DIFFERENTIATION AND PROLIFERATION
OF EMBRYONIC MAST CELLS OF THE RAT

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ABSTRACT
Histochemical reactions and radioautography were used to investigate the sequence of mast cell development in rat embryos. Mast cells arise ubiquitously in and are confined to the loose connective tissue in the embryo. The alcian blue–safranin reaction distinguishes between weakly sulfated and strongly sulfated mucopolysaccharides by a shift from alcian blue to safranin staining. Based on this reaction and morphologic characteristics, four stages were identified. Stage I mast cells are lymphocyte-like cells with cytoplasmic granules which invariably stain blue with the alcian blue–safranin reaction. In Stage II cells the majority of granules are alcian blue-positive, but some safranin-positive granules have appeared. Stage III mast cells are distinguished by a majority of safranin-positive cytoplasmic granules; some alcian blue-positive granules still remain. Stage IV cells contain only safranin-positive granules. Thymidine-H3 uptake and identification of mitotic figures indicates that mast cells in Stages I and II comprise a mitotic pool while those in Stages III and IV are mitotically inactive. The pattern of 3H incorporation and the sequence of appearance of histochemically identifiable mast cell constituents corroborates division of the proliferation and differentiation of embryonic mast cells into the four stages described above. The process of formation of mast cell granules is interpreted as reflecting the synthesis and accumulation of a heparin precursor in alcian blue-positive granules followed by the synthesis and accumulation of highly N-sulfated heparin along with mast cell chymase and finally histamine in safranin-positive granules.

INTRODUCTION
Mast cells are common elements of connective tissue in almost every vertebrate species examined. Historically the distinguishing characteristic of the mast cell has been its metachromatic cytoplasmic granules. Partial analysis and identification of the components of the granules have only been achieved in the past 30 years. The typical granules are now known to contain heparin, histamine, and an alkaline active protease or proteases; in two species 5-hydroxytryptamine is also a constituent of the granule. A systematic and comprehensive endeavor to study the source, mode of increase in numbers and differentiation of mast cells was made by Maximow and his associates between 1907 and 1924 (1, 9, 10, 28–30). Since then only partial and sporadic attempts have been made to characterize these processes. Usually this has been done in young and adult mammals by examining mitotic activity of typical mast cells with efforts to distinguish differentiation from some precursor cell type (2, 4, 8, 33–35, 39, 40). The availability of methods for the histochemical demonstration of heparin (37), histamine (21), and the characteristic proteases (19) coupled with the ability to examine specific chemical groups and the mitotic activity of cells with
radioisotope-labeled compounds provided the basis for a new survey of the problem.

METHODS AND MATERIALS

Embryos of Sprague-Dawley rats were collected either by surgical removal or following sacrifice of pregnant females under light ether anesthesia. Animals were killed postnatally by exsanguination under light ether anesthesia. Age was measured from the day on which sperm were observed in the vaginal smear (25); crown-rump length was used in some instances for assessing age utilizing the established correlation between this length and age (11).

The histochemical procedures and compounds employed with radioautography are listed in Table I. Tissues for the periodic acid-Schiff (PAS), diazotized p-bromoaniline (PBA) and phenyl propionyl-naphthol-AS (PPNAS) reactions were quenched in isopentane-propane solution at −190°C, freeze-dried, fixed in formaldehyde vapor for 48 hours at 60°C (21), embedded in 10 per cent piccolyte-paraffin, and sectioned at 5 μ. For other staining procedures, tissues were fixed at least 24 hours in 10 per cent neutral buffered formalin at 0°C, dehydrated in graded ethanol-water solutions and chloroform, cleared in xylene, embedded in 10 per cent piccolyte-paraffin, and sectioned at 5 μ.

S35O4, 3.0 μc per gram body weight, or tritiated thymidine, 0.7 μc per gram body weight, was administered to pregnant rats via tail vein. The time intervals prior to sacrifice were 48 hours for S35O4 and 20 minutes, 2, 4, 8, 12, 24, 48, and 120 hours for tritiated thymidine. Tissue sections were mounted on glass slides and coated with NTB-3 photographic emulsion (Eastman Kodak, Lot LSD 3-8-2). The coated slides were exposed for 14 days, developed in Dektol and the emulsion fixed in Kodak acid fixer followed by 10 minutes in 10 per cent formalin. The alcian blue-safranin reaction was utilized to stain the radioautographs. It was necessary to adjust the staining time in safranin to yield the same differential staining characteristics observed in uncoated sections.

