Respiratory failure resulting frequently in death is one of the complications in the course of post-hemorrhagic changes. A systemic inflammatory reaction plays a significant role in the pathogenesis of this syndrome. Mast cells also contribute to this effect. To broaden our knowledge of the pathogenesis of respiratory insufficiency, we evaluated morphometrically lung mast cells in hemorrhagically shocked rats. Lung sections were stained with alcian blue and safranin, and four separate locations were distinguished: under the lung pleura, around the bronchi and the large vessels, and in the interalveolar septa. A decrease in the area and volume of mast cells and an increase in their circularity index in interalveolar septa and around the bronchi was observed. An enlargement of mast cells around lung vessels was also found. There were no changes in the morphometric parameters of mast cells under pleura. The results suggest an activation and degranulation of mast cells and a role in the inflammatory process causing acute lung injury in hemorrhagic shock.

Key words: mast cell, lung, hemorrhagic shock, morphometry.

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of biologically active substances such as histamine, heparin, proteolytic enzymes, glycosidases, cytokines and the products of arachidonic acid — prostaglandins, leukotrienes, PAF (platelet activating factor) and others, which may be involved in the pathogenic processes of acute lung damage in hemorrhagic shock (Pittet et al., 1997). An attempt has been made to evaluate morphometrically mast cells in rat lung in order to broaden our knowledge of respiratory insufficiency in hemorrhagic shock, there being incomplete data about mast cells in hypovolemic shock.

Materials and Methods

The study was approved by the Regional Ethics Committee of the Medical University of Bialystok. The studies were conducted on 24 young female Wistar rats, of 180-200g body weight (average 190±10g), divided into two groups (n=12) and fed with a standard granular diet. The procedures involving the animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and Guidelines for The Use of Animals in Biomedical Research (Giles 1987).

The feeding was stopped at 12 hours before the experiment but there was free access to drinking water. The rats were anesthetized with ethyl ether and operated on under aseptic conditions. After fixing the animals in a supine position, tracheotomy was performed to keep the air ducts unobstructed. After exposing the cervical vessels, the left carotid artery was cannulated with a plastic cannula (0.6 mm in diameter) inserted 1 cm into the artery. The surgical manipulations took about 10 minutes. The measurement of systemic arterial blood pressure started after 5 minutes necessary to stabilize the circulation. A Statham P23 transducer, pre-amplifier and recorder (Gould, USA) were used.

The animals were divided into two groups (n=12 each): group I, sham operated (SO), which underwent anesthesia, tracheotomy and cannulation of the carotid artery; group II, shocked (HS), in which the initial procedures were conducted as mentioned above and then the hemorrhagic shock was evoked by the withdrawal of 25% of the circulating blood from the carotid artery over 3 minutes, which decreased the arterial blood pressure to 35±5 mm Hg. The volume of shed blood was calculated by the formula: v = body mass × 0.02. The shock duration was 75 minutes. After that the animals were decapitated.

Collection and fixation of the material

After dissection, the left lung was expanded with fixation liquid (absolute alcohol, chloroform, glacial acetate acid in a 6:3:1 ratio (Carnoy's solution) until the lung pleura was smoothed out. The bronchus was ligated and fixed in Carnoy's solution for 24 hours +4°C. The tissues were then embedded in paraffin blocks. Sections 7 µm thick were stained with alcian blue and safranin.

The cellular identification and measurements were carried out in a light microscope (Olympus Bx50), using ×400 magnification and examined simultaneously by two independent observers. Each pair of results was averaged. Morphometric computer analysis of the pictures was performed by means of Lucia G (Nikon) video channel and programming using PC Pentium 120.

Four separate typical locations were distinguished: directly under the lung pleura, around the bronchi and the large vessels and in the interalveolar septa. We analyzed around 200-300 mast cells in each compartment of each group. The following characteristics of mast cells were analyzed: area in mm², volume in mm³, the circularity index (the feature determining the shape of the object, i.e. the ratio of the shortest to the longest object diameter when it is approximated to an ellipse; this ratio equals 1 for a circle, and is less than 1 for all other shapes.

