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Methodology Article

Histological Methods in Life Science

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Abstract: Histology is the microscopic study of plant and animal tissues. Although all organisms are comprised of at least one cell, we will be focusing on observing cells and tissues of the human body. Histology is the study of tissue structure, extending from the level of the individual cell, through organs to organ systems. Histology is obviously related to Cell Biology and to Anatomy; it also forms the structural basis for understanding Function and is the preparation for the study of abnormal structure and function. In this methodology articles histology followed various methods clearly explain researchers and science innovators.

Keywords: Histology, Embedding, Tissues, Scanning Electron Microscope

1. Introduction

What is Histology?

Histology is the study of tissue structure, extending from the level of the individual cell, through organs to organ systems. Histology is obviously related to Cell Biology (Cytology) and to Anatomy; it also forms the structural basis for understanding Function (Physiology) and is the preparation for the study of abnormal structure and function (Pathology). Pathophysiological (clinical) examples will be utilized to illustrate aspects of functions and their significance. The principal aim of the course is to present knowledge of tissue structure which is sufficient for the understanding of Physiology. Technology continuously [1] tissues may be considered as aggregations of cells (of one, or more usually, several types) which serve a particular function or set of functions. From this definition it will be seen that the concepts of structure and function are essential to the study of Histology [2]

Particular skills which will be emphasized are:

A) Classification of tissues — essentially a verbal skill. Consequently it is important in order to pay close attention to the way tissues are described. This means concentrating on the precise and correct use of language — an essential skill in sciences, and the health-care professions.

B) Recognition of specific features and the application of criteria of classification — observational and reasoning skills.

C) Most importantly, the relationship between structure and function. This means bringing together knowledge from different fields (e.g. Anatomy, Biochemistry and Physiology) — synthetic and deductive skills.

Histology is the microscopic study of plant and animal tissues. Although all organisms are comprised of at least one cell, we will be focussing on observing cells and tissues of the human body. All organisms are composed of cells. Humanoid body cells are grouped by their similarities in structure and function into tissues. There are greater than 200 different types of tissues in the human body and all of these may be categorized into one of four groups: Epithelial Tissue, Connective Tissue, Muscle tissue and nervous tissue. In order to display these tissues, samples were taken from organs [1]. Organs are macroscopic structures which are composed of more than one tissue type and perform a definite function in a multi-cellular organism. Because you will be observing sections of organs, you will see several different tissues in each slide so it is important to scan the slide in order to find the tissue of interest. The thinly sliced sections of organs were mounted and stained with dyes (commonly hematoxylin and eosin) or stains that provide blue and red color to the tissues. In some cases, differential stains are used to stain specific

structures. As you view the different slides, it is tantamount to your advantage to view all of the slides within each group before observing slides of a discrete group. For example, you may start with connective tissues so you could view all of the slides or tissues that are grouped within this category identifying common characteristics before proceeding to the epithelial tissues.

Type of material obtained in laboratory

The human tissue comes from the surgery and autopsyroom from surgery two types of tissue is obtained.

1. As biopsy- a small piece of lesions or tumor which is sent for diagnosis before the final removal of the lesion or the tumor (Incisional biopsy).

2. If the whole of the tumor or lesion is transmitted for examination and diagnosis by the pathologist, it is called exceptional biopsy.

3. Tissues from the autopsy are directed for the study of disease and its course, for the advancement of medicine.

2. Material Methods

2.1. Light Microscopic Studies

Generally, most of the histological studies are conducted on the dead cells or tissues. For microscopically studies, cells or tissues have to pass through the process such as fixation, dehydration, embedding, sectioning, and staining [3]. Fixation methods, the term fixation means to immobilize. Fixing solution completes the following functions. It prevents bacterial decay and autolysis of the cells, renders the components of the cell stable, reduces the visibility of different cellular components and prepares the cells for staining. In the experimental study was followed on 10, 20 and 30th day, fish were taken out, sacrificed and the tissues of Gill, liver, kidney, and intestine were excised out. After cutting them into small pieces they were transferred immediately to the fixative.

2.2. Chemical Fixation Methods

The choice of suitable fixative generally depends on the type of analysis and the chemical nature of atypical cells [10]. In the present study of alcoholic brain's solution was used asfixative. The period of fixation was 1-3 days.

