STEREOLOGJCAL ANALYSIS OF MUCOSAL MAST CELL NUMBER IN THE RAT INTESTINE

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Abstract

Sections of rat jejunum, ileum and colon were stained with toluidine blue and acid fuchsin and analysed with the stereological methods. Three parameters of mucosal mast cells were calculated: volumetric density (Vv), surface density(Sv), and numerical density(Nv), separately for lamina propria mast cells surrounding the intestinal crypts, for that of villi as well as for intraepithelial mast cells of colonic epithelium. The results indicated that the most numerous mast cells occurred in lamina propria surrounding the crypts (almost 200,000 per cubic millimeter of the connective tissue) of jejunum, whereas the villus mast cells were significantly less numerous (about 55,000 per cubic millimeter of. the connective tissue). The number of intraepithelial mast cells was calculated as 41,500 per cubic millimeter of colonic epithelium. Similarly the volumetric density, i.e. the ratio of volume of mast cells tested to that of a given lamina propria situation within the intestine wall and the region of the intestine (JPMA 38: 78 , 1988).

INTRODUCTION

The mast cells are defined as cells containing large, electron-dense cytoplasmic granules[10] made up of heparin or related glycosaminoglycans and proteins[4,11] - They can take up and decarboxylate some amino acids, e.g., histidine 5 hydroxytryptamine, and to store the products of the decarboxylation, i.e., histamine or 5-hydrox. Ytryptamine[3,13]. The latter properties of the mast cells were the basic to classify them to APUD (amino-acid precursors uptake and decarboxylation) cell system. In the mucosa of both small and large intestines, numerous mast calls occur in the lamina propria surrounding the crypts and in that of villi[11,12]. The mucosal mast cells are recognised recently as a sub-population of the body mast cells[3]. They can easily be ‘discriminated from the tissue mast cells and blood basophils by localization and histochemical and functional criteria. Alike tissue mast cells and basophils the mucosal mast cells contain glycosaminoglycans, histamine, serotonine (rat), slow reacting substance of anaphylaxis (SRS-A), eosinophil chemotactic factor of anaphylaxis (ECF -A) and vasoactive intestinal peptide (VIP) [1,7]. All these substances are stored in specific granules of mast cell cytoplasm and can be released by an immunological mechanism mediated by IgE. Glucosaminoglycans of mucosal mast cells have relatively low molecular weight[13] and though they are coupled with proteins, they can easily be dissolved in routinely used fixatives. Therefore, the visualisation of mucosal mast cells requires the application of appropriate fixative and staining procedures[8,10]. Mucosal mast cells, in contrast to tissue mast cells and blood basophils[17] contain intracellular immunoglobulins and are believed to be engaged in an immediate type of hypersensitivity[9]. Within minutes of contact between offending antigens and mast cell there is release of histamine, serotonin, bradykinin, SRS, FCF and VIP causing smooth muscle spasm, vasoconstriction, capillary damage and oedema. IgE-mediated mast cell secretion, therefore, can regulate the permeability of mucosal vessels. The enhancement of vascular permeability can be considered as an attractive mechanism by which immunoglobulins can be transported to the mucosal surfaces. The frequency of occurrence of mucosal mast cells at various sites of mucosa, e.g. in

the villi and lamina propria surrounding the crypts as well as at various regions of the intestine is not known. The objective of the present study was to measure the mucosal mast cell numbers throughout the mucosa and at various regions of small and large intestine and for that the stereological method was applied. The results showed that the mucosal mast cells occur more frequently in the lamina propria surrounding the crypts than in that of villi. The mast cells occurred more frequently in jejunum than in ileum/colon.

MATERIALS AND METHODS

Animals and Specimen preparation

Adult male Wistar rats weighing 150-200 g each were used. The animals were maintained on standard laboratory diet and received water ad libitum. They were pronounced free from intestinal parasitic infection after a therapeutic coarse of an antthelrnitic drug with sub sequent faecal examination. These animals were bred and their progeny/litters were taken, maintained in the laboratory similarly till they matured. Adult male rats, selected for experimental work, were sacrificed by the blow on neck and exsanguinated. Pieces of jejunum at a distance of about 10 cm from pyloro-duodenal junction, the pieces of ileum about 5 cm from ileo-caecal junction and pieces of colon about 7 cm from caecum were taken.

