

## Changes in some collagen mucosal cells after irradiation

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*The aim of the present investigation was to study the histological and stereological changes in lymphocytes and mast cells after irradiation. For experimental model we used 20 Beagle dogs, 1–2 years old. Ten dogs were irradiated 20 days with 32 Gy onto the whole pelvis and tail. Another 10 dogs represented a control group.*

*Histological and stereological analysis were performed on a Wild sampling microscope M 501. In the nonirradiated group volume, numerical density and average volume of lymphocytes were significantly lower in comparison with the irradiated group. Volume density and average volume of mast cells were significantly lower in nonirradiated group. Numerical density of mast cells in this group was significantly higher.*

*The results of our experiments show that mast cells and lymphocytes in the intestinal mucosa are deeply involved in the tissue fibrosis occurring as the response to irradiation.*

*Key words:* colon-irradiation effects; intestinal mucosa-pathology

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### Introduction

Several years after irradiation severe fibrous changes of the colon often cause for a surgeon an unsolvable problem in surgical intervention. The purpose of our study was to investigate experimentally the histological and stereological changes in the colon occurring after irradiation in view of an early diagnosis and prevention of fibrosis. The intestinal epithelium is a tissue most sensitive to irradiation.<sup>1,2,3</sup> Individual cells in the intestinal mucosa such as lymphocytes and mast cells participate in the defense system of the body or in the protection against

tumor invasion. Therefore we paid special attention to them in our study. The literature data describe changes in the intestinal mucosa,<sup>1,4,5,6</sup> yet no stereological data could be found in the available literature.

### Materials and methods

20 Beagle dogs weighting 8 to 13 kg, 1 to 2 years old were included in our study. Ten dogs were irradiated (I) with  $\gamma$  rays on telecobalt (Phillips) with 32 Gy over the pelvis region and tail. The size of the irradiated region on the skin was 10 × 15 cm. Ten dogs represented the nonirradiated group (N). Ten days after concluded irradiation, a 1-cm wide piece of colon transversum was excised from the middle third of the colon of the anesthetized dogs. Tissue was fixed in Bouin's fluid, embedded in paraffin

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and cut in 5  $\mu\text{m}$  step serial sections. The step section was 20  $\mu\text{m}$  thick. The obtained preparations were stained with hematoxylin-eosin (HE), toluidine-blue and solution alcian blue (SAB) reaction.

An accurate histological analysis of the step serial sections was used to establish the changes in the mucosa of the colon in individual groups. The lymphocyte infiltration and presence, size, form as well as distribution of the mast cell's granules were studied.

Histological analysis<sup>7</sup> were performed on a Wild sampling microscope, using Weibel's test system. Volume density ( $V_v$ ) of lymphocytes and mast cells were estimated at an objective magnification  $\times 40$  using the M-42 test system. Numerical density ( $N_v$ ) of I and m were estimated according to the Weibel-Gomez method at an objective magnification  $\times 40$  using the M-100 test system. For these cells average volume was also calculated.

The results were statistically evaluated. Significant differences were determined by Student's "t" test.

## Results

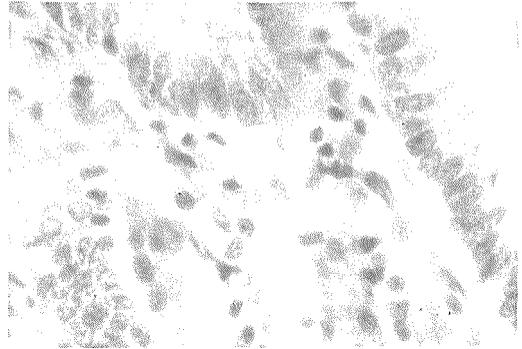
### *Histological analysis*

In the N group lymphocyte infiltration of lamina propria in the intestinal mucosa was well expressed while in the irradiated group (I) there were some rare lymphocytes only in the connective tissue. The individual ones infiltrated the epithelium (Figure 1).