OBSERVATIONS

Differentiation

STAGES OF DIFFERENTIATION

Sections in each series of embryos studied were stained with toluidine blue for the identification of mast cell granules, and the alcian blue-safranin staining reaction was used to differentiate strongly sulfated from unsulfated or partially sulfated mucopolysaccharides (15, 37). A combination of morphologic characteristics and staining properties with the alcian blue-safranin procedure permitted a division of the apparently continuous development of the mast cell into four arbitrary stages (Table II).

Stage I cells are round embryonic lymphocyte-like cells distinguishable from other mesenchymal cells only by the presence of a variable number of cytoplasmic granules which invariably stain blue with the alcian blue-safranin reaction and display a color intermediate between orthochromatic blue and metachromatic red-purple when stained with toluidine blue. The number of granules ranges from one to two scattered in the basophilic cytoplasm of the youngest cells of this stage to a number sufficient to crowd the cytoplasm (Fig. 3).

Stage II mast cells have scant, highly basophilic cytoplasm with granules which stain with safranin (after alcian blue) in numbers varying from a few to about half the number present in a given cell. Some granules show definite metachromasia with toluidine blue (Fig. 4).

Stage III mast cells are packed with granules. The majority of the granules stain red with the

<table>
<thead>
<tr>
<th>Substance or property demonstrated</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic mucopolysaccharides (36)</td>
<td>Toluidine blue, pH 3.5</td>
</tr>
<tr>
<td>Acidic mucopolysaccharides (37)</td>
<td>Alcian blue-safranin, pH 1.0</td>
</tr>
<tr>
<td>Polysaccharides (31)</td>
<td>Periodic acid-Schiff (PAS)</td>
</tr>
<tr>
<td>Nuclear morphology (38)</td>
<td>Feulgen-methylene green</td>
</tr>
<tr>
<td>Histamine (21)</td>
<td>Diazotized p-bromoaniline (PBA)</td>
</tr>
<tr>
<td>Chymotrypsin-like enzyme (18)</td>
<td>Phenyl propionyl naphthol-AS (PPNAS)</td>
</tr>
<tr>
<td>SO4 incorporation into heparin (20)</td>
<td>S35O4</td>
</tr>
<tr>
<td>Mitotic activity (4, 24)</td>
<td>Tritiated thymidine</td>
</tr>
</tbody>
</table>
 alcian blue–safranin procedure and metachromatically with toluidine blue (Fig. 5).

Stage IV is the fully mature stage. The cells are large and their relatively small nuclei are often obscured by the tightly packed granules which are brilliantly metachromatic with toluidine blue. With the alcian blue–safranin reaction all the granules are stained metachromatic brick red (Fig. 6).

**Nuclear Characteristics By Stage**

The Feulgen reaction as modified by Swift (38) was employed to study the morphology of the mast cell nuclei in sections from a 21 day old embryo and adult tongue. Methylene green, a nitrated analog of methylene blue (14), was utilized as a counterstain in 0.1 per cent aqueous solution adjusted to pH 3.5 with acetic acid. This stain is green in the orthochromatic and blue in the metachromatic state, allowing positive identification of mast cells with a color contrasting sharply with the magenta of the Feulgen-Schiff reaction.

