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Histological And Histochemical Study of Peritoneal Mast Cells of Albino Rat Using Two Types of Fixatives.

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Abstract:-
Introduction: *Mast cells have a widespread distribution and are found predominantly at the interface between the host and external environment.*
Aim and Objectives: *The main purpose of the present work is to histologically and histochemically study the properties of Peritoneal Mast cells of Albino Rat.*
Materials and Methods: *The stretch preparation of tissues were taken from albino rat and fixed in two types of fixatives; Formal Alcohol and Neutral buffered formalin.Then subjected to various histochemical stains.*
Results: *Peritoneal mast cells were found in good amount in Albino rat they are better stained in toulidine blue at ph 4.4 in neutral buffered formalin than in formal alcohol while with toulidine blue at ph 2 the reactions are similar with both the fixatives.Heparin Trisulphate was substantial whereas Heparin monosulphate and Neutral mucopolysaccaride were presentl in small amount in mast cells of albino rat.*
Conclusion: *Heparin Trisulphate is an active form of Heparin and is the main constituent of the peritoneal mast cell granules of Albino rat.*
Keywords: *Peritoneal mast cells, Albino rat, Heparin, Histochemistry.*

I. Introduction

Mast cells were first described by Paul Ehrlich in 1878[(1)].He belived in the functional importance of this cells and specially of its granule content whose staining reaction with basic aniline dyes led him to coin the word "Metachromasia".In 1937,Holmgren and Willander[(2)] observed that tissues that displayed a great number of Ehrlichschen Mastzellen"(Mast cells) were enriched in Heparin.The later years witnessed the establishment of relationship between mast cells, heparin and anaphylaxis which was supported by the discovery that histamine was present in the Mast cells (Riley and West 1952) [(3)] and released along with the heparin during anaphylactic shock(Rochae and Silva 1947) [(4)].

Heparin exists in mast cells in two forms as heparin monosulphate and heparin trisulphate.Heparin monosulphate falls in weakly sulphated group whereas Heparin trisulphate in highly sulphated group. According to Schiller 1963[(5)],Heparin is a heterogeneous polymer composed of alternatimg hexosamine and uronic acid moieties in equimolar quantity, the hexosamines is glucosmine and the uronic acid is a mixture of L- iduronic acid and D-glucornic acid .Ringertz[(6)] 1963 believes that there exists in cytoplasm of mast cells a number of different types of heparin some of which have their amino group,acetylated and some of which are sulphated.As such there are mast cells in different regions of different species and may be within the different regions of the same organ of the same species which have different biochemical properties.

II. Aim and Objectives

The aim of the present work is to histologically observe the distribution and histochemically study the properties of peritoneal mast cells of albino rat in two different fixatives using various histochemical stains.

III. Materials And Methods

- **Collection of specimen:**

The following animal comprise the study subject in present work.
Order Rodentia- Rattus Novegicus(Albino Rat)
Biopsy:-
Albino rat was anaesthetised and abdomen was open vertically from xiphoid process to pubic symphysis.Jejunum and ileum were indentified.The Strech preprations were made on chemically clean glass slides and immediately placed in fixatives.
Fixation: Following fixatives were used for fixation of stretch prepration and tissues
a) Neutral buffered(PH-7)Formalin
$NaH_2P0_42H_20$ -4.52gm
+

$Na_2HP0_42H_20$ -8.15gm
Analytic formalin-100ml
Distilled water-900ml

b) 10% Formalin in absolute alcohol

Formalin 10ml
Absolute Alcohol 90 ml

The stretch preparation of tissues were fixed for twenty four hrs in formal Alcohol and Neutral buffered formalin .Then they were directly subjected to different staining procedures after rising in distilled water.

- **For Histochemical Study, the following staining procedures were applied.**

1) For Highly sulphated acid mucopolysaccaride(Heparin Trisulphate)

a)Alcian Blue PH-1
b)Alcian Blue PH-1 after active methylation (Spicer,S.S,1960) [7]
C) Aldehyde fuschin

2) For weakly sulphated acid mucopolysaccaride(Heparin Monosulphate)

a) Alcian Blue PH-2.5(Spicer et al ,1967) [8]
b) Alcian Blue PH-2.5 after mild methylation(Spicer et al ,1967) [8]

3)For simultaneous distribution of highly and weakly sulphated acid mucoplysaccarides

a)Aldehyde Fuschin- Alcian Blue PH-2.5 Procedure

4) For simultaneous demonstration of sulphated and Neural mucoplysaccarides

a)PAS
b)Alcian Blue PH-1-PAS Procedure
c)Alcian Blue PH-2.5- PAS procedure

5) Toludine Blue for Metachromic properties at different PH

a)At PH-2
b) At PH-4.4

Histochemical reactions of mast cells granules were studied in order to determine the nature of reactive substance with respect to:
i)Heparin Trisulphate
ii)Heparin Monosulphate
iii)Neutral mucopolysaccaride

IV. Observations and Results

A) Morphology and distribution of peritoneal Mast cells of Albino Rat.

i) In Neutral Buffered Formalin- Mast cells are present 10-12/high power field(hpf) in toludine blue stained material but are about 20-22/hpf in Aldehyde fuschin stained mesentry.They are hardly 7/hpf in Alcian blue stained material at PH-2.5.The mast cells are mostly oval with central unstained nucleus and the cytoplasm is filled with metachromatic coarse granules. The granules are deep purple.

ii) In Formal Alcohol the mast cells are 12-13/hpf in Toludine Blue Stained material but are about 20/hpf in Aldehyde fuschin stained mesentry .Here also the mast cells are mostly oval with deep purple metachromic granules.In some mast cells the granules are seen outside the cell membrane which is probably due to mechanical handling during making strech prepration.

