



A Comparative Histological and Histochemical Study of Peritoneal Mast Cells of Albino Rat and Rabbit

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ABSTRACT

Introduction: Mast Cells Is Ubiquitously Present In Connective Tissue Of The Vertebrates And Has Numerous Functions And Plays The Foremost Role In Anaphylactoid Reaction.

Aim And Objectives: The Main Purpose Of The Present Work Is To Make A Comparative Histological And Histochemical Study Between Peritoneal Mast Cells Of Albino Rat And Rabbit.

Materials and Methods: The Stretch Preparation Of Tissues Were Taken From Albino Rat And Rabbit And Fixed In Two Types Of Fixatives; Formal Alcohol And Neutral Buffered Formalin. Then Subjected To Various Histochemical Stains.

Results: Peritoneal Mast Cells Were More Frequent In The Mesentery Of Albino Rat And Less Frequent In Rabbit. Heparin Trisulphate Was Substantial But Heparin M \ddot{o} n sulphate Was Present In Small Amount In Mast Cells Of Albino Rat Whereas Both Types Of Heparin Were Present In Equal Amount In Rabbit. Neutral Mucopolysaccharide Was Found In Small Amount In Mast Cells Of Albino Rat Whereas It Was Absent In Rabbit.

Conclusion: Heparin Trisulphate Is An Active Form Of Heparin And Is The Main Constituent Of The Peritoneal Mast Cell Granules Of Albino Rat Whereas In Rabbit Heparin Trisulphate And Heparin Monosulphate Are Present In Equal Amount.

Keywords: Peritoneal Mast Cells, Albino Rat, Rabbit, Heparin, Histochemistry.

I. INTRODUCTION

Paul Ehrlich 1878⁽¹⁾ Was First To Describe Mast Cells .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 Ehrlich'schen Mastzellen"(Mast Cells) Were Enriched In Heparin. Mast Cell Assumed Clinical Significance When Unna 1894⁽³⁾ Found That The Cutaneous Lesion In Urticaria Pigmentosa Consisted Almost Exclusively Of Mast Cells.

Heparin Exists In Mast Cells In Two Forms As Heparin Monosulphate And Heparin Trisulphate. Ringertz⁽⁴⁾ 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.Heparin Monosulphate Falls In Weakly Sulphated Group And Heparin Trisulphate In Highly Sulphated Group.According To Padawer 1963⁽⁵⁾, There Are Staining Differences Between Normal Mast Cells Of Different Species, Including Differences In Water Solubility Of Granules.Even Within A Single Species Number Of Mast Cells Vary Appreciably From One Organ To Another And Often Even Between Neighbouring Sites Within The Same Organ.

II. AIM AND OBJECTIVES

The Aim Of The Present Work Is To Make A Comparative Histological And Histochemical Study Between Peritoneal Mast Cells Of Albino Rat And Rabbit.

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III. MATERIALS AND METHODS

- **Collection Of Specimens:**

The Following Animals Comprise The Study Subject In Present Work.

1. Order Rodentia- Rattus Novegicus(Albino Rat)
2. Order Lagomorpha- Lupus Ruficavidatus(Rabbit)

Biopsy:-

Albino Rat And Rabbit Were Anaesthetised And Abdomen Were Open Vertically From Xiphoid Process To Pubic Symphysis. Jejunum And Ileum Were Identified. The Stretch Preparations Were Made On Chemically Clean Glass Slides And Immediately Placed In Fixatives.

Fixation: Following Fixatives Were Used For Fixation Of Stretch Preparation And Tissues

a) Neutral Buffered(PH-7)Formalin

Na₂HPO₄·2H₂O -4.52gm

+

Na₂HPO₄·2H₂O -8.15gm

Analytic Formalin-100ml

Distilled Water-900ml

b) 10% Formalin In Absolute Alcohol

Formalin 10ml

Absolute Alcohol 90 MI

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 Mucopolysaccharide(Heparin Trisulphate)

A) Alcian Blue PH-1

B) Alcian Blue PH-1 After Active Methylation (Spicer, S.S, 1960) ⁽⁶⁾

C) Aldehyde Fuschin

2) For Weakly Sulphated Acid Mucopolysaccharide(Heparin Monosulphate)

A) Alcian Blue PH-2.5(Spicer Et Al ,1967) ⁽⁷⁾

B) Alcian Blue PH-2.5 After Mild Methylation(Spicer Et Al ,1967) ⁽⁷⁾

3) For Simultaneous Distribution Of Highly And Weakly Sulphated Acid Mucopolysaccharides

a) Aldehyde Fuschin- Alcian Blue PH-2.5 Procedure

4) For Simultaneous Demonstration Of Sulphated And Neural Mucopolysaccharides

A) PAS

B) Alcian Blue PH-1-PAS Procedure

C) Alcian Blue PH-2.5- PAS Procedure

5) Toluidine 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 Mucopolysaccharide

IV. OBSERVATIONS AND RESULTS

A1) Morphology And Distribution Of Peritoneal Mast Cells Of Albino Rats.

i) In Neutral Buffered Formalin- Mast Cells Are Present 10-12/High Power Field(Hpf) In Toluidine 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 Toluidine 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.