Stage I and II mast cell nuclei varied considerably in size and morphologic detail. Since all the mitotic nuclei observed in the embryo appeared to fall in these stages, more variation in chromatin particle size and dispersion pattern was seen in Stages I and II than in later stages. The typical Stage I and II interphase nucleus was spherical, often lacked a definite nucleolus and had fine homogeneously dispersed chromatin with the exception of occasional peripheral chromatin clumps (Fig. 7). Stage III mast cells had nuclei which resembled the nuclei in surrounding mesenchymal cells. Often elliptical or kidney shaped, these nuclei uniformly had one or two well defined nucleoli, moderately clumped chromatin, and a clearly demarcated nuclear envelope (Fig. 8). The mature mast cell nucleus was poorly delineated by the Feulgen-Schiff procedure, and the usual metachromatic dyes used as identifying counterstains tended to obscure the nucleus by intense staining of the cytoplasmic granules. However, morphologic detail in Stage IV nuclei became evident in alcian blue–safranin preparations following the nitrous acid hydrolysis procedure described below. Stage IV nuclei were elliptical with large chromatin clumps abutting on the nuclear envelope and a centrally placed chromatin mass reminiscent of the "cartwheel" nucleus of the plasma cell (Fig. 9).
GRANULE CHARACTERISTICS

HEPARIN: The pattern of alcian blue-safranin staining of the heparin and/or heparin precursors of mast cell granules was outlined above. In order to clarify the results of the alcian blue-safranin staining reaction, hydrolysis designed to liberate sulfamido sulfate was performed on the tissue sections using nitrous acid (23). Hydrolyzed sections were stained simultaneously with sections pre-treated with 5 per cent acetic acid and with untreated sections. Hydrolysis for 60 minutes or longer at room temperature with dilute nitrous acid regularly abolished the affinity for safranin and the toluidine blue metachromasia of mature mast cell granules. Evidence for the specificity of the nitrous acid reaction was seen in the fact that this treatment did not affect the safranin staining or the toluidine blue metachromasia of chondroitin sulfate-rich embryonic cartilage.

The significance of the PAS reaction in mast cells was obscured by the finding that mature mast cell granules reacted positively with Schiff's reagent without prior periodic acid hydrolysis after freeze-drying and vapor fixation. The direct Schiff reaction first became positive in embryonic Stage III mast cells at about 19 or 20 days, so that data for the PAS reactivity of the mast cell were able to be interpreted only in Stages I and II. The first PAS positive cells morphologically like mast cells were seen in the region of the developing spinal column at about 16 days with both cytoplasm and granules staining. Cells corresponding to Stage I stained less intensely than those of Stage II.

Sulfate incorporation into the heparin and heparin precursors in mast cells was measured by radiosulfate uptake (5, 20). A series of embryos was examined after administration of $^{35}$O$_{4}$ and included embryos of approximately 19, 22, and 24 days postconception from three pregnant rats, with tongue and dorsal skin from the hind paws of the adult animals as control tissues. The average level of labeling of the mast cells in adult tongue was assigned a value of 1+ and the intensity of cellular labeling was graded from 0 to 4+ (Figs. 12 through 15). The sections were stained with the alcian blue-safranin reaction, and the intensity of labeling of each cell correlated with its maturational stage. The results of these experiments are given in Table III.

10.05 per cent NaNO$_{2}$ + 0.33 per cent acetic acid in equal parts.

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. of mast cells</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>++++</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>182</td>
<td>40</td>
<td>52</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>338</td>
<td>1</td>
<td>22</td>
<td>44</td>
<td>32</td>
</tr>
<tr>
<td>III</td>
<td>480</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>66</td>
</tr>
<tr>
<td>IV</td>
<td>100</td>
<td>27</td>
<td>73</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

HISTAMINE: The diazotized para-bromoaniline reaction for histamine was first positive in Stage IV mast cells at the time of birth (21 to 22 days postconception) and increased in intensity to a maximum by about the 33rd postnatal day.

MAST CELL PROTEASE: The characteristics of a protease with chymase-like activity present in rat mast cell granules have been reviewed by Lagunoff and Benditt (19). When the phenylpropionate ester of naphthol AS was used as the histochemical substrate for this enzyme in a limited series of embryos ranging from 15 to 26 days in age, a positive reaction was first observed at 17 days with little change in intensity in later embryos. As these preparations could not be counterstained satisfactorily, the stage of the earliest PPNAS-positive cells was difficult to assess, but must be Stage I and/or II, since Stage III and IV mast cells had not yet appeared at the 17-day level of embryonic development.

