The Mast Cell Reaction of Mouse Skin
To Some Organic Chemicals*

I. Estimation of the Relative Number of Mast Cells in Normal Mouse Skin

L.-G. Larsson, M. L., and Bengt Sylvén, M. D.

(From the Department of Radio-Pathology, Radiumhemmet, Stockholm, Sweden)

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Quantitative determination of the etheral sulphuric acids in the granules of the mast cells in small pieces of tissue would be of considerable value, but unfortunately no micromethods for such extractions and analyses have yet been devised. As matters stand, morphologists have thus to perform a rough estimation of the number of mast cells and their granule content from microscopic slides. The counting of mast cells per tissue unit presents no difficulties per se, but the estimation of the amount of metachromatic granules is rather subjective. However, when large differences exist in the number of mast cells and the granule content, such rough methods seem to give conclusive results (6, 7), but minor differences are apt to be overlooked. The methods outlined above are essential for studying the mast cell reaction to different agents. Without entering more closely into the rough quantitative data supplied by earlier authors (1, 2), a more detailed study will be presented in this paper, including a fairly reliable counting technic suitable for experimental work.

Whenever quantitative methods are applied for the demonstration of biological reactions, many questions will arise as to the validity of the control material. For instance, is it possible to determine a "normal" number of mast cells per cu.mm. in the skin of mice? How are they related to age, sex, body weight, nutritional conditions, and so forth? How large are the individual variations in litter mates? Are the individual variations small enough to permit a reliable "normal value" in the same region in the skin of mice, which in other respects are comparable?

Furthermore, we recall that some mast cells are rich in granules and will be heavily stained, whereas others are poor in granules and consequently are more difficult to discern. According to Hellström and Holmgren (3) it is desirable to perform the counting in thick sections (about 100 μ) in order to reduce the sources of error, but in such sections granule-poor mast cells are easily overlooked. Thus we are compelled to count these cells in sections of different thickness and the technic must be varied with regard to the material and scope of investigation. Many mast cells in the skin contain only a few small granules, and therefore comparatively thin sections have to be used.

Most skin lesions chemically induced involve inconvenient secondary changes such as edema and inflammatory cell reactions, resulting in an increase in volume of the dermis. A mechanical counting of the number of mast cells per cu. mm. of dermal connective tissue could easily lead to an erroneous decrease in the number of such cells. To avoid the error due to such secondary volume increments, we have correlated the total number of dermal mast cells with the measured length of the epidermis. This is apparently the method of choice under such circumstances.

The methods here reported are specially devised for experimental purposes, viz. for further studies on the effect of carcinogenic hydrocarbons, and will deal only with the number of mast cells in the skin of the interscapular region.

TECHNICAL DETAILS

A stock of common Swiss albino mice was registered as usual with regard to age, weight and general condition and fed the same mixed diet. Only animals free from abrasions, lice, vermin and fungi were used. With the help of a binocular loupe and small curved scissors the hairs were cut 1 to 2 days before death, and care was taken not to injure the epidermis. Cutting was performed in a rectangular field (1 cm. × 2 cm.) on each side of the spine in the interscapular regions, leaving a small strip of the coat between the fields. Flaps including the whole skin down to the deep external fascia were then excised. The skin flaps, measuring about 0.7 × 1.5 cm., were fastened with thin steel needles to pieces of cork. To
minimize distortion, faulty stretching and curling of free edges this was done before the last two edges of each flap were cut free. One strip from each cut skin area was then placed for 12 hours in a 4 per cent solution of basic lead acetate (4) and fixed in a mixture of equal parts of formaldehyde solution (14 per cent) and basic lead acetate (8 per cent) for 36 hours. For cytological examination similar flaps were fixed in a solution of formaldehyde, corrosive mercuric chloride and acetic acid. From each flap two sets of paraffin sections were prepared, measuring 4 and 10 μ in thickness respectively. All sections were perpendicular to the skin surface.

All sections of 10 μ were routinely stained in 0.1 per cent toluidine blue solutions in 1 per cent and 30 per cent alcohol (6, 7). The other set of sections was stained for cytological examination. Only sections of 10 μ treated with the basic lead acetate solutions and stained in 0.1 per cent toluidine blue in 30 per cent alcohol solution were accepted for mast cell count.