A2) Morphology And Distribution Of Peritoneal Mast Cells Of Rabbit.

I) In Neutral Buffered Formalin- Mast Cells Are Occasionally Seen And Are Never More Than 1/Hpf In Toulidine Blue Stained Material But In Aldehyde Fuchsin Stained Mesentry They Are About 3/Hpf. Here Also The Mast Cells Are Oval And Filled With Comparatively Smaller Granules Which Take Deep Purple Stain With Toulidine Blue But Light Purple With Aldehyde Fuchsin . Nucleus Is Hardly Discernible.

II) In Formal Alcohol- Mast Cells Are Present On An Average 10/Hpf But Only 4/Hpf Respectively With Toulidine Blue At Ph 2 And With Aldehyde Fuchsin. They Are Again Oval With Fine Metachromatic Granules In The Cytoplasm.

B 1) Occurence Of Different Types Of Mucopolysaccharides In Peritoneal 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 Alcian Blue PH 1 They Stain Light 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 2.5 PAS And Alcian Blue PH 1 PAS Procedure.
- These Staining Reactions Indicate The Presence Of Substantial Amount Of Heparin Trisulphate And Small Amount Of Heparin Monosulphate.

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 Cells Stain 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 Mucopolysaccharide.

B2) Occurence Of Different Types Of Mucopolysaccharides In Peritoneal Mast Cells Of Rabbit (Table No. 2)

I) Neutral Buffered Formalin

- With This Fixative The Mast Cells Stain Deep Purple With Toulidine Blue At PH 4.4 And Moderate Purple With Toulidine Blue At PH 2.
- With Alcian Blue PH 2.5 The Mast Cells Stain Light Blue And This Alcianophilia Is Abolished After Mild Methylation.
- With Alcian Blue PH 1 They Stain Faint Blue And This Alcianophilia Is Abolished After Active Methylation .
- With Aldehyde Fuchsin They Stain Light To Faint Purple And Are Unreactive To Aldehyde Fuchsin – Alcian Blue PH 2.5 And PAS Staining Procedure.
- With Alcian Blue PH 2.5- PAS, The Mast Cells Remain Unstained But Take Light Blue Stain With Alcian Blue PH 1- PAS Procedure.
- These Staining Reactions Point To The Presence Of Moderate Amount Of Both Heparin Trisulphate And Monosulphate And Absence Of Neutral Mucopolysaccharide.

II) Formal Alcohol

- Mast Cells Stain Light Blue Purple With Toluidine Blue At PH 4.4 And Moderate Purple With Toluidine Blue At PH 2.
- With Alcian Blue At PH 2.5 PAS The Mast Cells Stain Light To Faint Blue , This Alcianophilia Is Abolished By Mild Methylation.

- The Mast Cells Are Unreactive To Alcian Blue At PH 1. They Stain Light Purple With Aldehyde Fuchsin And Aldehyde Fuchsin-Alcian Blue PH 2.5
- They Are Unreactive To PAS Staining Procedure As Well As To Alcian Blue PH 1- PAS Staining But Stain Light Blue With Alcian Blue PH 2.5- PAS Staining Procedure.
- These Staining Reactions Indicate The Presence Of Moderate Amount Of Heparin Trisulphate And Monosulphate But Absence Of Neutral Mucopolysaccharide.

Table No1:Histochemical Reactions Of Mast Cells In Mesentery 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

Table No2: Histochemical Reactions Of Mast Cells In Mesentery Of Rabbit 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	2B	0	1B	0	1-2P	0	0	0	2B
FA	2P	3BP	1-2B	0	0	0	2P	2P	0	2B	0

Abbreviations:NBF=Neutralbufferedformalin,FA=Formalalcohol,TB=Toluidineblue,AB=Alcianblue, AF=Aldehyde Fuchsin,PAS=Periodic Acid Schiff,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⁽⁸⁾ 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.

