THE EVALUATION OF MAST CELLS OF RAT URINARY BLADDER UNDER STRESS CONDITIONS

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ABSTRACT

Objective: Stress, is associated with many diseases of unknown etiology.This study demonstrates the effects of stress on distribution of mast cells of rat urinary bladder.

Methods: Three experimental groups were designed as control, stress and recovery. Mast cells (MCs) in both mucosa and detrusor of the urinary bladder were both morphologicaly and quantitatively examined.

Results: The statistical analyses showed a significant increase in the number of mast cells in the mucosa ($p<0,001$) of the stress group when compared with the control group.

Conclusion: The increase in the number of mast cells and the highly degranulated mast cell morphology in stress conditions were in accordance with the histopathological findings of many diseases of unknown etiology such as Interstitial Cystitis (IC), neurogenic bladder disease and bladder cancer.

Key Words: Experimental stress, urinary bladder, mast cell

INTRODUCTION

Mast cells (MCs) were first described by Echrlich in 1887. They are present throughout the body and function in allergic, inflammatory and immune reactions (1,2). They take origin from the bone marrow and leave as undifferentiated cells and maturate under the influence of tissue factors as the c-kit ligand (stem cell factors) and interleukins 3 and 4 (1).

MCs are classified into three types as mucosal mast cells (MMCs), connective tissue mast cells (CTMCs) and intracranial mast cells based on their histochemical properties, quantity of stored histamine, proteoglycans and granule associated neutral proteases, sensitivity to secretagogues, susceptibility to inhibitory drugs and their location (3). The MMCs are found mostly in the bladder and gastrointestinal tract. The CTMCs are present in the skin and lung. The intracranial ones, share the characteristics of the other two types (4).

The MCs synthesize various substances in the form of granules in the cytoplasm such as heparin, histamine, proteases, phospholipases, chemotactic substances and cytokines. In addition, there are other molecules within the MCs, that are synthesized de novo namely leukotrienes, prostaglandins, platelet-activating factor, vasoactive intestinal polypeptide and tumor necrosis factor (2,5).

The conventional histochemical staining technique for detection of MCs is acidified toluidine blue (ACTB) or alcian blue counterstained with safranin. A proper staining and fixation are critical for adequate preservation of MCs secretory granules and differentiation between mucosal and connective tissue mast cells (5).

The secretion of MCs is triggered by various stimuli like anaphylatoxins, lymphokines, lectins, drugs, solar radiation, some toxins and viruses, exercise (especially in cold), hormones (ACTH, estradiol and etc), immunglobulin-E and antigen, the neuropeptides as neurotensin, somatostatin and substance P (SP) and the neurotransmitter acetylcholine (2,6,7).

However, increased numbers of MCs are also found in many diseases like migraine headaches, multiple

sclerosis, neurofibromatosis, scleroderma, breast and bladder carcinoma (2,8-10).

MCs also play a role in many inflammatory diseases especially of the lung and intestine. Increased number of MCs is also regarded as a potential marker of interstitial cystitis (IC) and they are thought to have a role in the pathogenesis of IC (5,9,11-17).

Clinicians, described a striking bladder condition with urinary frequency, urgency, burning and suprapubic pain as IC. It is regarded as a multifactorial disease characterized by mononuclear inflammation, mucosal hemorrhage, epithelial disruption and the most strikingly increased number of MCs in the bladder wall (5,15,18-25).

The aim of this study is to quantify the number of MCs in mucosa and detrusor muscle of the rat urinary bladder in response to stress conditions and also compare the stainings of mast cells by using two different techniques.

MATERIAL AND METHODS

Animals

Adult female Wistar-Albino rats weighing 180-200 gr were used in this study. They were housed individually in light and temperature controlled room on a 12:12 hour light/dark cycle, and fed on a standard pellet lab cow and water ad libitum.

Experimental Groups

Three groups were designed as control (n=4), stress (n=8) and recovery (n=8). The standard lab conditions were applied to the animals of the control group during the experimental procedure. The animals in the stress and recovery groups were fasted for 48 hours and following the fasting they were put into restraint cages for 3 hours at 4°C temperature. After these stress conditions, the animals in the recovery group were put in standard lab conditions for 48 hours. All animals were then sacrified under ether anesthesia and their bladders were removed for light microscopic investigations.