**METHODS OF COUNTING**

Mast cell counts in thin sections (10 μ) were done by the following methods:

1. One side of a square-ruled ocular micrometer, (described below,) was placed as close as possible to the borderline between epidermis and dermis. Mast cells were then counted separately in that part of the slide corresponding to the upper half of the micrometer net as well as in that corresponding to the lower half. In other words, the number of mast cells was estimated, in the first instance, in the superficial portion of the dermis, and in the second, in the lower dermis and in the hypodermal tissue (Fig. 1). The slide was then moved laterally and a new pair of half-squares were counted as before. Manipulations and counting procedures were repeated 60 times in each case, and the mast cell count thus obtained gave an average value of the number per tissue volume corresponding to the calibrated square rule. The average numbers derived from the right inter-scapular region of the animals were denoted by A and B respectively, and refer to the upper and lower halves of the micrometer. Correspondingly, the average numbers of mast cells derived from the left side of the animals have been called a and b.

Thus,

\[ A \text{ and } a = \text{ the average number of mast cells (60 observations) in a piece of the superficial dermis measuring 0.01 mm. } \times 0.0044 \text{ sq. mm.} \]

\[ B \text{ and } b = \text{ the average number of mast cells (60 observations) in a similar piece of deep dermal and hypodermal connective tissue from the left side of the animal.} \]

2. The other method implies that we determine the number of dermal mast cells per 1.0 mm. of epidermal length. This method is designed to avoid the error due to secondary volume increment. Accordingly, a linear calibrated ocular micrometer measuring 1.00 mm. in length is used to apply this standard to the microscopic slides. Thus, 1.00 mm. is plotted with small dots on the slide along the epidermal basement membrane, and then the number of mast cells is counted in the underlying dermal connective tissue. In this way a total of 40 to 50 mm. of skin is examined in sections 10 μ in thickness. To get such a long area of skin, 6 different non-serial sections have usually been used. The average numbers of mast cells in the dermis per mm. of epidermal length was denoted by C and c corresponding with the designations used above.

\[ C \text{ and } c = \text{ the average number of dermal mast cells per 1.0 mm. of epidermal length, regardless of dermal thickness. (Sections, 10 μ. Number of observations 40 to 50.)} \]

The following standard microscopic equipment was used throughout: Zeiss achromatic objective No. 20, ocular No. 7, and one ocular, square-ruled micrometer net, measuring 0.3 mm. X 0.3 mm. ( = 0.09 sq. mm.) with reference to the object.

**RESULTS**

For the estimation of the number of mast cells, the first method was applied to 10 mice 8 weeks old (Table I). Forty-two additional mice 2 to 3 months old were further examined but for the sake of brevity these results are not recorded in detail. However, it may be mentioned that the
number of mast cells in this material showed the same average value \( (A \text{ and } a = 10.3) \) and also very large individual variations (Table I). Both methods were used in two groups of litter mates (Table II). Only the second method was applied to 2 groups of litter sucklings (Table III).

The figures in Tables I, II and III and those of the additional material mentioned above justify the following conclusions.

The **individual variations** of the average numbers of mast cells in the different age groups are very large, both in the dermal \((A, a, C, \text{ and } c)\) and in the hypodermal \((B, \text{ and } b)\) material. Even if we could present a "statistically correct" average number of mast cells in mice of different age groups, these figures would be of little or no value to experimental research, because they do not permit any conclusions as to the actual number of mast cells in the individual mouse.

On the other hand, a comparative dermal mast cell count obtained in symmetrical skin areas shows remarkable similarity for both sides as manifested by the reported quotients \( A/a \) and \( C/c \). The corresponding quotients \( B/b \) do not express as great conformity.

Thus, we have found a method suitable for experimental purposes, viz. for studying the effect of different agents on the number of dermal mast cells, provided that symmetrical skin areas from

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**Table I:** Relative Number of Mast Cells in the Interscapular Skin of Normal 8 Weeks Old Swiss Albino Mice