Statistical analysis

The results were analyzed statistically with the calculation sheet of Microsoft Excel (Microsoft) and a statistical packet of Statgraphics plus (Statistical Graphic Corp.). None of the variables fitted the normal distribution, so a non-parametric test, Mann-Whitney, was used to evaluate numerical data presented as the mean ± of standard deviation (±SD). The level of statistical significance equaled p<0.05.

Results

The computer morphometry of lung mast cells of HS rats compared to that of the SO rats revealed the most significant difference in morphometric parameters, such as a decrease in the area and volume and an increase in the circularity index of mast
cells accumulating in interalveolar septa (Figure 1a and b). Small sizes and a significant change in mast cell shape indicate immature and young forms or/and their degranulation. Equally significant changes were observed in mast cells concentrated around the bronchi (Figure 2). There was a significant decrease in the area and volume. The circularity index increased; however, the shape of mast cells remained elongated, which was typical of mast cells located in the connective tissue. Mast cells accumulating loosely in the adventitia of lung blood vessels were the most spherical. The increase in morphometric parameters was observed in these cells which had the largest measurements in the population examined. The relatively smallest difference in morphometric parameters was observed in the

subpopulation of mast cells located under the pleura (Figure 3). There were no significant changes in their shape and size. Taking into consideration migration capabilities of mast cells, it can be assumed that they migrated to the pleural cavity, which would explain the lack of quantity and quality changes in this subpopulation.

Based on the computer analysis of the microscopic picture of mast cells in their typical location in the lung, numerous morphometric data of chosen parameters were obtained and are presented in Tables 1-3.
Discussion

The present observations differ from the results of our previous investigations on the same experimental model (Kasacka et al., 2001). In that study, we observed no changes in the morphometric parameters of mast cells in the pleural cavity except for a decrease in the circularity index (Kasacka et al., 2001). This is partly in agreement with the results of other authors who observed a decrease in the shape index of mast cells in the peritoneal cavity during hemorrhagic shock (Debek et al., 1995), an increase in the cell size and a decrease in the shape of mast cells in the peritoneal cavity after exposure to activating compounds. The application of a mast cell specific stabilizer (sodium chromolyn) normalized the mast cell morphometric parameters except for the circularity index. This change in shape seems to be a permanent morphometric feature and confirms mast cell activation (Levi-Schaffer et al., 2000). The anatomical location and concentration of mast cells depended upon their phenotype, but prevailed around the bronchi, vessels and pleura. The characteristic accumulation of mast cells around the bronchi and vessels in the lungs, both denervated and hypoxemic, may be caused by a functional relation of mast cells and nerve endings through neuromediators — CGRP, SP and NGF (nerve growth factor). These peptides induce, directly or indirectly, an increase in the number of mast cells through an enhanced degranulation and release of inflammatory mediators, mainly histamine, but also NGF (Tozzi et al., 1998). In hemorrhagic shock, the phenomenon of ischemia/reperfusion may be an early factor influencing mast cell activation, and at a later stage, the proliferation of fibroblasts, which are a rich source of SCF (stem cell factor) (Vural et al., 2000) might be a factor influencing their activation.

The distribution of particular mast cell phenotypes in the lung structures depends on local growth factors. Bronchial hyper-reactivity (Liakakos et al., 1997) and reconstruction of the wall of the pulmonary vessels thickened by chronic hypoxia and pulmonary hypertension may result from these distribution processes (Kushimoto et al., 1996; Hamada et al., 1999).

Surface adhesion molecules located on the surface of endothelium play a significant role in mast cell migration. Mast cells possess functional receptors for adhesion molecules of the endothelium (Palecanda et al., 1997). A growth factor (SCF), and IL-3, IL-4, IL-9 and IL-10 belong to other agents regulating mast cell migration and maturation (Meininger et al., 1992; Frenz et al., 1997). Additionally, inosine, a product of adenosine deamination, is a potent inducer of mast cell degranulation with all its consequences (vascular trans-exudate, bronchial spasm, inflammatory agent recruitment and further damage of hypoxemic tissues) (Xiaowei et al., 1997).