Histological procedure: The intestinal fragments each 1—2 cm long were fixed in a methanol-formalin-glacial acetic acid (85:10:5) mixture[3,14]~, dehydrated, embedded in paraffin and cut at 10 urn thick sections. These sections after deparaffinisation were stained with toluidine blue (pH 1.0) and counter-stained with acid fuchsin/mordant. The light microscope was utilized to identify and study the general distribution of mast cells in the intestinal mucosa.

$$N_v = \frac{K}{B} \sqrt{\frac{NA}{Vv}} \,,$$

$$P_P = P_M / P_C \text{ (for lamina propria mast cells) or}$$

$$P_{P_1} = P_N / P_E \text{ (for intraepithelial mast cells)}$$

adapting the basic stereological equation:

$$V_V = P_P \text{ [16]}.$$

Stereological procedure: The method of stereology described for ultrathin sections[15,16] was adapted for the light microscopy level. For all measurements the graticule consisting 100 squares was placed into eyepieces of the microscope. Using the immersion objective, 100 x and eyepiece 10 x the graticule surface area was calculated as 1451.6 um^2. All measurements were done by placing the graticule, pT, on a section image and recording the numbers of intersections of graticule points with: 1) the mast cells (PN); the sections of mast cell with clear nuclei were exclusively taken into consideration, 2) fragments of epithelium (RE) and 3) fragments of connective tissue of lamina propria (PC) of either villi or that surrounding the crypts. The volumetric density, Vv, i.e. the fraction of mast cells to the volume of tissue which surrounds the mast cells (either connective tissue of lamina propria or epithelium), was estimated from fraction:
From these equations the volumetric Vv, of either lamina propria mast cells of density the viii or those of lamina propria surrounding the crypts were calculated and expressed as the fractions. The surface density Sv, of mast cells was calculated from the formula: Sv = rn/PS, where m = number of mast cell profiles (each containing nucleus) per counted field, PS = number of intersection points of fragments of mucosal connective tissue or epithelium expressed in square micrometers of the graticule. Surface density of mast cells repiesents, therefore, the number of their profiles per unit of surface area e.g. square milli meter. The numerical density Nv, of mast cells was calculated according to the formula 15,16 Where NA = number of profiles of mast cells per tested area, K = size distribution coefficient of the mast cells; the latter was estimated for mucosal mast cells as about 1.2, Bshape coefficient of mucosal mast cells estimated as about 2.5, and Vv = volumetric density of mast cells per cubic unit of either connective tissue of lamina propria or epithelium. All parameters obtained at present, i.e . Vv, Sv and Nv of mast cells were calculated separately for sections of jejunum, ileum, and colon as well as for lamina propria of viii and that surrounding the crypts. More than 6000 fields of mucosa from at least 100 villi and larnina propria surrounding at least 100 crypts were analysed. Separately, the Vv, Sv and Nv were calculated for intraepithelial mast cells situated in the lining epithelium of the colon. In computer-assisted processing the results were arranged, the standard errors of means were calculated. The chisquaretest was applied to verify the results.

RESULTS

The numerous mast cells of the lamina propria in both small and large intestine were observed after application of appropriate fixation and staining (see Material and Methods). In addition to lamina propria mast cells, numerous intraepithelial mast• cells were observed in the lining epithelium of the dolon (Figure 3) while the former occurred in the wall of both the small and large intestine, the latter occurred exclusively in the lining epithelium of the colon. Both the lamina propria and intraepithelial mast cells were identified by dark blue colour of their cytoplasmic granules stained with toluidine blue at pH 1.0 and by red, round nucleus stained with acid fuchsin (Figures 1,2 and 3).