Microscopic Preparation

The specimens were fixed in normal 10% buffered formalin for 48 hours, dehydrated in increased alcohol series and embedded in paraffin wax. 5 µm thick sections were stained with Haematoxylen-Eosin (H-E) for general morphology. 1% Aqueous Toluidine Blue (ATB) for five minutes or 0,5 % Toluidine Blue in 0,5 M hydrochloric acid (pH 0,5, for 30 minutes or five days-ACTB) stains were used for the identification of mast cells (11,13,26,27). To observe the migration of MCs towards the epithelium some of the specimens were fixed in 4% phosphate buffered gluteraldehyde (0,13 M, pH 7,4) for 4 hours and postfixed in 1% OsO_4 for 1 hour. The specimens were dehydrated in graded alcohol series and embedded in Epon 812. Then were stained with 1% Toluidine Blue (28).

Mast Cell Counts

MCs containing metachromatic granules were counted separately at x400 magnification in the mucosa and detrusor muscle. An eyepiece was used to define the counting area. Fifteen different areas were randomly selected in both mucosa and detrusor muscle and MC density was expressed as number of cells per unit area.

Quantitative Analysis of Mast Cells

Kruskal-Wallis nonparametric median test and Dunn's multiple comparing test were used to evaluate MCs. Results were regarded statistically significant when $p<0.001$.

RESULTS

Control Group

ATB and ACTB stainings showed few MCs in the mucosa and detrusor muscle (Fig.1a, 1b). When ACTB stained sections were examined with light microscope, MCs gave violet color while other tissue elements were in pale-blue. Darker MCs and pale background were obtained when the number of staining days was extended. The EPON embedded sections showed mast cells in the mucosa both in lamina propria and epithelium (Fig.2).

Stress Group

Compared with the control group, the stress group H-E stainings showed polymorphonucleated leukocytes in lamina propria (Fig.3). Both ATB (Fig.4a, 4b) and ACTB (Fig.4c, 4d) stainings showed an increased granulated and degranulated MCs in the mucosa (Fig.4a, 4c) -mostly in lamina propria- and detrusor muscle (Fig.4b, 4d). They were generally located around the blood vessels and were mostly degranulated (Fig.4c and inset). The EPON embedded sections showed swollen urothelial cells, increased number of MCs in both urothelium and lamina propria (Fig.5a, 5b).

Recovery Group

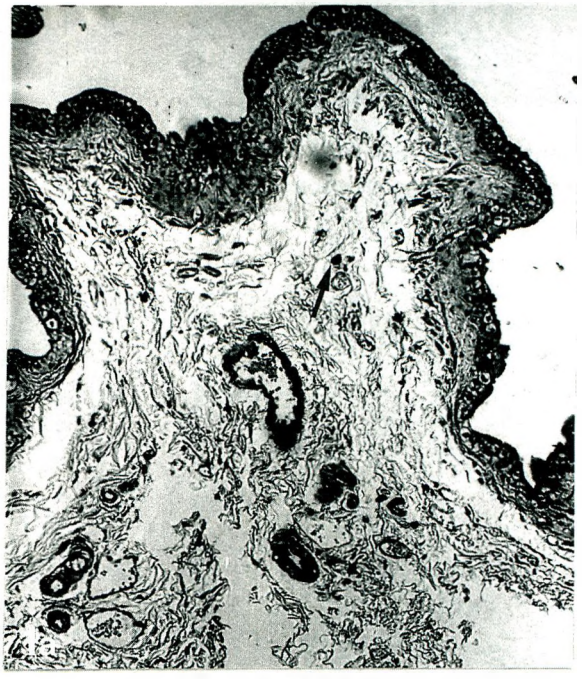
ATB (Fig.6a) and ACTB (Fig.6b) stainings showed decreased number of MCs when compared with the stress group, but increased number of MCs when compared with the control group. The cytoplasms of MCs have reduced number of granules, giving a pale violet appearance (Fig.6b inset). Similar to the stress group the MCs were also identified in the urothelium (Fig.7).