PROLIFERATION

EARLY DISTRIBUTION: Stage I mast cells were first seen in head mesenchyme ventral to the developing brain in embryos of about 15 days of age. The early mast cell population of the embryo increased rapidly in number, and Stage II cells appeared in developing loose connective tissue around the dorsal vertebrae on the 16th day. At 18 or 19 days post-conception, Stage III cells

TABLE III

$^{35}$O$_{4}$ Incorporation of Mast Cells

According to Stage

Expressed as per cent of cells in each stage at the indicated level of intensity of labeling based on the average labeling of Stage IV mast cells taken as 1+, 2+ equal to twice the labeling intensity of stage IV, etc. See Figs. 10 to 13. Values obtained from one thousand consecutively counted Stage I, II and III mast cells in hind limbs of two newborn rats from the same litter (crown-rump ca. 45 mm) and 100 consecutively counted Stage IV mast cells in adult tongue and dorsal foot skin.

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TABLE IV
Mast Cell Stage versus Crown-Rump Length and Age

Values represent per cent of cells in each stage based on one hundred consecutively counted mast cells in subcutaneous mesenchyme in one parasagittal section from one embryo at each indicated age/length.

<table>
<thead>
<tr>
<th>Crown-rump length in mm</th>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage III</th>
<th>Stage IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.5</td>
<td>93</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>50</td>
<td>38</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>28</td>
<td>42</td>
<td>47</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>32</td>
<td>34</td>
<td>50</td>
<td>16</td>
<td>0</td>
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<tr>
<td>39</td>
<td>23</td>
<td>50</td>
<td>27</td>
<td>5</td>
</tr>
<tr>
<td>45</td>
<td>21</td>
<td>33</td>
<td>41</td>
<td>5</td>
</tr>
</tbody>
</table>

became evident generally in subcutaneous connective tissue, but cells of Stage IV were not present until just prior to birth (Table IV).

Mitotic counts: Utilizing the Feulgen-Schiff reaction with methylene green counterstain described above, sections from a twenty-one day old embryo and adult tongue were examined for morphologic evidence of mitotic activity. Nuclei of Stage I and II mast cells were found in each stage of mitosis except the telophase (Fig. 11). Binucleate and daughter cells were also seen in embryonic tissue; however, no mitotic nuclei or binucleate cells were observed in mast cells in adult tissue. In the results presented in Table V, only clearly identifiable nuclei were included in the mitotic group. Prophase and reconstruction phase nuclei were included as a single category since no clear distinction could be made between them. The “not identified” category includes mast cells whose nuclei were obscured by granules or were otherwise unable to be unclassified.

TABLE V
Mitotic Activity in Mast Cells Observed with the Feulgen-Methylene Green Technique*

Values obtained from 1103 consecutively counted mast cells with visible nuclei in the hind limb of 21 day rat embryo.

<table>
<thead>
<tr>
<th>Prophase-reconstruction phase</th>
<th>Not identified</th>
<th>Interphase</th>
<th>Metaphase</th>
<th>Anaphase</th>
<th>Telophase</th>
<th>Binucleate cells</th>
<th>Daughter cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>276</td>
<td>702</td>
<td>101</td>
<td>3</td>
<td>4</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Per cent</td>
<td>25</td>
<td>63.6</td>
<td>9.2</td>
<td>0.27</td>
<td>0.36</td>
<td>0.91</td>
<td>0.63</td>
</tr>
</tbody>
</table>

* cf. text, page 579.