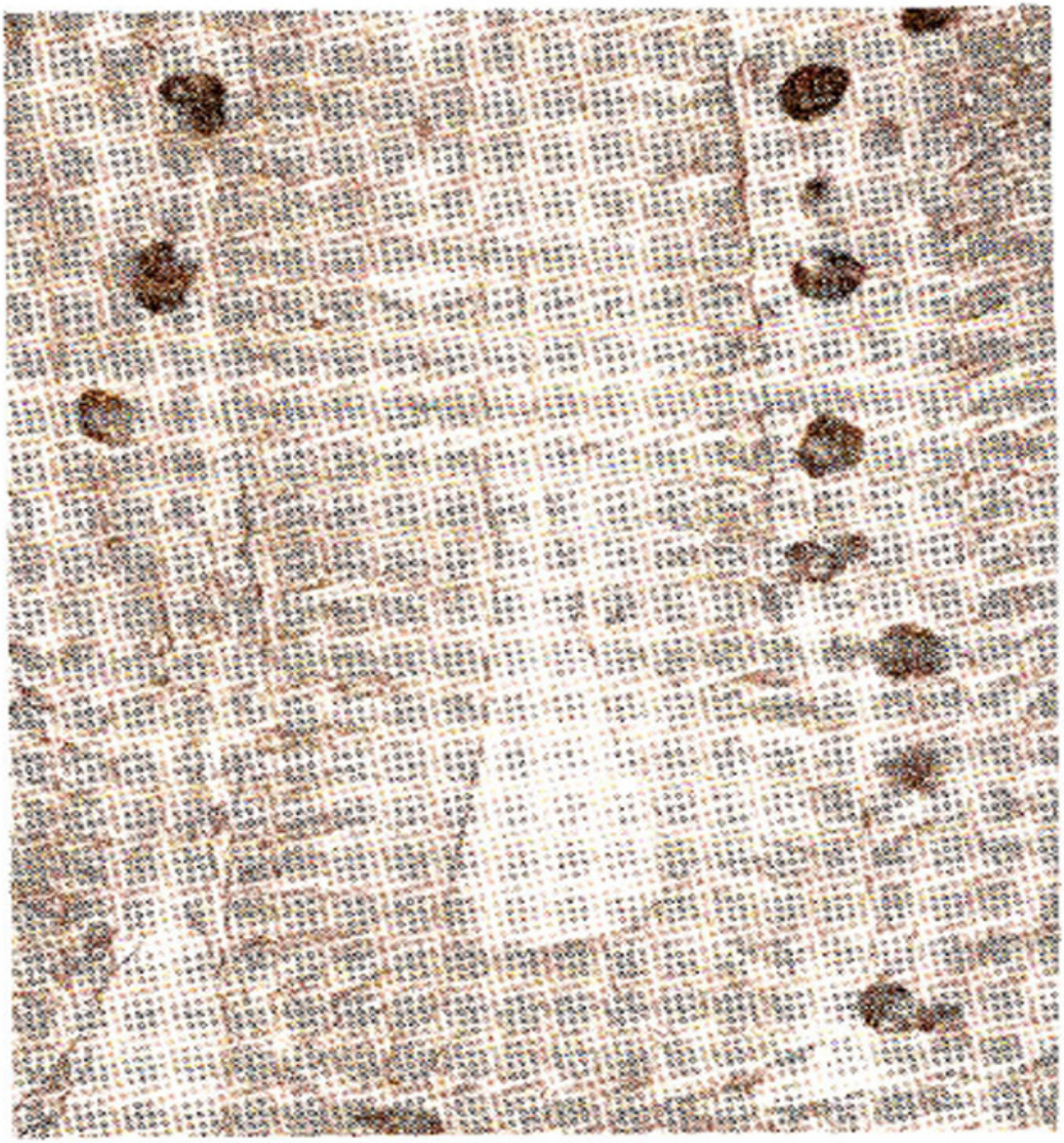

Figure 1. Mucosal mast cells in the lamina propria surrounding the crypts of Jejunum. Toluidine blue and acid fuchsin staining. Obj. 40 x; eyepiece 10x.

Figure 2. Mucosal mast cells of the lamina propria of jejunal villus (A) and crypt-villus junction.