Picric acid – 1gm

5% Formaldehyde -60 ml

Glacial Acetic acid – 15 ml

80% Alcohol – 150 ml

The tissues were as shown in freshly prepared fixative and after the period of fixation. They were moved directly to 70% alcohol. Dehydration methods, after the fixation, water molecules from the cells or tissues were removed. These processes are recognized as dehydration. In the experimental study, progressively higher grades of alcohol prepared from rectified spirit were used to dehydrate the tissues. Gruesomebe used for dehydration were 30%, 50%, 70%, 90% and 100% (absolute alcohol). In the stepwise dehydration, the tissues were kept along with the progressive alcohol grades for the following duration: 30% and 50% grades for 5 to 10 hours: in 70% grade for 10 to 12 hours: 90% grade for 10 to 12 hours: in 90% grade for about 15 hours and in 100% absolute alcohol grade for 6 to 8 hours [9].

2.3. Clearing and Fixation Methods

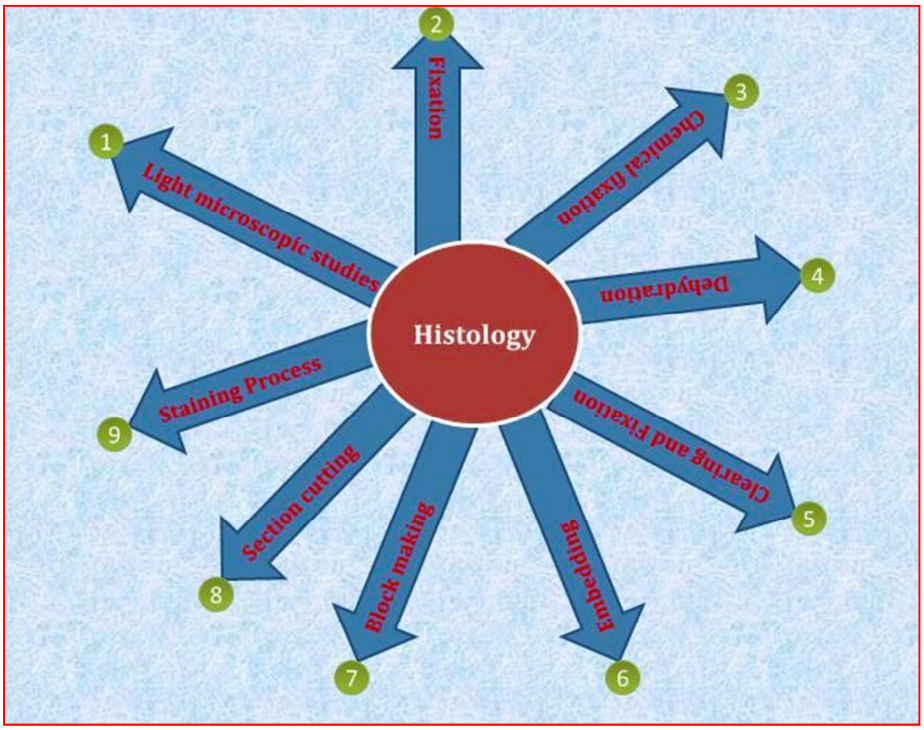


Figure 1. *Histological process schematic diagram.*

Xylene was used as the clearing agent because the tissues contain alcohol so it has to be removed from the tissue to make it firm for the purpose of section cutting [4]. The tissues were kept in xylene for about 2 to 4 hours. Xylene was not only bringing about the infiltration of paraffin into tissues but also make them transparent by removing their opacity. Hot and cold infiltration was done in under to ensure that all the xylene/ airspace are replaced by paraffin wax (Figure 1).

2.4. Embedding Methods

In the process of embedding the material was inserted into certain supporting media of sufficient hardness for cutting thin sections with the microtome. Paraffin wax was used in the process of embedding. The embedding process involves soaking of the tissue in molten wax at a standard temperature, coinciding with the melting point of the embedding medium used.

