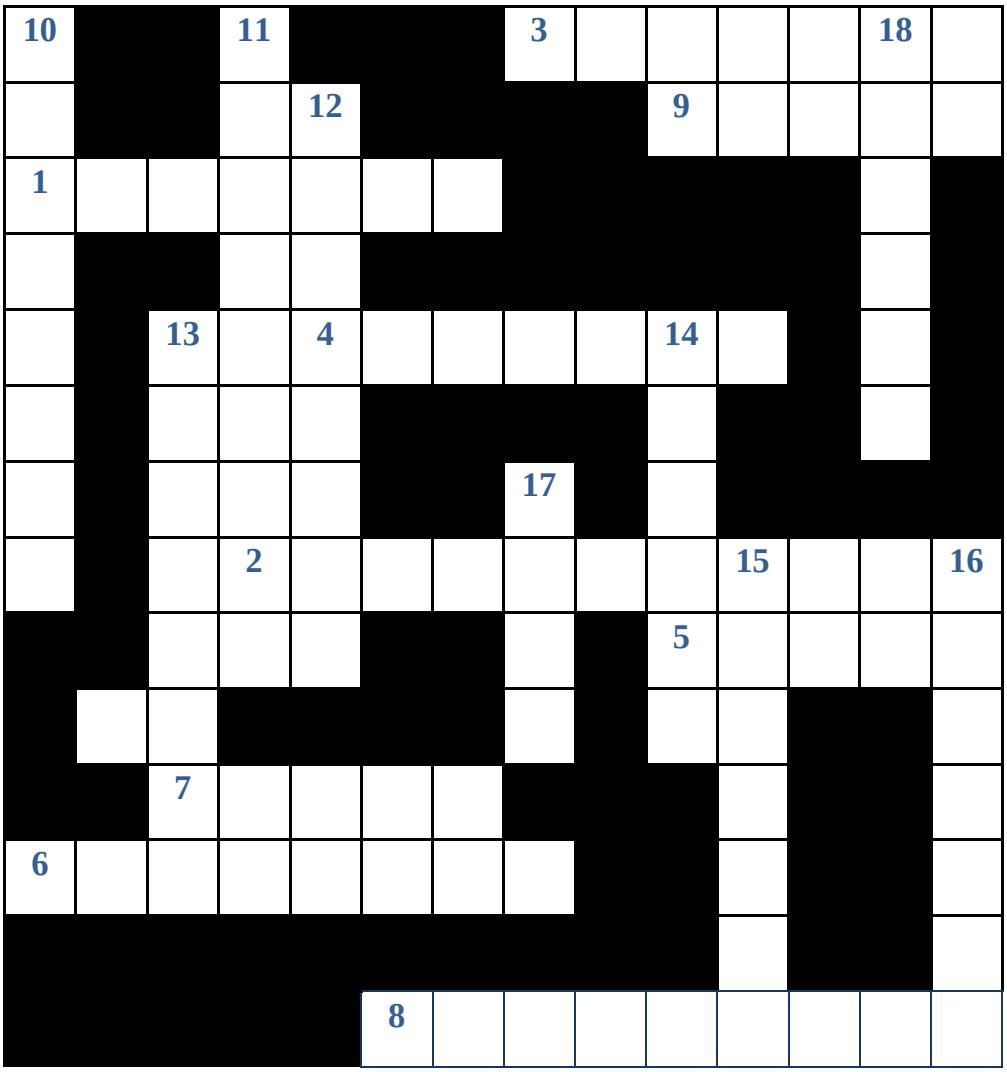
The STPI expresses our collective hope that all of our members and their families are safe and well during this challenging time.