Quantification of Mast Cells

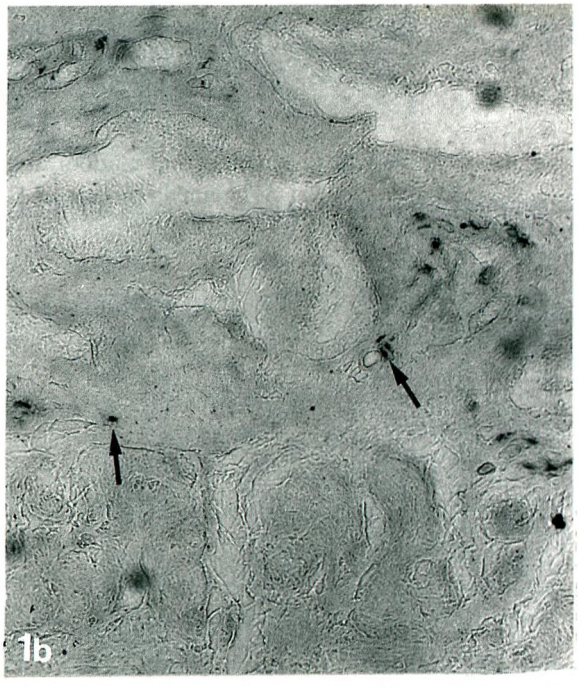
The mean number of MCs per square milimeter in the bladder stained with ATB were counted as follows. The control group: 20 cells/mm^2 in mucosa, 12,5 cells/mm^2 in detrusor muscle. The stress group: 64 cells/mm^2 in mucosa, 32,25 cells/mm^2 in detrusor muscle. The recovery group: 27,33 cells/mm^2 in mucosa, 13,33 cells/mm^2 in detrusor muscle (Fig.8). The number of MCs in the bladder stained with ACTB were as follows. The control group: 27,5 cells/mm^2 in mucosa, 17 cell/mm^2 in detrusor muscle. The stress group: 74 cells/mm^2 in mucosa, 62,75 cells/mm^2 in detrusor muscle. The recovery group: 36,66 cells/mm^2 in mucosa, 43 cells/mm^2 in detrusor muscle (Fig.9).

According to the statistical analysis, both staining techniques (ATB and ACTB) showed significant increase ($p<0.001$) in the number of MCs in the stress group compared with the control group. Comparison of the stress and the control groups with the recovery revealed no statistical significance ($p>0,001$). The difference in the number of MCs in detrusor of all three groups was also insignificant ($p>0,001$).

However, the ACTB stained sections showed an increased number of MCs in all groups when compared with the ATB stained sections.



1a



1b

Fig.1.: Control bladder specimen stained with ATB (A) and ACTB (B) showed several mast cell in mucosa. Note that mast cells stained dark blue on pale ground (B). A:x100, B:x100 magnification.

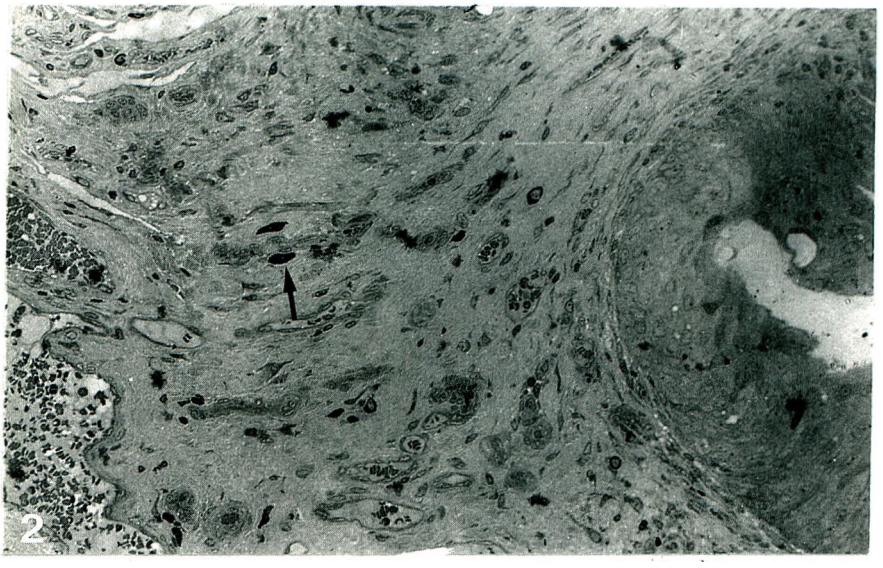


Fig.2.:

EPON embedded sections from the control bladder specimens revealed mast cells closely localized to the blood vessels. Toluidine blue staining. x 400 magnification

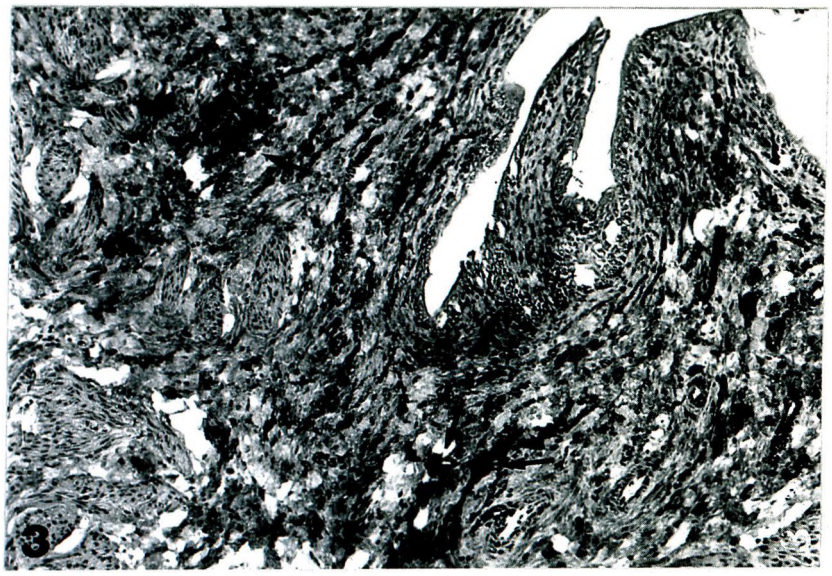


Fig.3.: Stress group bladder specimens showed leukocyte infiltration (→) in lamina propria. H-E staining, x100 magnification