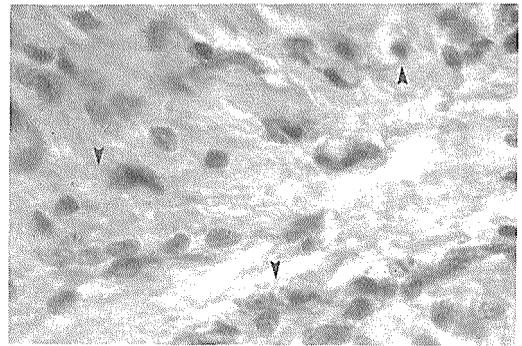
In the N group the mast cells were equally distributed in the connective tissue. Their cytoplasm was full of metachromatically stained granules. Oval nuclei were visible in the middle of cells (Figure 2). In the I group the mast cells were mainly in the connective tissue at a basal part of the cryptes. Their shapes were mainly irregular. Numerous metachromatic granules were dispersed around the cells (Figure 3).

### *Stereological analysis*

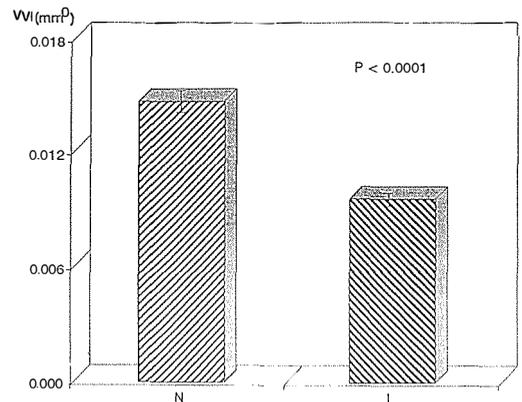
In the N group all the measured stereological values of lymphocytes such as  $V_v$  (Figure 4),  $N_v$  (Figure 5) and average volume of the indi-



**Figure 1.** Rare lymphocytes in the irradiated group (HE, obj. 40 $\times$ ).



**Figure 2.** Degranulated mast cells in the intestinal mucosa in the irradiated group (SAB, obj. 40 $\times$ ).



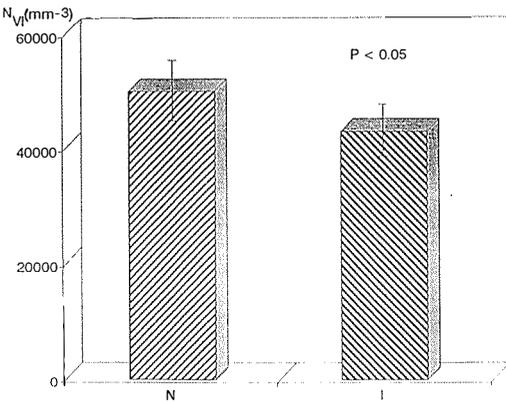
**Figure 3.** Volume density ( $V_v$ ) of lymphocytes in the nonirradiated (N) and the irradiated (I) group ( $V_v \pm 2SE$ ).

vidual lymphocytes  $V_I$  (Figure 6) were significantly lower in comparison with the I groups.

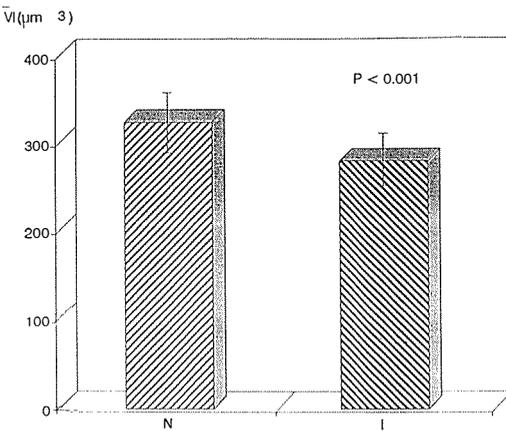
$V_v$  of mast cells (Figure 7) and  $V_m$  mast cells (Figure 8) were significantly lower, while the  $N_v$  (Figure 9) was significantly higher in the N group compared to the I group.

### Discussion

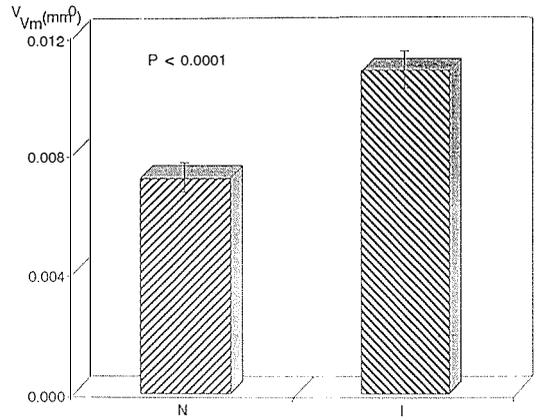
Stereological analysis of lymphocytes after irradiation revealed reduction of  $V_v$ ,  $N_v$  and  $V$  of lymphocytes. This demonstrates that irradiation reduces the number and the size of the cells.