2.5. Block Making Methods

After embedding the tissue with wax, it was cast into a block of paraffin. This process is called block making or casting of the block. Mold of 'L' pieces was adjusted in such a way to accommodate the object. The mold was full of molten paraffin wax. The impregnated tissue was placed in the mold according to the plane of section needed. Immediately fiery tip of the needle was transferred in the molten wax on all the slides of the tissue. This was done to remove the air bubbles. The label carrying all the details of the tissue was attached on one side of the mold. Gently the air was blown into the mold; the wax forms a thin layer on the surface. The mold was gently immersed in cold water so as to ease the wax rapidly. When the block becomes solid it was taken from the water [5].

2.6. Trimming the Block Methods

The prepared block was trimmed into correct shape for section cutting: Mounting the block. The trimmed block was included with a holder and inserted into the jaw of the microtome.

2.7. Methods of Removing Bubbles Trapped Beneath the Sections

Bubbles may get trapped under a section while in the tissue floatation bath. This need to remove before the section is picked on the slides this may be done by:

i. Which the edge of the slide.
ii. Can be teased out with bent dissecting needle.
iii. Place the sections on the slide and run 2% alcohol under them. Any folder or bubble is removed.

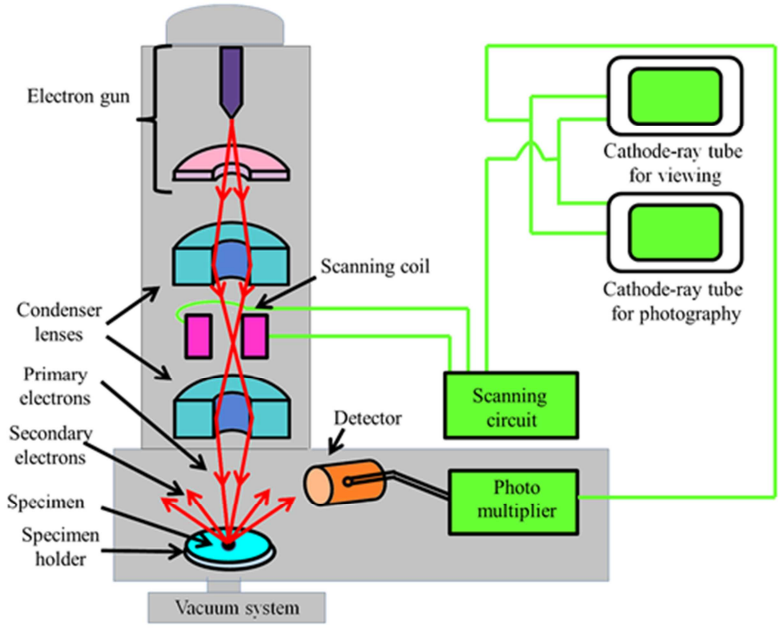
To cut a tissue which has a tendency to crumble or fragment while cutting. With the mouth open and sounding a soft long drawn 'H' thus 'h ' exhale gently on to the section, as it leaves the knife and cut very slowly. This also serves to reduce the effect of static electricity. If sections to fragment due to a large amount of blood in the tissue, the block should be coated with colloid in between sections. The surface of the block should be wiped dry and painted with a camel hair brush which has been dipped in 1% in Celloidin. After allowing a few seconds for the Celloidin to dry a section in acut in the usual way [6]. It must be borne in mind that when floating the sections to remove the creases. Thecolloidal layer must be uppermost, and the water should be a little hotter than usual to counteract the effect of colloids. Following drying in the usual way, the collodion is removed with equal parts of ether and alcohol before removing wash with xylol.

2.8. Serial Sectioning Methods

Serial sectioning may be needed to study the tract of some structure or to find the extent of lesions. Sections are collected from the very first cut that includes any tissue. Ribbons of ten - 1-10, 11-20, 11-20, and 21-30 soon are picked up and installed on the slides. Step sections methods: Thistakes the place of serial sections and for the same reasons. Sections are taken at periodic level through the block. The request is made for every 'n the section for a total of 'n' sections. Intermittent sections or sections on either side of each step sections for a total of 'n' sections. Cooling block and knife, in general, keep ice cubes ready at hand and cool the surface of block and knife before cutting, always dry and block and knife and block after the application of the knife.