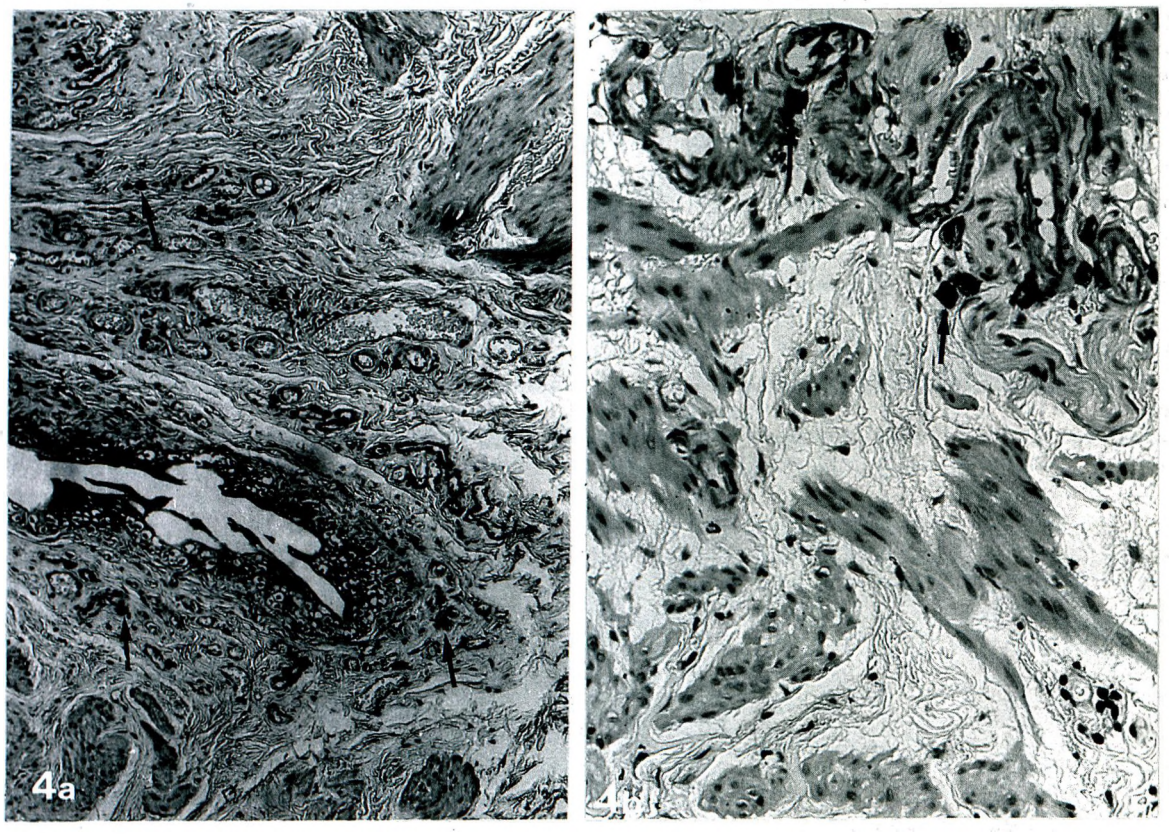
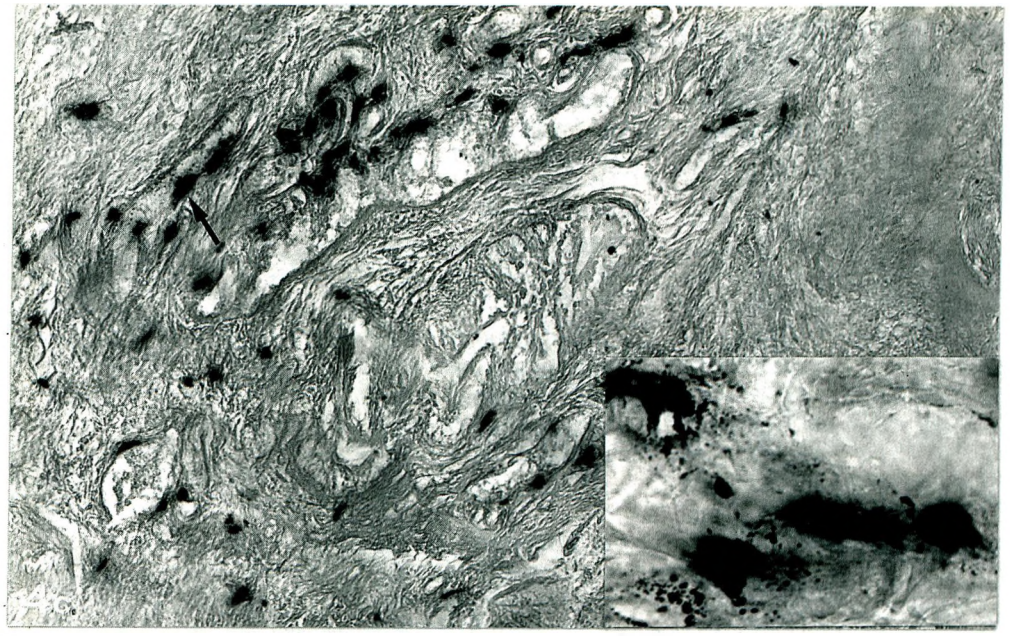
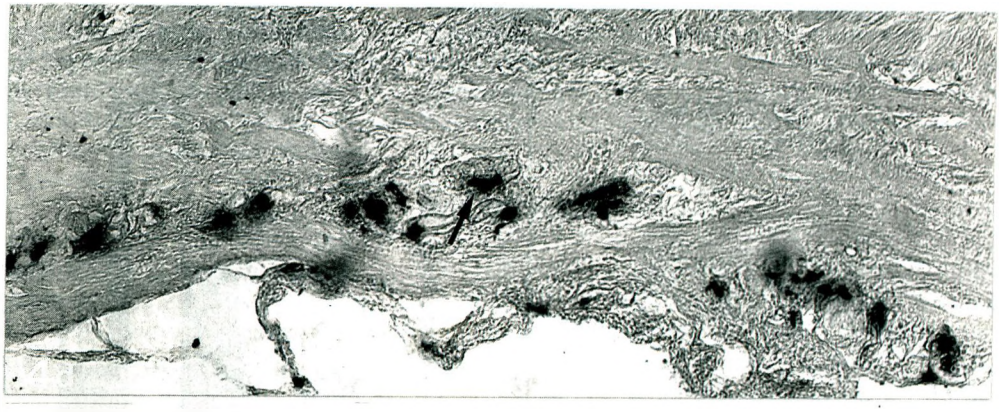