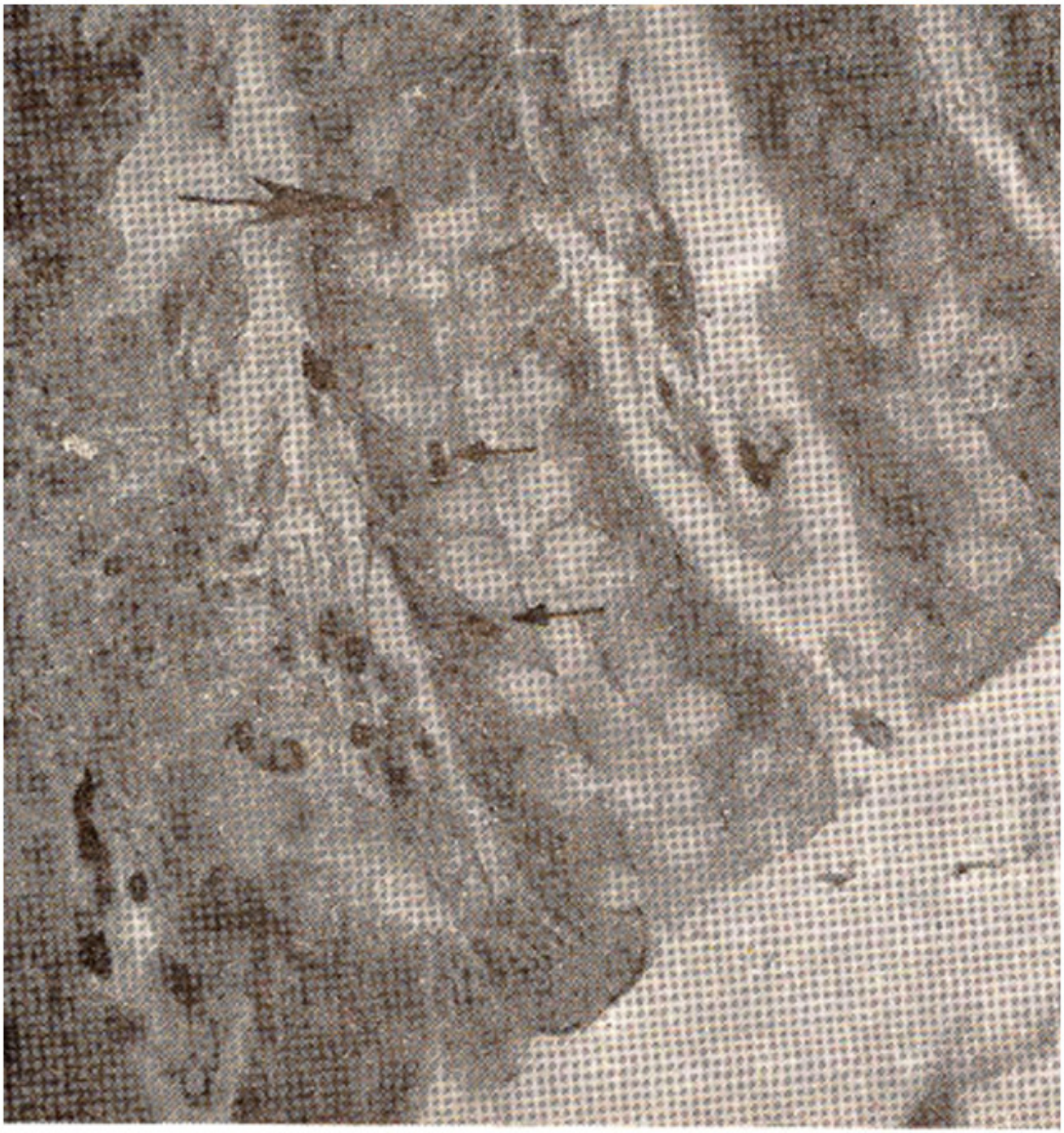

Figure 3. Intraepithelial mast cells in the crypts of rat colon (arrows). Toluidine blue and acid fuchsin staining. Obj. 40x eyepiece 10x.

The general appearance of mast cells o lamina propria surrounding the crypts and those of villi were similar to each other (Figure 1 and 2). The stereological analysis of more than 6000 counted (test) fields revealed the volumetric density, Vv, of lamina propria and intraepithelial mast cells. The parameter Vv represents the ratio of the volume of a given mast cell population to that of either connective tissue of lamina propria or epithelium which form the environment of the mast cells. The Vv of the mast cells of lamina propria surrounding the crypts has 0.12 with the standard error of 0.009,

i.e. it was four times as much as that of the villus lamina propria mast cells which was 0.03 with standard error of 0.0025 (Table I and Figure 4).

TABLE

Volumetric density (Vv), Surface density (Sv) and numerical density (Nv) of mucosal and intraepithelial mast cells at various regions of intestine of the rat.

Site of mast Cell Occurrence	M	Vv	Sv (Cells/ mm^2)	Nv (cells/ mm^3)
Lamina propria surrounding crypts				
Jejunum	458	0.181 (0.017)	2.720 (270)	190,000 (32,000)
Ileum	802	0.079 (0.007)	1,200 (100)	102,000 (14,000)
Colon	1235	0.087 (0.005)	1370 (80)	115,000 (11,000)
Lamina propria of villi:				
Jejunum	1538	0.052 (0.004)	780 (50)	72,300 (8,000)
Ileum	2099	0.0141 (0.001)	280 (20)	40,000 (5,000)
Lining epithelium colon	1235	0.023 (0.002)	364 (25)	41,500 (5,000)

Note: Figures in parenthesis are the standard errors of mean calculated despite the distribution of data are unknown.