The changes in pulmonary mast cells described above can be regarded as processes of their activation and migration to the sites of typical location induced by chemotactic agents. These result in the secretion and participation of mast cell mediators in

**Table 1. Area of lung mast cells of sham operated and hemorrhagically shocked rats.**

<table>
<thead>
<tr>
<th>Location</th>
<th>Sham operated rats [µm²]</th>
<th>Shocked rats [µm²]</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interalveolar septa</td>
<td>30.18±1.43</td>
<td>18.59±0.80</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Perivascular</td>
<td>32.12±1.51</td>
<td>35.50±1.43</td>
<td>p &lt; 0.02</td>
</tr>
<tr>
<td>Subpleural</td>
<td>28.93±1.99</td>
<td>27.85±2.45</td>
<td>N.S.</td>
</tr>
<tr>
<td>Peribronchial</td>
<td>40.99±1.97</td>
<td>29.55±1.40</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

Data represent the means ± SD.

**Table 2. Volume of lung mast cells of sham operated and hemorrhagically shocked rats.**

<table>
<thead>
<tr>
<th>Location</th>
<th>Sham operated rats [µm³]</th>
<th>Shocked rats [µm³]</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interalveolar septa</td>
<td>135.53±8.88</td>
<td>64.31±4.37</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Perivascular</td>
<td>148.51±10.00</td>
<td>168.84±10.18</td>
<td>p &lt; 0.002</td>
</tr>
<tr>
<td>Subpleural</td>
<td>131.26±12.94</td>
<td>122.43±12.05</td>
<td>N.S.</td>
</tr>
<tr>
<td>Peribronchial</td>
<td>214.40±14.97</td>
<td>130.86±9.34</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

Data represent the means ± SD.

**Table 3. Circularity index of lung mast cells of sham operated and hemorrhagically shock rats.**

<table>
<thead>
<tr>
<th>Location</th>
<th>Sham operated</th>
<th>Shocked rats</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interalveolar septa</td>
<td>0.71±0.15</td>
<td>0.75±0.15</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Perivascular</td>
<td>0.76±0.14</td>
<td>0.80±0.13</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Subpleural</td>
<td>0.62±0.19</td>
<td>0.64±0.18</td>
<td>N.S.</td>
</tr>
<tr>
<td>Peribronchial</td>
<td>0.71±0.17</td>
<td>0.75±0.15</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

Data represent the means ± SD.
inflammatory reactions, bronchial spasm and increased mucus secretion, edema and damage to the endothelium. The enlargement of mast cells around the vessels may be consistent with the results of studies on their role in angiogenesis, fibrousness and the reconstruction of pulmonary vessel structure in lung hypertension (Mitani et al., 1999).

Biologically active substances released from mast cells have the ability of degrading numerous structural and enzymatic proteins, and glycosaminoglycans. This causes the patho-genic changes resulting in acute lung injury in hemorrhagic shock. A degradation of such proteins, such as collagen types IV, V and VI, fibronectin, and pro-stromelysine induces con-nective tissue stroma destruction and base- ment membrane damage of both blood vessels and epithelium. Histamine and kinins released from mast cells are responsible for capillary vessel dilatation, whereas cytokines and other chemotactic agents cause further mobilization and activation of proinflammatory cells. Moreover, the ability of mast cells to synthesize oxygen reactive forms, TNF-α and PAF may suggest a significant role of mast cells in proinflammam-tory process stimulation. By contrast, heparin released from mast cells inhibits numerous ele-ments of the inflammatory reaction and influences the maintenance of organic homeostasis (Humphries et al., 1999; Zehnder and Galli, 1999). It also inhibits bronchial spasm and hyper-sensitivity, and mast cell degranulation (Martinez-Salaz et al., 1999).

Mast cells are currently regarded as the cells directly involved in the inflammatory re-action in lungs. Hemorrhagic shock initiates an inflammatory reaction cascade and triggers an interdependent system among its elements, such as the release of numerous proinflammatory cytokines (TNF-α, IL-4, IL-5, IL-6, IL-8), anti-inflammatory cytokines (TGF-β, IL-10), neutrophil recruitment and the activation and overproduction of free radicals (Anderson et al., 1990; Kuhnlie et al., 1995; Galli and Wershil, 1996; Hierholzer et al., 1997; Hierholzer et al., 1998). In our study, the changes in mast cell parameters may suggest that they are activated and play a certain role in the modulation of the inflammatory processes that cause acute lung injury in hemorrhagic shock.

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