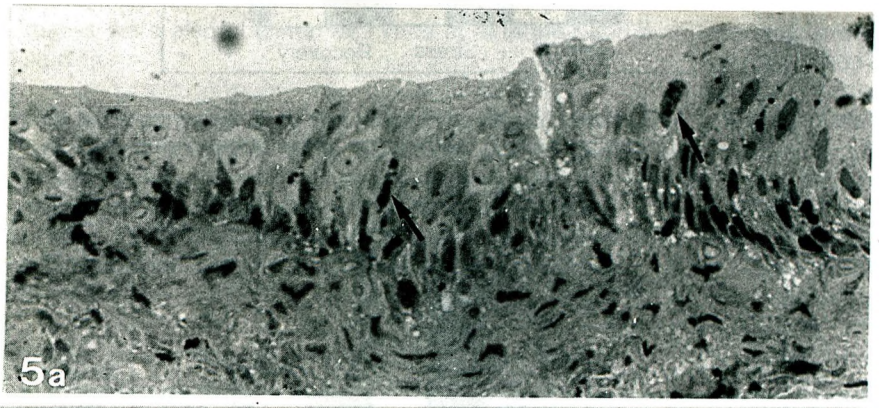
Fig.4.: Stress group bladder specimens stained with both ATB (A;B) and ACTB (C,D) showed increased mast cells (→) in the mucosa (A,C), and the detrusor (→) (B,D). Note that mast cells are fully or partially degranulated (inset). A:x100 B:x200, C:x100, D:x100, inset x400 magnification



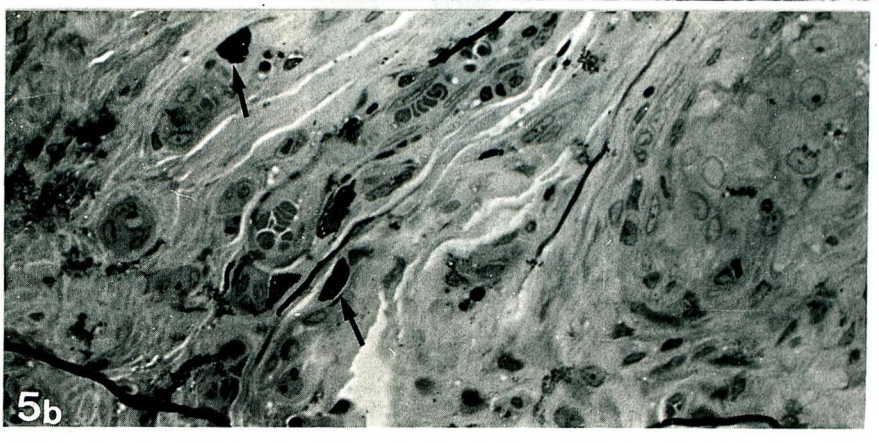
4 c



4 d

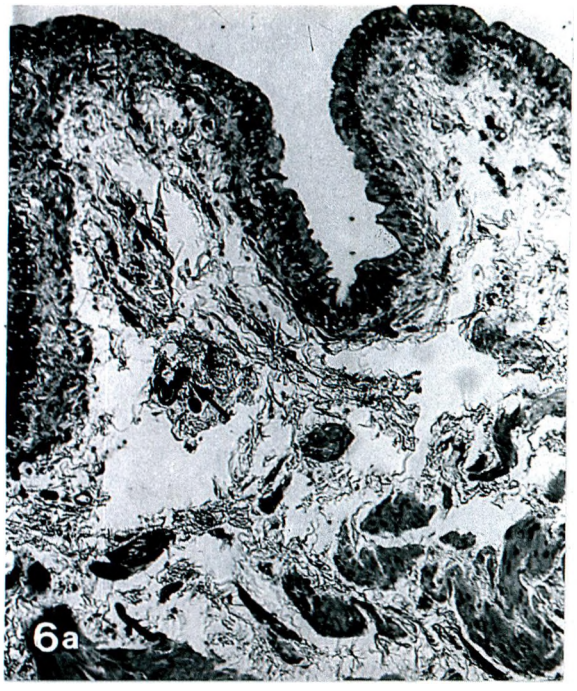


5 a



5 b

Fig.5.:
EPON embedded sections from the bladder of stress group, showed mast cells (→) localized in the urothelium (A) and in the lamina progria (B). A:x400, B:x200 magnification.