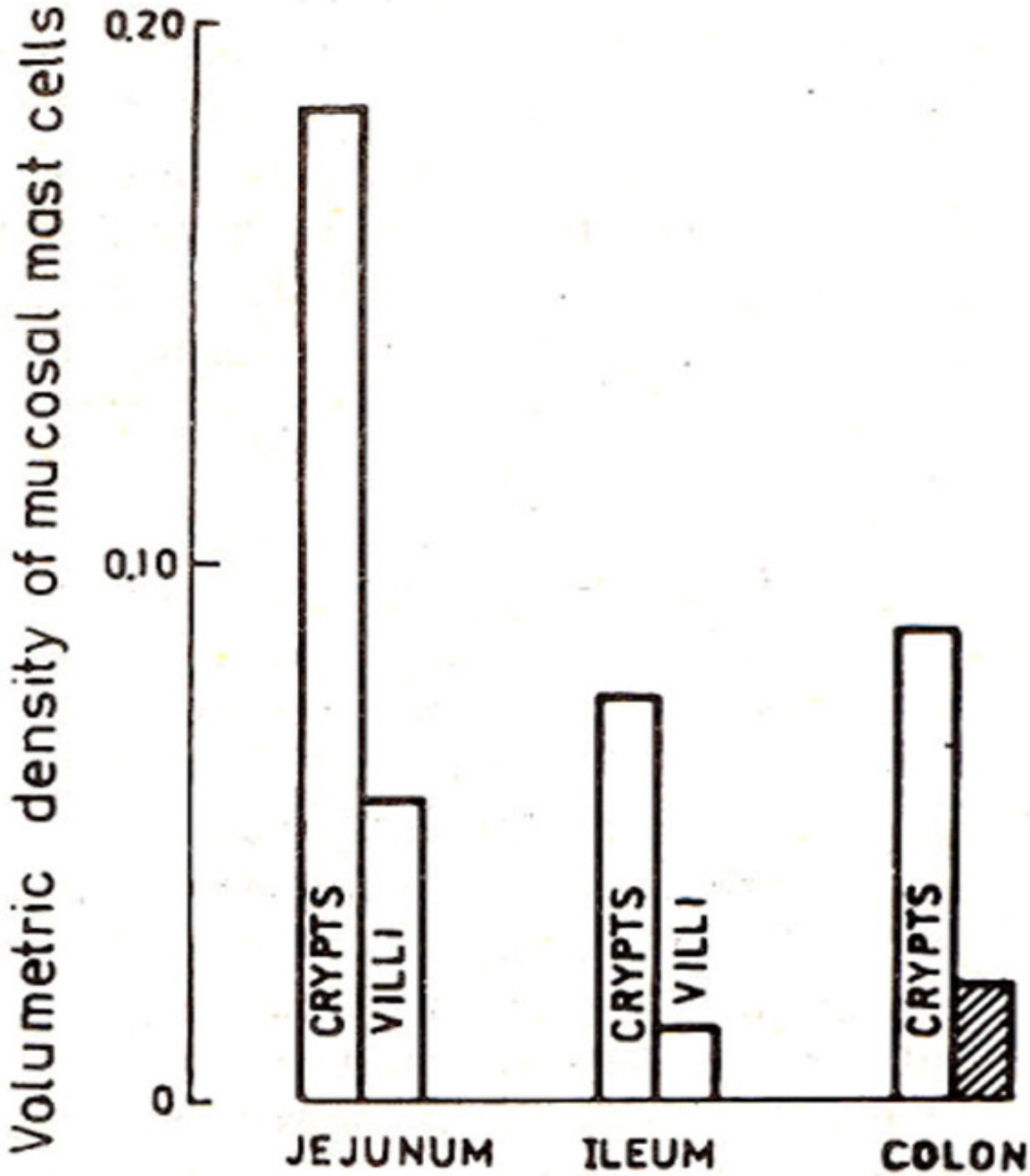

Figure 4. Diagram presenting the values of volumetric density of mast cells of lamina propria surrounding the crypts and that of villi of jejunum, ileum and colon (open bars). The dashed bar represents volumetric density of intraepithelial mast cells of colonic epithelium.