2.9. Mounting and Spreading the Ribbon Methods

For installing the material, glass slides were accustomed. Slides were smeared with adhesive Mayer's albumen because the section should remain fixed to the slide while staining subsequently [7]. The tiny piece of ribbon was given to a slide and floods it with water by dropper. Then the slide was placed on the hot plate to heat the water; thereby the paraffin ribbons begin to stretch. Soon after the ribbon was completely stretched the slides were derived from the hot plate and the water was drained off and permitted to dry. Labeling: By thinking I need a diamond pencil; the slides were labeled after the slide was completely dried: Staining Process. The slide was retained in xylene for 30 minutes to 1 hour to deparaffinized. Then the slides were to be sent through the low-grade series of 100%, 90%, 80%, 70%, 50% and 30% ethyl alcohol and water. First, the slides were stained with hematoxylin for 2 to 5 minutes and then they were washed in water. After that, the slides were dehydrated by passing through ascending order alcohol series up to 70%alcohol. Secondly, the slides were stained with eosin for 2 quick dips and then washed in fresh grade 70% alcohol. Dehydration was done through ascending grades of 80%, 90% ethyl alcohol and absolute alcohol I and II for 5 to 10 minutes. Slides were admitted to absolute alcohol for complete dehydration. Then slides were passed into xylene I for clearing. One or two changes of xylene were given and then finally mounted in DPX Mountain.



Source: National programme on technology enhanced Learning

Figure 2. *Scanning electron microscope Schematic diagrams.*

2.10. Scanning Electron Microscopic Study

The tissues were dissected out, washed repeatedly in 0.2M phosphate buffer and then set at 3% glutaraldehyde. Dehydration was done in acetone grades and was accompanied by critical point drying. Ultimately dried tissues were mounted on the stub and were sputtercoated with gold in a gold coating unit (thickness 100A) and were examined and photographed using JEOL JSM 6360 scanning electron microscope (SEM) Japan [8].

3. Conclusion

In the present methodology paper have provide deals with medical and life science researchers, drug design and histopathology field to concern promote clear structural methods. In current scenario histopathology most developed essential medical and life science researches hence we provide novel customized methodology paper use to other.

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References

[1] Tamizhazhagan V, Pugazhendy K, Sakthidasan V, Jayanthi C. The toxicity effect of monocrotophos 36 E. C% on the histological changes in Gill of *Labeo rohita* (Hamilton, 1882) International Journal of Innovative Research in Multidisciplinary, 2, (11) 435-439- 2016.

[2] Alan and Tuffery, (2007) Histology Information on Textbooks. 1-5. chapters www.coursehero.com/file/12219349/AllMedSciManual07/.

[3] Gurr, E. Methods for analytical histology and histochemistry. Leonard Hill (Books) Ltd. London, 1959.

[4] Lab Exercise: Histology (Revised Spring, 2012), Page 6 of 17.

[5] Bozzola JJ, Russell LD. Electron microscopy: principles and techniques for biologists. Boston: Jones and Bartlett, 1992.

[6] Wilson KS, Christiansen TA, Quick C. External fixation in maxillofacial surgery, Otolaryngol Clin North Am. 1976, 9(2):523-32.

[7] Eltoum I, Fredenburgh J, Grizzle WE. Advanced concepts in fixation: 1. Effects of fixation on immunohistochemistry, reversibility of fixation and recovery of proteins, nucleic acids, and other molecules from fixed and processed tissues. 2. Developmental methods of fixation. *J Histotechnol* 2001; 24; 201-210.

[8] Roy, P. K. Munshi, J. S. D. and Datta Munshi, J. (1986): Scanning electron microscopic evaluation of the effects of saponin on the gills of the climbing perch, Anabas testudineus (Bloch) (Anabantidae: Pisces). Indian J. export. Bio I. 24:511-516.

[9] Peter jamieson, (1928). An Introduction to the technique of section -cutting reimburse E. & S. livingstone r6-r7 teviot place pp-70-73.

[10] Tamizhazhagan V, Pugazhendy K, Sakthidasan V, Jayanthi C, Barbara Sawicka, Shuuduv Gerlee, Ramarajan K, Manikandan P,(2017), The toxicity effect of pesticide Monocrotophos 36% E. C on the enzyme activity changes in liver and muscles of *Labeorohita* (Hamilton, 1882), 8 (5), 60-67.