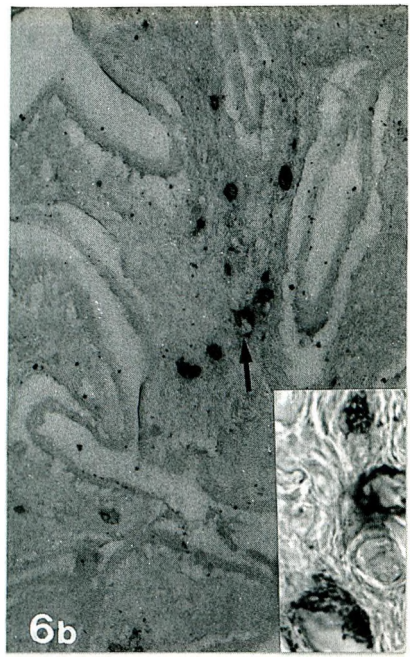


Fig. 6.: Bladder specimen of recovery group stained with both ATB (A) and ACTB (B) showed reduced number of mast cells (→). Note the mast cells with less granules and reduced densities of cytoplasm (inset). A:x100, B:x100, inset x400 magnification

6 a **6 b**

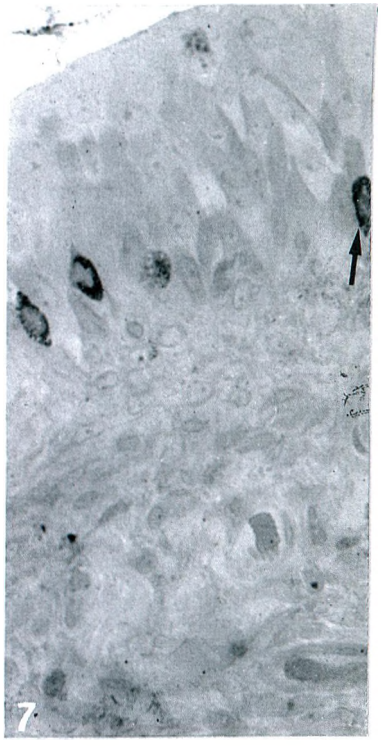


Fig.7.: EPON embedded sections of bladder of recovery group showed that mast cells were mainly localized in the urothelium (→). Toluidine blue staining. x400 magnification

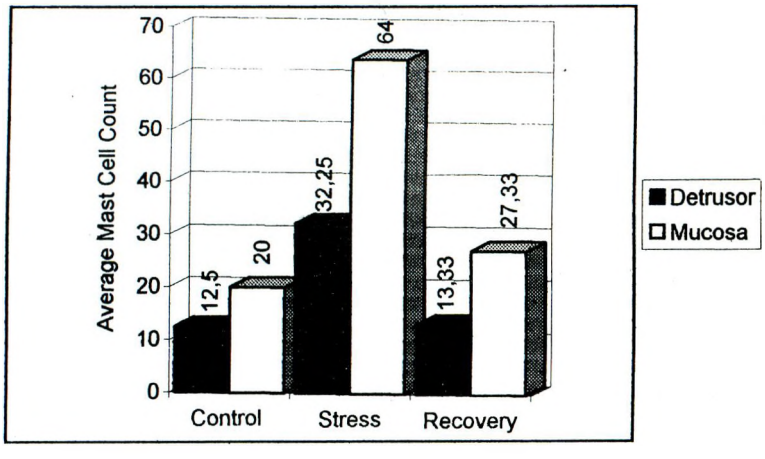


Fig.8.: ATB staining. showed significantly ($p<0.001$) increased number of mast cells in the mucosa of the stress group