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Age, weeks</th>
<th>Weight, gm.</th>
<th>Number of mast cells Right side</th>
<th>Quotients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>( A \pm E ) Right side ( B \pm E )</td>
<td>( a \pm E ) Left side ( b \pm E )</td>
</tr>
<tr>
<td>1†</td>
<td>8</td>
<td>19.5</td>
<td>10.5 ± 0.5 2.7 ± 0.3</td>
<td>9.9 ± 0.6 3.0 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>17</td>
<td>9.0 ± 0.5 2.5 ± 0.3</td>
<td>9.2 ± 0.6 2.6 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>14.5</td>
<td>15.4 ± 0.8 4.2 ± 0.4</td>
<td>16.8 ± 1.0 3.9 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>17.5</td>
<td>7.1 ± 0.4 3.3 ± 0.3</td>
<td>6.7 ± 0.4 3.0 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>20</td>
<td>9.4 ± 0.6 2.1 ± 0.2</td>
<td>10.4 ± 0.7 2.4 ± 0.2</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>18</td>
<td>19.7 ± 0.9 5.1 ± 0.4</td>
<td>21.5 ± 1.0 5.0 ± 0.4</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>19.5</td>
<td>8.2 ± 0.4 3.2 ± 0.2</td>
<td>8.4 ± 0.5 3.3 ± 0.3</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>12.5</td>
<td>11.1 ± 0.7 3.5 ± 0.3</td>
<td>10.0 ± 0.6 3.9 ± 0.3</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>25</td>
<td>9.2 ± 0.5 2.1 ± 0.2</td>
<td>8.7 ± 0.5 3.0 ± 0.2</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>13.5</td>
<td>10.3 ± 0.6 3.8 ± 0.3</td>
<td>10.3 ± 0.6 3.2 ± 0.3</td>
</tr>
</tbody>
</table>

**Average numbers:**

\( A \text{ and } a = 11.0 \)
\( B \text{ and } b = 2.6 \)
\( C \text{ and } c = 34.3 \)

**Standard deviation for quotients:**

\( \pm 0.08 \pm 0.14 \)

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**Table II:** Relative Number of Mast Cells in the Interscapular Skin in a Second Series of 6 to 8 Weeks Old Swiss Albino Mice

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Age, weeks</th>
<th>Weight, gm.</th>
<th>Number of mast cells Right side</th>
<th>Quotients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>( A \pm E ) Right side ( B \pm E )</td>
<td>( C \pm E )</td>
</tr>
<tr>
<td>11†</td>
<td>6</td>
<td>19</td>
<td>9.9 ± 0.5 3.8 ± 0.3 40.7 ± 3.5</td>
<td>10.7 ± 0.6 3.9 ± 0.3 42.0 ± 3.5</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>16</td>
<td>8.6 ± 0.5 1.8 ± 0.2 28.4 ± 3.0</td>
<td>9.5 ± 0.5 2.2 ± 0.2 27.4 ± 3.1</td>
</tr>
<tr>
<td>13</td>
<td>6½</td>
<td>19</td>
<td>7.1 ± 0.4 2.9 ± 0.3 27.2 ± 2.6</td>
<td>7.4 ± 0.4 2.5 ± 0.3 25.0 ± 2.5</td>
</tr>
<tr>
<td>14</td>
<td>6½</td>
<td>16</td>
<td>12.8 ± 0.6 3.6 ± 0.3 36.6 ± 3.2</td>
<td>11.9 ± 0.6 3.0 ± 0.3 35.0 ± 3.5</td>
</tr>
<tr>
<td>15</td>
<td>7</td>
<td>17</td>
<td>9.6 ± 0.5 3.4 ± 0.3 35.3 ± 3.5</td>
<td>8.4 ± 0.5 3.0 ± 0.3 34.4 ± 3.1</td>
</tr>
<tr>
<td>16</td>
<td>7</td>
<td>17</td>
<td>8.0 ± 0.4 2.6 ± 0.3 38.5 ± 4.0</td>
<td>8.7 ± 0.3 2.3 ± 0.2 38.5 ± 3.5</td>
</tr>
<tr>
<td>17</td>
<td>7½</td>
<td>18</td>
<td>10.4 ± 0.6 1.9 ± 0.2 30.3 ± 3.1</td>
<td>11.8 ± 0.6 1.9 ± 0.2 35.1 ± 3.4</td>
</tr>
<tr>
<td>18</td>
<td>8</td>
<td>19</td>
<td>10.6 ± 0.6 3.2 ± 0.3 40.2 ± 3.9</td>
<td>10.3 ± 0.6 3.0 ± 0.3 37.5 ± 3.7</td>
</tr>
<tr>
<td>19</td>
<td>8½</td>
<td>22</td>
<td>8.4 ± 0.5 1.6 ± 0.2 35.3 ± 3.0</td>
<td>7.5 ± 0.4 1.3 ± 0.2 32.4 ± 3.5</td>
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<td>20</td>
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<td>18</td>
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<td>9</td>
<td>18</td>
<td>6.0 ± 0.4 3.6 ± 0.3 28.3 ± 3.0</td>
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<tr>
<td>22</td>
<td>9</td>
<td>18</td>
<td>7.8 ± 0.4 1.8 ± 0.2 30.5 ± 2.8</td>
<td>9.3 ± 0.5 1.9 ± 0.2 34.0 ± 3.1</td>
</tr>
</tbody>
</table>