DISCUSSION

Proliferation

The first recognizable mast cells of the rat embryo arise in the head mesenchyme near the ventral portion of the developing brain at about 15 days postconception. The number of mast cells increases reaching a maximum in the 2 days just prior to birth, with those in the deep (more axial) connective tissue appearing last. In the newborn rat the distribution of the mast cell popu-
In the studies of the incorporation of thymidine-H\(^3\) into mast cells 2 hours after administration of a single dose, 20 per cent to 30 per cent of Stage I and II cells were labeled. Since the total number of mast cells in Stages I and II increases rapidly in embryonic connective tissue during the labeling intervals studied, it is plausible to assume that the high rate of incorporation of thymidine-H\(^3\) indicates mitotic activity in Stage I and II mast cells. Precursor cells also presumably proliferate mitotically, and would increase the apparent incorporation rates of Stage I and II, the magnitude of their contribution depending on the rapidity with which they develop recognizable cytoplasmic granules. The alternative possibility, that only mitotically active precursor cells incorporate thymidine into newly formed DNA, divide, and synthesize mast cell granules in a period of 2 hours in sufficient number to give up to 30 per cent labeling of the Stage I and II cells, is highly unlikely. The delayed labeling of Stages III and IV and the reduction in the proportion of labeled cells in Stages I and II during the period when the proportion of labeled cells in Stages III and IV is rising, is consistent with a sequence of maturation from mitotically active Stages I and II to mitotically inactive Stages III and IV. Accordingly, the embryonic mast cell population is divisible into mitotic, maturation, and end-stage subpopulations: Stage I and II cells comprise a mitotic pool; Stage III cells comprise the maturation pool and attain the end-stage of development (Stage IV) without significant intervening

<table>
<thead>
<tr>
<th>Stage</th>
<th>0 min</th>
<th>10 min</th>
<th>40 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>0</td>
<td>74</td>
<td>205</td>
<td>27</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>0</td>
<td>109</td>
<td>463</td>
<td>27</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>133</td>
<td>5</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>122</td>
<td>3</td>
</tr>
</tbody>
</table>

All stages: 0 min 190/1000, 10 min 136/1000, 40 min 128/1000, 60 min 124/1000, 120 min 127/1000.
mitotic proliferation. The data presented do not rule out the possibility of mitosis occurring at some low rate in Stage IV cells; however, the rapid relative and absolute increase in the mast cell population accompanied by the maturational shift described above (Table IV) indicates that the contribution of mitosis in Stage IV cells, if it occurs, would be small in comparison to the number of Stage IV cells arising by maturation from Stage III. If in fact no mitosis occurs in Stages III and IV, the arrival in Stage IV of the most rapidly maturing labeled Stage II cells and/or incorporation of thymidine-H3 without mitotic activity as discussed below may account for the 3 per cent labeling observed in Stage IV at 12 and 24 hours following dosage (Table VI). This scheme is presented diagrammatically in Fig. 2.

Pelc (32) divides cells incorporating tritiated thymidine at rates detectable in our experiments into two “metabolic stages,” those incorporating thymidine at rates indicating doubling of DNA with subsequent mitotic division, and cells incorporating thymidine at comparable rates without subsequent mitotic division. He further suggests that in certain cell populations incorporation of thymidine largely represents DNA synthesis in non-dividing cells, leading to a high value for the ratio of the per cent labeled nuclei to the mitotic index, whereas in cell populations incorporating thymidine with subsequent cell division, this ratio has a value in the range of 15 or less. Although other investigators report that some mast cells in adult rodents incorporate thymidine, the incidence of these cells is low (2, 4, 40). Allen has presented information obtained with young rats (30 days) for which the percentage of labeled cells and the mitotic index give a ratio of 10 which is in the range of a mitotically active population (3). In our study, the mitotic index derived from data presented in Table IV, assuming that 50 per cent of the cells in the category labeled “Prophase-Reconstruction Phase” are in prophase, is slightly greater than 5 per cent. All of the mitoses were observed in Stage I or II cells. Since approximately 20 to 30 per cent of Stage I and II cells was labeled at the maximum incorporation interval, the ratio of mitotic index of embryonic mast cells to the per cent of labeled cells in the mitotic population is approximately 5; this is consistent with DNA synthesis leading to mitosis. The observation of mitotic figures in Stage I and II cells directly confirms the occurrence of mitosis in these stages (Fig. 11). The population of mast cells in young rats and those experimentally stimulated mast cells (4) in older rats which undergo mitosis could well correspond to a pool of immature cells comparable to the Stage I and II cells of the embryo. The mast cells of the adult may also largely come from such a reservoir.

