Cytomorphic Effects of Chemical and Hormonal Agents, and Electronic Stimulation on the Peritoneal Mast Cells of the Rat

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ABSTRACT

After the intraperitoneal injections of alloxan, carbon tetrachloride, cortisone acetate, adenocorticotrophic hormone, morphine hydrochloride, toluidin blue, physiological saline solution, distilled water, and direct stimulation with electronic current, the peritoneal mast cells of the rat were observed in order to document and study the cytomorphic changes.

Adult Sprague-Dawley strain albion rats were used. The substances tested were dissolved in physiological saline solution and injected into the abdominal cavity. Three to twenty-four hours later the rats were sacrificed and the morphological changes of the peritoneal mast cells were observed by means of phase contrast microscopy and ordinary light microscopy.

Cytomorphic effects of alloxan on the mast cells were comparatively marked and those effects of CCl₄, cortisone, ACTH, morphine-HCl, and physiological saline solution were slight and similar to each other. But the destructive effects of toluidin blue, distilled water, and electronic stimulation on the mast cells were severe and noticeable in this study.

These results indicate that the intraperitoneal mast cells of rats show more sensitive reactions to a metabolic poison alloxan, a low osmotic pressured-material distilled water, and a histamine liberator toluidin blue, and a physical stimulus electronic stimulation than the other similar chemical agents.

INTRODUCTION

The intraperitoneal mast cells of the rat are unusually favorable material for in vivo experiments upon mast cells because experiential solutions injected intraperitoneally have access to a very large member of these cells and the effects of chemical agents upon them can be rapidly evaluated in stained slides.

Cebra and Mold (1962) observed that in vivo pretreatment with insulin does not influence the production of mast cells in tissue cultures of subsequently removed thymus tissue of the rat. Padawer (1960) also reported that a metabolic poison, alloxan, was tested as an inducer of deformation but this agent did not elicit any morphological response. Piccinelli (1956) reported that the inhalation of carbon tetrachloride causes mast cell degranulation, followed by a secondary increase in the number of mast cells of the rat. Smith and Lewis (1955) found that in the rat, hypophysectomy or administration of ACTH or cortisone elicited a small increases in the number of abnormal mast cells as evidenced by vacuolation and clumping of cytoplasmic granules, and suggested that the pituitary-adrenal system exerts a controlling influence on the mast cell. Monkhouse and Baker (1963) reported that cortisone treatment caused an increase in plasma clearing factor lipase levels in...
both intact and adrenalectomized rats, and no significant differences were found between the groups, but the wide variation in mast cell count, even in control animals, made the results difficult to evaluate. Johansson and Norn (1963) reported that exposure of rat peritoneal mast cells in vitro was found to be 300 to 400 times weaker as a liberator of histamine than compound 48/80 or polymyxin "B". Lee (1968) reported that fairly significant degranulation of mesenteric mast cells in the rat treated with morphine hydrochloride was observed, which was probably associated with the concomitant liberation of tissue histamine partly derived from the mast cells. Bloom (1957) reported that electron and phase contrast microscopy revealed that in the rat, toluidin blue intraperitoneal injection increased the formation of granule-containing vacuoles in the peritoneal mast cells, at the same time causing disintegration and release of granules. Smith (1958) reported that administration of the histamine liberating compound 43/80, stilbamidine, proamine sulfate, and toluidin blue brought about the release of histamine from the mast cells, and the mesenteric mast cells swelled and were shortly after followed by fading changes of granules. Fawcett (1954) observed that intraperitoneal injection of Tyrode solution alone was not particularly damaging to the mast cells and little or no histamine was released. Fawcett (1955) reported that the mesenteric mast cells of the rat were disrupted by the intraperitoneal injection of distilled water, thereafter they were slowly replaced. Riley (1955) discussed the effect of electronic current on mast cells, and Ceaba et al. (1961) reported that when a galvanic current is passed through a rat, many mast cells accumulated in the thymus. The present study attempts to observe the cytomorphic effects of the metabolic poisons alloxan and carbon tetrachloride (CC14), hormonal agents cortisone acetate and adrenocorticotrophic hormone (ACTH), histamine liberators morphine hydrochloride (morphine-HCl) and toluidin blue, physiological saline solution (0.85% NaCl) and distilled water, and a physical stimulus by electronic stimulation (galvanic current).