B Occurence of different types of mucoplysaccarides in peritonel mast cells of Albino Rat(table no.1)

i**) Neutral buffered formalin**

- With this fixatives the mast cells Stain deep purple with toludine blue at PH-4.4 and moderate purple with toludine blue at PH-2
- With alcian blue at PH-2.5 the mast cells stain moderate blue and this alcianophilia is reduced after active methylation.
- With aldehyde Fuschin-Alcian Blue PH-2.5 the mast cells stain moderate purple.They are unreactive to PAS staining procedure but stain light blue with Alcian Blue PH 2.5PAS and Alcian Blue PH 1 PAS procedure.

These staining reactions indicate the presence of highly sulphated form of heparin but also small amount of weakly sulphated form.

ii)Formal Alcohol

- With toludine blue PH 4.4 the mast cells stain deep purple and similar reaction is obtained with toludine blue at PH 2
- With Alcian Blue Ph -2.5 the mast cellsstain moderate blue and also give similar reaction with Alcian Blue at PH-1. This Alcianophilia is reduced to faint blue after mild methylation in the former and active methylation in the later.
- With aldehyde fuschin they stain moderate purple with Aldehyde fuschin –alcian blue Ph-2.5 also they give moderate purple reaction. The mast cells are unreactive to PAS staining procedure but stain moderate to red with Alcian Blue PH-2.5 PAS stain.
- With Alcian Blue PH-1 Pas Staining some mast cell stain light blue while others give the moderate red reaction.

These staining reactions indicate the presence of substantial amount of Heparin trisulphate and small amount of heparin monosulphate and neutral mucopolysaccaride.

Table No1: Histochemical reactions of mast cells in mesentry of Albino Rat fixed in different fixatives.

Fixatives	TB at pH 4.4	TB at pH 2	AB at pH2.5 UT	AB at pH 2.5 mild MeoH	AB at pH1 UT	AB at pH1 active MeoH	AF	AF-AB pH2.5	PAS	AB pH 2.5-PAS	AB pH 1-PAS
NBF	4P	3P	3B	1B	2B	1B	4P	3P	0	2B	2B
FA	4P	4P	3B	1B	2B	1B	3P	3P	0	3R	2B,3R

Abbreviations:NBF=Neutralbufferedformalin,FA=Formalalcohol,TB=Toloudineblue,AB=Alcianblue, AF=Aldehydefuschin,PAS=PeriodicacidSchiff,UT=Untreated,MeoH=Methylation,P=Purple,B=Blue,R=Red. Staining colour of mast cells: 0= unstained, 1= Faint,2=Light,3=Moderate,4=Deep

V. Discussion

Selye 1965[(9)] has defined mast cell as connective tissue element which possesses cytoplasmic granules that stain metachromatically under ordinary condition.This definition demarcates the mast cells from epithelial elements containing metachromatic granules of mucin and even from closely related basophils which are blood born and myelogeneous.He found alcoholic formalin highly satisfactory in preserving the metachromatic granules as well as trace amounts of various metal in mast cell granules.

Ehrlich (1878) [(1)] Coined the term "metachromasia" to designate staining reactions of structures with certain cationic aniline dyes in a tone different from that possessed by the dye as seen in the reaction of mast cell granules.

In the present work the identification of the mast cell is based on metachromatic reaction of its granules with acidified toludine blue at PH-2 buffered Toludine blue at PH-4.4 ,Aldehyde Fuschin and Alcian Blue at PH-2.5. Toludine blue has been used at two different levels so as to identify both types of mast cell granules containing both lower and higher sulphates of Heparin.The distribution of peritoneal mast cells have been studied only with the purpose of showing the frequency distribution so as to study the histochemical reaction that reflects upon the patternof distribution of mast cells in the mesentry of albino rat.We found 10-12/hpf in Aldehyde fuschin,7/hpf in Alcian blue whereas with formalin alcohol 12-13/hpf in toludine blue 20/hpf in Aldehyde form,mast cells were found.

This finding correlates with that of Hardy and Weshbook(1895) [(10)] who found mast cells in good amount in the peritoneum of rat.The present study revealed the presence of highly sulphated form of heparin in substantial amount and weakly sulphated heparin in small amount in peritoneal mast cells of Albino rat.Lennert 1959 [(11)] and Combs et al 1965[(12)] suggest that the purple to red purple colour of rat mast cell granule is due to higher sulphate of heparin.The staining reaction in the present work indicate the presence of substantial amount of heparin Trisulphate and small amount of heparin monosulphate and neutral mucopolysaccarides.Comptom1982[(13)] noted that reactivity to PAS in formal alcohol may be due to presence of neutral mucopolysaccaride or Heparin mono-sulphate.

VI. Conclusion

A comparative histochemical reaction of mast cells in two fixatives showed that mast cells are better stained in toulidine blue at ph 4.4 in neutral buffered formalin than in formal alcohol while with toulidine blue at ph 2 the reactions are similar with both the fixatives.Heparin Trisulphate is an active form of Heparin and is the

main constituent of the peritoneal mast cell granules of Albino rat.Heparin Monosulphate and neutral mucopolysaccaride is present in small amount.

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