**Average numbers:**

\( A \text{ and } a = 9.3 \)
\( B \text{ and } b = 11.0 \)
\( C \text{ and } c = 2.6 \)

**Standard deviation for quotients:**

\( \pm 0.08 \pm 0.14 \)

---

The **individual variations** of the average numbers of mast cells in the different age groups are very large, both in the dermal \((A, a, C, \text{ and } c)\) and in the hypodermal \((B, \text{ and } b)\) material. Even if we could present a "statistically correct" average number of mast cells in mice of different age groups, these figures would be of little or no value to experimental research, because they do not permit any conclusions as to the actual number of mast cells in the individual mouse.

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Thus, we have found a method suitable for experimental purposes, viz. for studying the effect of different agents on the number of dermal mast cells, provided that symmetrical skin areas from the same animal always serve as controls. We hardly need repeat that this method so far is applicable only to dorsal skin areas, and is valuable chiefly for studying the strictly dermal mast cells. Furthermore, because of the apparent discrepancy between section thickness and mast cell size, the methods are unsuitable for the estimation of the total numbers of mast cells per tissue unit. If absolute numbers are desired, counting must be performed on thicker sections (3).
constantly larger in young animals (Table III) than in adults (Table II), and thus the statement by earlier investigators could not be corroborated (1, 2). For lack of additional data this fact will not be discussed (3). In most mice high and low numbers of mast cells in the dermis were found to be consistent with respectively high and low numbers of hypodermal mast cells (Table I and II).

The errors inherent in both methods presented above depend upon two different types of error, viz. (a) biological and individual variations in the frequency of mast cells, and (b) technical errors due to the methods of preparation. Our primary values are influenced by both groups of errors simultaneously. Due to the fact that our methods imply comparisons between the relative numbers of mast cells in symmetrical skin areas from the same animal, we have avoided the error caused by individual variations. The biological variation in the number of mast cells in the same animal is of course not eliminated. We have to mention the following important technical errors: overstretching of flaps, swelling, shrinkage, faulty section angles, and thickness.

A statistical expression of the distribution of our primary values is obtained by calculating the standard error of the mean for A, B, C, a, b, and c, called $E_A$, $E_B$, etc. (Eq. 1). The resulting standard errors for the quotients are calculated, (Eq. 2) and amount to ± 10 per cent for $A/a$, and ± 15 per cent for $C/c$. These standard errors do not include systematic technical errors.

If under extremely unfavorable conditions all sources of error go in the same direction, we would get a considerable total error. But fortunately the actual error usually is much smaller, as evidenced in Tables I to III. The standard deviation of quotients $A/a$ and $C/c$ amounts to ± 10 per cent (Eq. 3). This expression includes all possible sources of error. Thus, we are justified in stating that these counting methods are fairly applicable to experimental purposes.

The methods reported above will be used in serial studies on changes in the frequency of visible dermal mast cells induced by different agents. When these standard methods are applied to frequent serial observations resulting in uniform numerical changes, we accept deviations in quotients exceeding $2\sigma$ as evidence of true changes in the number of granule-bearing mast cells.

### SUMMARY

In the interscapular skin areas on the dorsum of mice of different age groups, the individual variations in the number of dermal and hypodermal mast cells were found to be so large, that it proved impracticable to determine an average standard number. On the other hand, in each individual the numbers of mast cells were found to be about the same in symmetrical skin areas. Using this fact, two simple methods are described for the quantitative assay of the relative number of mast cells in thin tissue sections (10 μ). The methods afford ample possibilities for studying the mast cell reactions to different experimental agents, provided that counts for absolute cell numbers per tissue unit are not attempted.

### REFERENCES


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