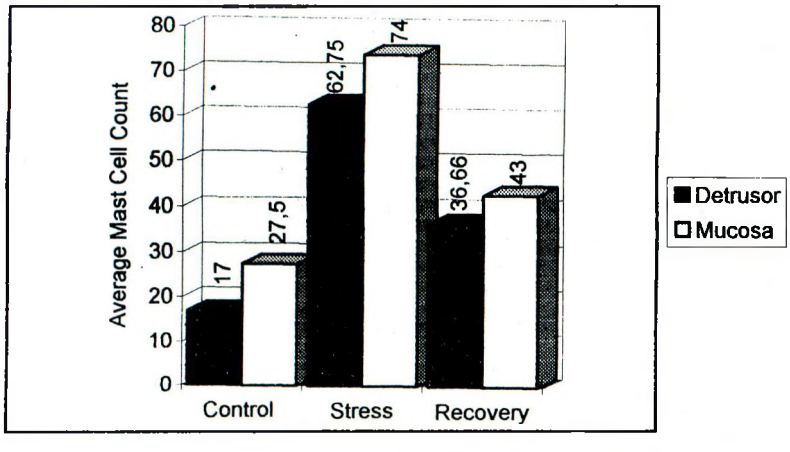


Fig.9.: ACTB staining, showed significantly ($p<0,001$) increased number of mast cells in the mucosa of the stress group

DISCUSSION

Stress can be a cause in diseases of unknown etiology. There are many diseases that are triggered by stress, ulcerative gastritis and psoriasis are two of well-known examples (29-33).

Stress conditions effect various organs and systems in the body via endocrine, immune or neural mechanisms. Many authors have put forward a neural stimulation of MCs in the case of stress (5,35). It has been reported that SP is considered to release histamine from mast cells and its synthesis was increased in various types of stress (34). Increased SP levels in stress conditions have been reported with the aid of experimental studies (6,30).

Researchers have counted MCs of patients with IC in both detrusor muscle and mucosa. They have obtained various increase in the number of mast cells per mm^2. Theoharides et al (9) counted 33,8 cells/mm^2 in mucosa, Feltis et al (14) counted 34,5 cells/mm^2 in submucosa, Larsen et al (5) counted more than 28 cells/mm^2, Lynes et al (5) counted 57 cells/mm^2 -more than half were degranulated-, Aldenborg et al (11) and Hanno et al (21) both counted more than 10 cells/mm^2 in detrusor muscle of IC patients (5-9,11,14,21,36).

In our experimental study we also observed increased number of MCs mostly in the mucosa (64 cells/mm^2) and in the detrusor (32,25 cells/mm^2) of urinary bladder. These findings were in accordance with the results obtained by the above authors. In addition to the increased number of MCs we have also found that they were highly degranulated. Similar results were also obtained by Lynes et al in their IC studies.

The recovery group showed reduced number of mast cells when compared with the stress group. The absence of stress conditions abolished the stimulation of mast cells in the urinary bladder.Therefore, stress can be one of the factors which stimulates both the increase and degranulation of mast cells.

This study showed that, the ACTB technique can be preferred for morphometric and morphological studies related to the MCs.

An elevated concentration of histamine in bladder biopsies was also demonstrated in IC patients (7,14,16,17).

The increase in MCs was also observed in various types of carcinoma such as breast and bladder cancer (9,10). The increased MCs in carcinoma are highly granulated whereas in IC they are highly degranulated (9).

Schwartz et al suggested a biopsychosocial model for the development of IC that is life stress, genetic vulnerability plus an intervening factor such as an infection could initiate IC (19).

The IC is thought to be a disease with endocrine, immune and neural components in which activated MCs play a central role (5). Hand based his studies on neurogenic etiology of IC (20). He reported increased density of submucosal nerves in IC. Other authors have reported a proliferation of nerve fibers in IC (37,38) and correlated the number of MCs with nerve fiber proliferation and histamine level (16). Therefore, the proliferation and the exocytosis of the MCs are tought to be stimulated by autonomic afferent nerve fibers (36). In the present study, the increased number of MCs and their degranulation under stress conditions can be related to the proliferation and stimulation of nerve fibers.

IC is regarded as multifactorial disease of unknown etiology. Although MCs alone may not explain the certain etiology, a common mechanism may play a role in both IC and stress conditions leading to an increase in the number of MCs and thus we believe that stress can be one of the factors causing IC.

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