MATERIALS AND METHODS

Healthy adult rats (Sprague-Dawley strain albino rats), weighing about 200 gm. were used. Food and water were allowed ad libitum. Substances to be tested were dissolved in appropriate solvents just before use and injected intraperitoneally into the left abdominal region. Three to twenty four hours later, the rats were anesthetized with nembutal (5 mg./100 gm. body weight) and the abdominal cavity was opened.

<table>
<thead>
<tr>
<th>Substance (Group)</th>
<th>Dose/100 gm. body weight</th>
<th>Solvent</th>
<th>pH</th>
<th>Osmolarity (mOsm/liter)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Alloxan</td>
<td>20 mg</td>
<td>Physiol. sal.</td>
<td>3.9</td>
<td>332</td>
<td>1#</td>
</tr>
<tr>
<td>2. Carbon tetrachloride</td>
<td>0.15 ml</td>
<td>40% Olive oil</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>3. Cortisone acetate</td>
<td>3 mg</td>
<td>Physiol. sal.</td>
<td>6.8</td>
<td>311</td>
<td>2#</td>
</tr>
<tr>
<td>4. ACTH</td>
<td>3 IU</td>
<td>&quot;</td>
<td>6.7</td>
<td>298</td>
<td>3#</td>
</tr>
<tr>
<td>5. Morphine-HCl</td>
<td>5 mg</td>
<td>&quot;</td>
<td>—</td>
<td>—</td>
<td>4#</td>
</tr>
<tr>
<td>6. Toluidin blue</td>
<td>0.2 mg</td>
<td>&quot;</td>
<td>6.2</td>
<td>282</td>
<td></td>
</tr>
<tr>
<td>7. Physiol. sal. sol.</td>
<td>2.5 ml</td>
<td>—</td>
<td>6.6</td>
<td>284</td>
<td></td>
</tr>
<tr>
<td>8. Distilled water</td>
<td>2.5 ml</td>
<td>—</td>
<td>7.0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>9. Electronic stimulator</td>
<td>2V (5.7mA)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5#</td>
</tr>
</tbody>
</table>

# 1) Alloxan WAKO Pure Chemical Co., Ltd.). 2) Cortisone acetate saline suspension, Koen Wha Antibiotic Medicine Co., Ltd. 3) MARCOTON, ACTH-Corticotrophic, USP XVI, Manning & Co. Ltd. 4) Evans Medical Supply Co. Ltd. 5) Electronic stimulator manufactured by American Electronic Labs., Inc.
Peritoneal fluid was sampled with a clean dropper then was studied for cytomorphic changes with a phase contrast microscope (AO Spencer). An ordinary light microscope was also used for observation of slides stained with toluidin blue aqueous solution (1:1,500).

The doses of the substances and electronic stimulation administered in this study was as follows (Table 1). Using the electordes the electronic stimulation (frequency - 5 pulses/sec, duration - 0.5 milliseconds, and 4 volts, 11.4 mA) was for three seconds administered to the peritoneal fluid separated from the abdominal cavity and spread over the slide.

**RESULTS**

The shape and size of rat peritoneal mast cells varied from conditions to conditions. The normal intact mast cells are large and round or slightly ovoid in shape. Free floating peritoneal mast cells have a large round nucleus centrally located in the cytoplasm. The cytoplasm of the peritoneal mast cells was filled with fine or coarse metachromatic granules. Generally two kinds of mast cell in size were distinguished; One was the large-sized cells (20.5 micron in diameter) and the other was the small-sized ones (15.7 micron in diameter). The other cellular elements of the rat peritoneal fluid were generally round in shape. (e.g. macrophages, 10.9 micron in diameter) and far smaller than the mast cells. The incidence of peritoneal mast cells to all of the peritoneal cell elements was 2.3% in the physiological saline-treated group. Hardy any morphological changes were found in these cells (Table 2).

1. Effects of alloxan on the mast cells were seen in almost half of the number of the mast cells. The cytomorphic deformations, e.g. nuclear displacement to the periphery, slightly irregular contour of the cell, and swelling and degranulated changes (Fig. 6) frequently observed in the large-sized cells, occurred in this group. The numerical decrease of the peritoneal mast cells was found compared with the physiological saline-treated group.

2. The effects of carbon tetrachloride on the mast cells was slight. The appearance of large cytoplasmic vacuoles (Fig. 2) and displacement of dark large granules were observed in this group. Swollen mast cells derived from the large-sized mast cells were uncommonly found by the phase contrast microscopy. The numerical decrease of the mast cells was slight and similar to the ACTH-treated group.