STP-I CENTRAL COUNCIL

TEST YOUR KNOWLDEGE

CROSS WORD :

PREPARED BY : Dr. Jomy Jose, M.V.Sc., DIBTP



Across

1. Most common skin OR subcutis tumor of rat
2. Dilation of pre-existing blood vessels
3. Birth defect
4. Waste away
5. Swelling
6. Affecting the whole body
7. Sudden onset, short course
8. Loss of cell differentiation
9. Lack of breathing

Down

10. Abnormal collection of fluid in body space
11. Gray-colored gland; lies intraorbitally
12. Prolactin-inhibiting factor
13. Has exocrine and endocrine functions
14. Elicit immune response when attached to large carrier protein
15. Benign tumour of glands
16. Tumour of bone, muscle, soft tissues
17. A unit of heredity
18. An abnormality in tissue

Answers on page number 12

IBTP exam 2020

INDIAN BOARD OF TOXICOLOGIC PATHOLOGY (IBTP)

IBTP board of directors have decided to postponement of 2020 IBTP examination till further notice. This is due to our inability to implement IBTP training schedule due to COVID-19 pandemic impacting travel. IBTP training workshops fulfilled the primary objective of providing adequate practical training to prepare candidates for the examination.

IBTP Webinar Series

IBTP initiated webinar series on Continuing Education in Toxicologic Pathology from July 2020

Webinar #1 : What is an artifact in Pathology? What is real and how to interpret?

- Dr. S. K. Vijayasarathi President, IBTP, Expert Pathologist, Department of Safety Assessment, Eurofins Advinus Limited, Bengaluru presented the first webinar on **What is an artifact in Pathology? What is real and how to interpret? on** 4th July 2020, Saturday at 7 PM

Webinar #2 : Continuing education in Toxicologic Pathology - Male Reproductive System

- Dr. Shekar S. Chelur, M. V. Sc., DABT, DIBTP, Director, Preclinical Safety Evaluation, Aurigene Discovery Technologies Ltd, Bengaluru presented the second webinar on **Continuing education in Toxicologic Pathology- Male Reproductive System on** 18th July 2020, Saturday at 7 PM.

Webinar # 3 : Continuing education in Toxicologic Pathology- Urinary System

Dr. Kamala Kannan, MVSc., DIBTP; Head Pathology, Eurofins Advinus Ltd., Bengaluru. will be presenting third webinar on **Continuing education in Toxicologic Pathology- Urinary System on** 15th August 2020, Saturday 7 PM.

Register for webinar by clicking following link

https://toxpathindia.com/evrplus_registration/?action=evrplusegister&event_id=20

STP-I SPONSORED WEBINAR

STP-I in association with IATP recently held a webinar on Digital Pathology on July 31, 2020. The Topic of Webinar : "What Toxicologic Pathologists Need to Know About Digital Pathology: The Basics to Get Started" delivered by Aleksandra Zuraw, DVM, PhD.

The webinar covered the basic concepts like digital pathology terminologies, including such concepts as artificial intelligence, machine learning, deep learning and image analysis and dependencies between them. Aleksandra Zuraw, DVM, PhD is a board certified veterinary pathologist at Charles River Laboratories in Frederick, MD, USA

Dear STP-I Members
Together we can make 'Toxpath' better...!!!

You are requested to communicate your recent professional updates such as

1. Update on educational qualifications
2. Participation in international seminars (symposium overview of 3-4 paragraphs)
3. Oral and poster presentation in international symposiums
4. Guest lectures in symposiums other than STPI conferences
5. Your recent publications in national and international journals
6. Advanced training in field of Toxicologic Pathology
7. Articles, short communication and abstracts
8. Histomorphological illustrations and diagnosis

We will also consider to post

1. Job Postings in Toxicology and Toxicopathology
2. Poll and survey results (Relevant to Toxicopathology).
3. Reviews of books, chapters, new instruments and techniques.
4. Mini Review (Relevant to Toxicology and Toxicopathology)
5. Fun-Time: Cartoon/ pictures/PJ's relevant to our Domain

Send communications and feedback to Editor through
E-mail : stpi.india@gmail.com

CROSSWORD ANSWERS

Across	Down
1. Fibroma	10. Effusion
2. Angiectasis	11. Harderian
3. Anomaly	12. Dopamine
4. Atrophy	13. Pancreas
5. Edema	14. Hapten
6. Systemic	15. Adenoma
7. Acute	16. Sarcoma
8. Anaplasia	17. Gene
9. Apnea	18. Lesion

*****END OF DOCUMENT*****