**Differentiation**

Heparin is known to be present in high concentration in mast cells (7, 17) and is responsible for the characteristic metachromatic staining of the granules. The presence of metachromatic granules, although not entirely sufficient to identify the cell, is an invariable characteristic of mast cells. Thus the synthesis of heparin or an identifiable heparin precursor may be taken as the biochemical event that marks off the mast cell from its precursor. The metachromasia of heparin depends on the presence of a high concentration of acidic sulfate groups distributed along the polymeric chain (6, 36). The sulfates in the case of heparin are present both as sulfamido groups and as the more common sulfate esters (12). Spicer has postulated that the alcian blue-safranin reaction differentiates degrees of sulfation, the more highly sulfated the polysaccharide, the greater its affinity for safranin (37). Since nitrous acid hydrolysis is known to selectively remove sulfamido sulfate from heparin (23) and nitrous acid abolishes safranin staining of mast cell granules but does not alter the safranin staining of the N-sulfate poor polysaccharides of cartilage, some confidence is placed in the histochemical selectivity of this treatment sequence. It is proposed then that those mast cells having safranin-negative, alcian blue-positive granules (Stages I, II, III) contain a polysaccharide poor or totally lacking in N-sulfate, presumably a heparin precursor. This polysaccharide could also be responsible for the bona fide PAS staining seen in the early stages of mast cell differentiation. Similarly, those cells having safranin-positive granules contain highly N-sulfated polysaccharide, probably chemically mature heparin.

Labeling of mast cells with radiosulfate in the rat embryo (13) and in adult tissues (13, 20, 26, 27) has previously been described. $^{35}$SO₄ incorporation may represent either sulfation of newly synthesized heparin or merely the turnover of labile sulfate in preformed heparin. There is a striking difference between the moderate labeling of embryonic Stage II, the intense labeling of...
Stage III cells, and the low levels of incorporation in Stage I and adult mast cells (Figs. 12 through 15). This is taken as indicating a high rate of heparin synthesis in the Stage II and III cell population, consistent with the increasing number of granules present in these cells and the increasing proportion of granules which stain with safranin in the AB-S staining sequence.

The appearance of chymase activity in embryonic cells at a time when only those cells considered to be immature mast cells are present in the embryo on the one hand confirms the identification of these cells, and on the other hand demonstrates that this enzyme is present in the cell at essentially maximal concentration during the process of active heparin synthesis. The early appearance of this enzyme is consistent with evidence that the enzyme constitutes a significant part of the granule matrix (5, 22). This is in contrast to the situation with respect to histamine, as histamine does not become evident in embryonic mast cells until after the appearance of N-sulfated mucopolysaccharide and is very slow in reaching maximal levels. Although the determination of precisely when histamine is first bound in mast cells is limited by the sensitivity of the method employed, the difference between the concentrations of chymase and histamine relative to the maximal concentration of each in adult mast cells is indicative of different maturational patterns.

Based on the data presented, the process of granule maturation can be tentatively analyzed in terms of histochemical landmarks and incorporation of radioisotope-labeled compounds. Granules containing alcian blue-positive material giving a valid PAS reaction and failing to bind safranin are present in cells of Stages I, II, and III. These characteristics of the granules probably indicate the synthesis and storage of a heparin precursor. The low level of $^{38}O_4$ incorporation in the alcian blue-positive granules of Stage I mast cells strengthens this interpretation. Granules containing material which binds safranin appear in Stage II, increase in numbers through Stage III, and become metachromatic with safranin in Stage IV. This pattern, along with the increasing $^{38}O_4$ incorporation in Stage II, III, and IV cells, may indicate the synthesis and accumulation of highly N-sulfated heparin in the safranin positive granules.

The presence of chymase during Stages II and III would allow the interaction of heparin and this enzyme to form part or all of the framework around which the mature mast cell granule is organized. Recent evidence (5, 22) favors the concept that histamine is associated with the mast cell granule by relatively weak physicochemical forces. Its late appearance in the mast cell granule may indicate that the chemical scaffolding of the granule must be completed before binding can occur.