3. The intraperitoneal injection of cortisone acetate caused also slight cytomorphic changes. But the diminution of stainability with toluidin blue, swollen cells, and slightly elongated mast cells were characteristic in this group. The

<table>
<thead>
<tr>
<th>Substance</th>
<th>% of Normal mast cells</th>
<th>% of Affected mast cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small</td>
<td>Large</td>
</tr>
<tr>
<td>1. Alloxan</td>
<td>43.3</td>
<td>8.2</td>
</tr>
<tr>
<td>2. CC14</td>
<td>81.4</td>
<td>13.3</td>
</tr>
<tr>
<td>3. Cortisone</td>
<td>81.7</td>
<td>17.4</td>
</tr>
<tr>
<td>4. ACTH</td>
<td>66.1</td>
<td>26.9</td>
</tr>
<tr>
<td>5. Morph-HCl</td>
<td>98.4</td>
<td>0</td>
</tr>
<tr>
<td>6. Tolu.-blue</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7. Physiol.-sal.</td>
<td>89.3</td>
<td>9.3</td>
</tr>
<tr>
<td>8. Dist. water</td>
<td>10.1</td>
<td>12.6</td>
</tr>
<tr>
<td>9. Electr. stim.</td>
<td>3.8</td>
<td>0</td>
</tr>
</tbody>
</table>

# Both normal or the affected mast cells were hardly found in the peritoneal fluid.
The cytomorphic effect of ACTH was comparatively marked from the view point of changes in the cellular contour. A number of the ACTH-treated mast cells had eccentric nuclei, elongated cytoplasm (Fig. 3), and irregular large granules around the nuclear area. Some swollen mast cells were also found here and there. Numerical fluctuation of the peritoneal mast cells was slight.

5. Morphine hydrochloride also induced cytomorphic changes similar to the hormonal agents. Eccentric nuclei (Fig. 1.) irregular contour of the cell, and swollen cytoplasm were easily found in this group. There was a slight decrease of the number of peritoneal mast cells and similar to the cortisone-treated group.

6. There were notable changes not only in the cytomorphic aspect but also in the numerical distribution of the peritoneal mast cells treated with toluadin blue. After administration of this dye, most of the mast cells were identified with difficulty due to disruption and degranulation (Fig. 5) scattering of the cells. The solitary mast cell granules discharged from the cells, black dye particles, and the other irregular-sized cell debris were sporadically observed.

7. After the intraperitoneal injection of normal physiological saline solution, hardly any changes were found in the mast cells. Slight deformation of large-sized mast cells (e.g. swollen contour of the cell) was observed but such a change was negligible and similar to the intact normal mast cells.

8. The disruptive effects of distilled water on the peritoneal mast cells was remarkable in this group. Both small-sized mast cells and large-sized mast cells were damaged (Fig. 4), degranulated, and disappeared due to osmotic disruption. Large-sized mast cells were rarely found and a few only small-sized ones remained in the peritoneal fluid. But this treatment caused no enduring damage to other cell elements of the peritoneal fluid. The intact mast cells were remained in the incidence of 22.7% to the peritoneal mast cells.

9. The effects of electronic stimulation on the peritoneal mast cells were remarkable not only in cytomorphic changes but also in stainability of mast cell granules. Almost all of the peritoneal mast cells were notably affected by ever a low dose of galvanic current (4 volts, 11.4 mA). This dose altered the morphology of the mast cell by displacement of the nucleus, severe elongation of the cytoplasmic mass, swelling and hypertrophy of the cell and extreme degranulation. The nuclei of the affected mast cells were stained a pale blue colour and the cytoplasmic granules which were originally metachromatic did not show their specific metachromasia. Only 3.8% to the total peritoneal mast cells were normal. Most of the affected mast cells contained large vacuoles in their cytoplasms.

**DISCUSSION**

Padawer (1960) reported that rat peritoneal mast cells revealed considerable cytomorphic changes in response to colchicine and some colchicine derivatives. Choi et al. (1966) also reported that after the intraperitoneal injection of colchicine or colcemid the peritoneal mast cells showed an altered morphology and they agreed with the results suggested by Padawer (1957, 1960).