The Vv of intraepithelial mast cells of the colon was 0.023 with standard error 0.002 (Table 1). The surface density Sv of mast cells expresses the cell profile number per square unit. The Sv values for mast cells of lamina propria surrounding the crypts (1200—2720 cells per square millimeter of connective tissue was higher than that of lamina propria of the villi (280—780 cells per square

millimeter of connective tissue). Surface density of lamina propria mast cells were particularly numerous in the jejunum and relatively less numerous in ileum and colon (Table, Figure 5). Intraepitheial mast cells of the colonic epithelium occurred with the frequency of 364 cells per square millimeter of epithelium. The most interesting results of our stereo-logical analysis are those of numerical density of mucosal mast cells of various parts of lamina propria as well as of various regions of the intestine from jejunum through lleum to colon. The numerical density Nv, of mast cells present the number of cells in three-dimensional system of either connective tissue of lamina propria or epithelium. Tables and Figure 5 present these results. It is apparent that the highest number of mast cells occur in lamina propria surrounding the crypts of jejunum. There were as many cells as 190,000 mast cells per cubic millimeter of connective tissue. In ileum and colon the mast cell number fell to 102,000 — 115, 000 per cubic millimeter of the connective tissue. In the lamina propria of villi of jejunum and ileum the respective numbers of mast cells were calculated as 72,000 and 40,000 per cubic millimeter of connective tissue. The number of intraepithelial mast cells of the colonic epithelium was 41,500 cells per cubic millimeter of the lining epithelium. Not all figures presented in Table and Figures 4 and 5 were found significantly different to each other. To verify such differences the Chi-square test was applied. For the distributions of values of Vv, Sv and Nv, the Chi-square values were calculated. The latter values were divided by theoretical Chi-square values at confidence level of 0.05. If obtained, fractions were higher than 1.0, the difference between a given parameter calculated for various parts of lamina propria and for various regions of the intestine was significant at 0.05 level of confidence. If the respective values were lower than 1.0 there was no significant difference. Figure 6 presents the ratios of experimentally obtained values of Chi-square to theoretical values of Chi-square at 0.05 confidence level, versus the parameters (Vv, Sv, Nv) obtained for various parts of lamina propria and various regions of the intestine. It is relevant that the volumetric density of mast cells as well as their numerical density in the lamina propria of jejunum villi are significantly higher than that of the ileum ($p < 0.01$). Similarly, the number of mast cells as well as thoir volumetric density in lamina propria surrounding the crypts were highly different than those of villi ($p < 0.005$). The differences between cell numbers and volumetric density of mast cells situated in lamina propria surrounding the crypts of jejunum, ileum and colon were more variable (Figure 6) although the respective parameters, e.g. Sv or Nv, of mast cell stereology of jejunum had tendency to be higher than those of the ileum and colon.

DISCUSSION

The stereological analysis of quantitative distribution of mucosal mast cells indicated non randomness of mast cell number both across the lamina propria of a given region of intestine as well as along the various regions of intestine. Both the volumetric density (the ratio of mast cell volume to connective tissue volume) and the numerical density (mast cell number per cubic unit) of mast cells situated in lamina propria surrounding intestinal crypts have been found at present investigation significantly higher than those of villus lamina propria. Similarly, the mucosal mast cells were found to occur more frequently in jejunum shown in ileum and colon. On the basis of present study we can only speculate the possible causes of non-random distribution of mucosal mast cells. It may be assumed that such a non-randomness reflects the variability of alimentary tract requirements for mast cells functions. The mucosal mast cells were reported to contain intracellular IgE[8,9] the suggestions were made that mast cells mediate an immediate type hypersensitivity reactions. IgE-mediated mast cell secretion of histamine, serotonine, VIP and SRS—A may regulate mucosal vascular permeability. Therefore, mast cell secretion resulting in the increase of vascular permeability within the mucosa can be an attractive mechanism of faciiated transportation of immunoglobulins to the mucosal surface to prevent action of luminal antigens. Such a notion is strongly supported by the reaction of mucosal mast cells to the

Nematodes infestation[5,8,9]. The latter generates a localized anaphylactic reaction that coincides at first with the reduction of mast cell number followed by their proliferation. Both reactions of mast cells are considered as a reflection of an immediate-type hypersensitivity reaction. It may be assumed, therefore, that the fadiiated transport of immunoglobulins toward the surface of mucosa is higher at the level of the crypts (presumably close to the villus base) than in the villi themselves. The same cause, i.e, the tendency to occupy the more strategic positions against luminal antigens, leads to the occurrence of the mast cells in the colonic epitheium. The ameboid activity of mast cells[2] may facilitate their positioning with the epithelium.

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