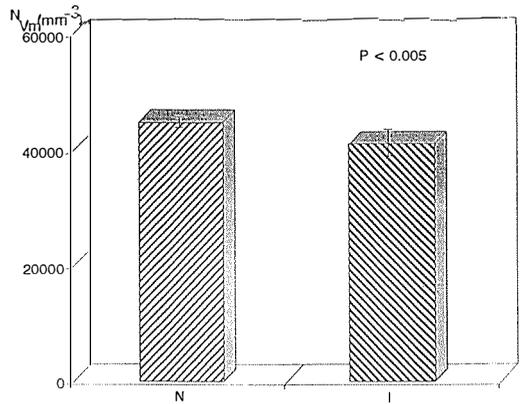
**Figure 4.** Numerical density ( $N_v$ ) of lymphocytes in the nonirradiated (N) and the irradiated (I) group ( $N_v \pm 2SE$ ).



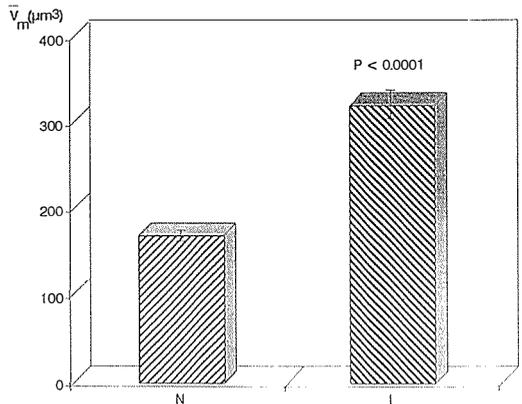
**Figure 5.** Average volume ( $V_I$ ) of lymphocytes in the nonirradiate (N) and the irradiated (I) group ( $V_I \pm 2SE$ ).



**Figure 6.** Volume density ( $V_v$ ) of mast cells in the nonirradiated (N) and the irradiated (I) group ( $V \pm 2SE$ ).



**Figure 7.** Average volume ( $V_b$ ) of mast cells in the nonirradiated (N) and the irradiated (I) group ( $V \pm 2SE$ ).



**Figure 8.** Numerical density ( $N_b$ ) of mast cells in the nonirradiated (N) and the irradiated (I) group ( $N \pm 2SE$ ).

The findings of Black<sup>8</sup> and Breiter<sup>9</sup>, who investigated histological changes of lymphocytes in the intestinal mucosa, agree with our findings. The authors state that numerous lymphocytes and plasma cells, penetrating the intestine with blood or being produced by proliferation of the lymphocytes in the mucosa, are smaller after irradiation. This most probably reflects the change in the immune system resulting in the progression of irradiated tissue impairment. It is also possible that X rays damage the chromosomes and so diminish the capacity of lymphocytes. The consequence is the reduction of their number and the inhibition of normal response to antigenic stimulation.<sup>10,11</sup> It is also possible that the irradiated lymphocytes release nucleoproteins which affect proliferation of fibroblasts and formation of collagen fibers in the irradiated tissue.<sup>3-5</sup>

Our stereological analysis of the irradiated mast cells revealed a significantly increased  $V_v$  and  $V$  of an individual cell and reduced  $N_v$ . Irradiation also caused degranulation. These results agree with the findings of Grand<sup>12</sup> and Sedgwick<sup>13</sup>, who investigated mast cells in the mucous membrane of the small intestine of irradiated mice and rats. The literature data demonstrate that histamine is released from the mast cell granules during irradiation.<sup>3,14</sup> The literature data state that mast cells are the only cells in the body with receptors for IgE and can therefore bind IgE.<sup>15,16</sup> Literature data and our results suggest that new antigens are produced in the damaged intestinal mucosa after irradiation. Binding of neo-antigens to the antibodies IgE fixed to the mast cells caused the degranulation and the release of vasoactive amines and other inflammatory mediators so as leukotrienes.<sup>17</sup> Such leukotrienes can induce long-term contraction of the intestinal vessels. All mentioned changes cause poorer oxygenation of intestinal tissue and finally fibrosis of the intestinal wall.

The results of our experiments have confirmed our hypothesis that mast cells and lymphocytes in the intestinal mucosa are deeply involved in the tissue fibrosis occurring as the response to irradiation.

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