As is seen from the data presented above, the embryonic tissue mast cell arises, proliferates, and matures in a manner analogous to the process of granulopoiesis as postulated by Maximow (28). The mast cell arises from a relatively undifferentiated cell of mesodermal origin which cannot be distinguished from other primitive mesenchymal cells, and is first recognizable by the appearance of specific cytoplasmic granules. In both mast cells and granulocytes mitotic proliferation continues until many cytoplasmic granules are present, but essentially ceases at a certain point after which maturation is completed by a process of granule formation and morphologic alteration. Unlike the granulocytes, mast cells in embryos do not arise in hematopoietic tissue but rather in the loose connective tissue. The end result of mast cell genesis is the formation of a mature cell population which has lost its proliferative capability but has acquired an unique functional capacity.

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Figure 1  Tritiated thymidine labeling of mast cell nuclei by stage expressed as the per cent of mast cell nuclei with greater than twice background density of hits. Data taken from Table VI.

Figure 2  Schematic representation of mast cell proliferation and maturation. Immature granules are represented by circles, mature granules by dots.
FIGURE 3  Stage I daughter mast cells containing only a few relatively large alcian blue positive granules. Alcian blue-safranin stain. × 1400.

FIGURE 4 Stage II mast cell. Fig. 4a depicts the cell photographed with a green filter (Wratten XI Filter No. 11) allowing visualization of both red and blue granules. Fig. 4b is the same cell photographed with a strong red filter (Wratten A Filter No. 25) which allows only blue granules to be seen. Fig. 4c is again the same cell photographed with a strong blue filter (Wratten C5 Filter No. 47) which allows only red granules to be seen. Note the greater number of blue granules represented by Fig. 4b and the distinctly different location of granules in Figs. 4b and 4c. Alcian blue-safranin stain. × 1400.

FIGURE 5 Stage III mast cell. Figs. 5a, 5b, and 5c were photographed using filters as described for Fig. 4. In Fig. 5a both red and blue, in Fig. 5b only blue, and in Fig. 5c only red granules are seen. Note the greater number of red granules as represented by Fig. 5c and the different distribution of granules in Figs. 5b and 5c. Alcian blue-safranin stain. × 1400.

FIGURE 6 Stage IV mast cell containing only red granules. Alcian blue-safranin stain. × 1400.
FIGURE 7 Stage I mast cell, Feulgen-Schiff stain with methylene green counterstain. Note the spherical nucleus with relatively dispersed chromatin. This nuclear morphology is typical of interphase nuclei of either Stage I or Stage II mast cells. × 1400.

FIGURE 8 Stage III mast cell, Feulgen-Schiff reaction with methylene green counterstain. The nucleus is ovoid with some aggregation of chromatin as compared with Fig. 7, and two prominent nucleoli are present. × 1400.

FIGURE 9 Stage IV mast cell alcian blue-safranin stain following 2 hours hydrolysis in dilute nitrous acid. Characteristic granules have largely disappeared revealing the nuclear contents. The chromatin clumps abutting the nuclear envelope and a central chromatin mass give an appearance closely resembling the plasma cell nucleus. × 1400.

FIGURE 10 Stage I mast cell in subcutaneous tissue of a 27 mm rat embryo. Nuclear labeling by tritiated thymidine four hours following administration. Alcian blue-safranin employed as counterstain. × 1400.

FIGURE 11 Anaphase in a mast cell in subcutaneous connective tissue of a 41 mm rat embryo. Feulgen-methylene green preparation. × 1400.
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FIGURE 12 Stage I mast cell S\textsuperscript{35}O\textsubscript{4} radioautograph stained with alcian blue-safranin. The intensity of labeling is 1+ on the scale presented in Table V. × 1400.

FIGURE 13 Stage II mast cell S\textsuperscript{35}O\textsubscript{4} radioautograph stained with alcian blue-safranin. Intensity of labeling is 2+ on the scale presented in Table V. × 1400.

FIGURE 14 Stage III mast cell S\textsuperscript{35}O\textsubscript{4} radioautograph stained with alcian blue-safranin. Intensity of labeling is 4+ on the scale presented in Table V. × 1400.

FIGURE 15 Stage IV mast cell S\textsuperscript{35}O\textsubscript{4} radioautograph stained with alcian blue-safranin. Intensity of labeling is 1+ on the scale presented in Table V. × 1400.
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