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 Toluidine Blue At PH-2 Buffered Toluidine Blue At PH-4.4 ,Aldehyde Fuchsin And Alcian Blue At PH-2.5.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 Mesentery Of Albino Rat And To Compare It With That Of Rabbit.

In Albino Rat We Found Mast Cells 10-12/Hpf In Toulidine Blue,20-22/Hpf In Aldehyde Fuchsin,7/Hpf In Alcian Blue With Neutral Buffered Formalin Whereas With Formalin Alcohol 12-13/Hpf In Toluidine Blue 20/Hpf In Aldehyde Fuchsin Were Found.This Finding Correlates With That Of Hardy And Washbook(1895)⁽⁹⁾ Who Found Mast Cells In Good Amount In The Peritoneum Of Rat.

In Rabbit We Found Mast Cell 1/Hpf In Toulidine Blue,3/Hpf In Aldehyde Fuchsin With Neutral Buffered Formalin Whereas With Formalin Alcohol 10/Hpf In Toluidine Blue 4/Hpf In Aldehyde Fuchsin Were Found. Padawer And Gorden, 1956⁽¹⁰⁾ Found Great Species Variation In Mast Cell Content Of Peritoneum As Judged By Comparative Observation On Rat,Guinea Pig And Rabbit.This Supports The Present Findings.

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. Mono And Trisulphate Forms Of Heparin In Mast Cells Have Been Reported By Spicer Et Al 1967⁽¹³⁾. Staining Reaction In The Present Work Indicate The Presence Of Substantial Amount Of Heparin Trisulphate And Small Amount Of Heparin Monosulphate In Mast Cells Of Albino Rat Whereas Both Were Present In Equal Amount In Rabbit. Neutral Mucopolysaccharides Is Absent In Mast Cells Of Rabbit But Present In Small Amount In Albino Rat.Comptom1982⁽¹³⁾ Noted That Reactivity To PAS In Formal Alcohol May Be Due To Presence Of Neutral Mucopolysaccharide .

VI. CONCLUSION

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 Mucopolysaccharide Is Present In Small Amount Whereas In Mast Cells Of Rabbit Both Heparin Triphosphate And Heparin Monophosphate Is Present In Equal Amount. Neutral Mucopolysaccharides Is Absent In Rabbit Mast Cells But Are Present In Small Amount

In Albino Rat.This Study Might Help In Making Comparative Histochemical Study Of Peritoneal Mast Cells With Other Mammalian Species.

REFERENCES

- [1]. Ehrlich P.(1878) Beitrage Zur Theorie And Praxis Der Histologischen Färbung (Leipzig University)
- [2]. Holgren H,Willander O (1937), Beitrag Zuekenntnis Der Chemie Und Funktoin Der Ehrlichs Chen Mastzellen Z, Mikrosk ,Anat, Forsch.42;242-278.
- [3]. Unna,P.G.(1894):Die Spezifische Färbung Der Mast Zellen Kornung.Monatsh,Prakt.Derm.19:367.
- [4]. Ringertz N.R 1963 :Polysaccarides Of Neoplastic Mast Cells Ann.N.Y Acad.Sc, 103:209
- [5]. Padawer, J.(1963):Mast Cells And Basophils. Ann. N.Y.Acad. Sci.103:1
- [6]. Spicer S.S (1960):A Correlative Study Of The Histochemical Properties Of Rodent Acid Mucopolysaccarides.J.Histochem, Cytochem,8:18
- [7]. Spicer S.S , Horn,R.G ,And Leppi, T.J 1967: The Connective Tissue,The William And Wilkins Co.Baltimore, P;251-303
- [8]. Selye ,H(1965):The Mast Cells Washington Butterworths
- [9]. Hardy ,W.B And Wesh Book 1895;Mast Cells Are Found In The Peritoneum Of Rat D28,474.Wandering Cell Of Alimentary Canal.J.Physiol.(Lond),18,478
- [10]. Padawer, J. & Gorden, A.S.(1956): Cellular Elem. In Perit Mast Cell In Mammals.Anat.Rec.12:209
- [11]. Lennert ,K And Schubert ,J.C.F (1959); Unterschuchungen Uber Die Sauren Mucopolysaccaride De Gewemastzellen Immenschilichen Knechenmark.Fankf Ztscher.Pathol.69,579
- [12]. Combs.J.W; Laugunoff,D And Benditt,E.P (1965);Differentiation Of Embryonic Mast Cells Of Rat.J Cell.Biol 25:577-592
- [13]. Compton,A.S (1982); A Cytochemical And Cytological Study Of Connective Tissue Mast Cells;Amer.J.Anat.91 :301-329