In the present investigation the peritoneal mast cells treated with alloxan revealed some cytomorphic changes and such a result was different from that of Padawer (1960). The administration of carbon tetrachloride showed a slight morphological change and a decrease of the peritoneal mast cells, which was in contrast to the results reported by Piccinelli (1956). But the morphological influences of both chemical agents on mast cells were similar to each other.
Smith and Lewis (1955) reported that cortisone or ACTH elicited small increases in the number of abnormal mast cells as evidenced by vacuolation and clumping of cytoplasmic granules. Caballero and Braccini (1951) also reported that the adult rats injected with cortisone constantly showed a marked reduction in the mast cell counts of the muscular tissue, together with a decrease of hyaluronic acid of the connective tissue. In the authors' results both cortisone and ACTH elicited not only morphological changes but also slight diminution of stainability as the results of Padawer and Gordon (1956).

Johannesson and Norn (1963) reported that morphine-treated mast cells revealed some degranulation and such a affect of the drug was much weaker than that of the other histamine liberators. In this study there was also a slight decrease of the mast cells and cytomorphic change. The cytoplasmic extrusions of the affected mast cells were characteristic in this group as the observations of padawer (1966). Lee (1968) reported that the rat mesenteric mast cells treated with morphine hydrochloride showed fairly significant degranulation but in this group no remarkable changes were found. It is deduced that the rat peritoneal mast cells are more resistant and tolerable to carbon tetrachloride, cortisone acetate, adrenocorticotropic hormone, and morphine hydrochloride than the mesenteric mast cells.

The effect of toluidine blue injection on the mast cells was noticeable in view point of morphological level. As in the experimental results of Bloom (1957), Smith and Lewis (1957), and Smith (1958), disintegration, release of the granules, and swelling and fading changes of granules were also observed in this group. It was confirmed that toluidin blue induced considerable cytomorphic changes in the peritoneal mast cells and most of the mast cells disappeared from the peritoneal fluid. Such a remarkable changes also occurred in peritoneal mast cells treated with distilled water. But in the distilled water-treated group, the intact mast cells of the peritoneal fluid maintained an incidence of 22.7% to the peritoneal mast cells.

Casba et al. (1961) reported that galvanic current led to accumulation of mast cells in the thymus, and Bercovici and Graham (1964) reported that in mice subjected to ionizing radiation, the mast cells in the peritoneal fluid increased. While Smith and Lewis (1954) reported that in the rat, a different forms of stresses had no effect on the total number of cutaneous and mesenteric mast cells but increased the percentage of abnormal forms (cells with vacuolation and granule conglomeration). In the present study the peritoneal mast cells were very sensitive to this physical vitimuli and revealed remarkable cytomorphic changes accompanied by elongation of the cell, which was just similar to polymorphism or cytoplasmic extension induced by colchicine (Padawer, 1960:1966). But despite the intensive cytological effects, the peritoneal mast cells did not lose their abilities to stain metachromatically with toluidin blue. It is probable that the effects of electronic stimulation on stainability of mast cells are different from those of streptomycin and various basic colloids such as protamine, lysozyme, thymus histone, and hemoglobin, which are known metachromasia-inhibiting factors. Caselli et al. (1960) reported that if smears of rat peritoneal fluid were brought in contact with various electropositive colloids including cytochrome C and ACTH, the metachromasia of the mast cells disappeared. In the present study the stainability of the cells influenced by cortisone, ACTH, and the electronic stimulation were generally weaker than those of the mast cells contacted with the other substances.

REFERENCES
YUNG KEUN OH, KUM DUCK CHOI, HYUCK BANG AND MAN SOO PAK — 57 —


LEGEND FOR FIGURES

Fig. 1. Rat peritoneal fluid treated with morphine-HCl. A large mast cell with an eccentric nucleus is visible. Phase contrast. Oil immersion.

Fig. 2. Rat peritoneal fluid treated with CC14. A mast cell with many large vacuoles is seen. Phase contrast. Oil immersion.

Fig. 3. Rat peritoneal fluid treated with ACTH. An elongated mast cell with fine granules is visible in the center. Phase contrast. Oil immersion.

Fig. 4. Rat peritoneal fluid treated with distilled water. A deformed mast cell with dark coarse granules is visible. Oil immersion.

Fig. 5. Rat peritoneal fluid with toluidin blue. A disrupted mast cell and white cells are visible. Notice the dirtied background with various debris. Toluidin blue stain, Oil immersion.

Fig. 6. Rat peritoneal fluid treated with alloxan. An intact mast cell and many disintegrated mast cells without granules are visible. Toluidin blue stain, Oil immersion.