Characteristic organelles of follicle cells, the so-called 'lining-bodies'[13] were also found in the surface epithelium of the left gonad of chick embryos from the 6th day onwards[14]. These ultrastructural observations are likewise in favour of the classical hypothesis of the origin of avian follicle cells. RAHIL and NARBAITZ[14] found 'boursouflures' (emerging from the somatic cells of the ovarian epithelium[15] together with a network of fine microfilaments, especially concentrated at the apical poles of the surface epithelium cells. According to the latter authors, these organelles perhaps play a rôle in the change of cell shape, and may be related to the formation of sex cords. Our observations seem to afford additional evidence for this supposition.

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Association of Mastopoiesis with Haemopoietic Tissues in the Neonatal Rat

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Summary. Mast cells in the newborn rat occur in haemopoietic foci of liver and spleen, but disappear from those foci as extramedullary haemopoiesis ceases during the initial postnatal month. At the same time, mast cells increasingly populate bone marrow and connective tissues of heart, lung, stomach and portal tract of liver.

In the foetal rat, mast cells appear on the 15th or 16th day of gestation in caudal mesenchyme near the ventral portion of the developing brain[2–7]. They subsequently become widely distributed, being particularly associated in the adult with connective tissues, but are sparse in parenchymal organs such as liver and kidney[8]. On the other hand, there is little information on the distribution and population density of mast cells in the mature foetus, or on the proliferation of these cells in the neonatal rat to achieve their abundance in the adult[9, 10].

To compare the distribution and density of mast cells in the newborn rat and young adult, batches of 4 albino rats (each animal from a different litter) were sacrificed at intervals of $^1/_2$ to 90 days after birth. Pieces of heart, lung, thymus, spleen, liver, stomach, kidney, abdominal skin and tibia from each animal were fixed in Lillie's neutral buffered formalin, and 5 µm paraffin sections were stained for 5 sec with 0.25% eosin Y in 80% ethanol containing 0.25% acetic acid, rinsed briefly in tap water and then stained for 10 min with 0.01 or 0.1% toluidine blue in aqueous 0.3% acetic acid. Mast cells were enumerated in 100 fields per section with a Carl Zeiss N.A. 0.95 × 40 objective and × 8 compensating oculars (field diam = 440 µm), the number of cells being expressed as the corresponding number per mm³ of tissue. No cell corresponding to the basophil leucocyte of non-murine mammals has been identified in the present work, a result consistent with reports of the very low incidence of basophils in the bone marrow of adult rats[11]. In the present investigation, mast cells have been distinguished from basophils by a histochemical technique based on the vigorous hydrolysis of naphthol AS-D chloroacetate obtained with mast cells and the feeble hydrolysis with basophils[12]. Distinction of mast cells from 'mucosal mast cells' has been verified by the inability to demonstrate metachromasia in the latter when fixed in buffered 10% formalin[13].

Enumeration of mast cells in neonatal rats has revealed that the above organs fall into 4 groups (Figure). In the 1st, which comprises skin and thymus (Figure, block 1), the count at birth is relatively high and remains so throughout the initial 90 post-natal days. In skin, mast cells occur mainly in the dermis and panniculus adiposus, while in the thymus 80–90% lie in or near the interlobular connective tissue.

In the 2nd group – viz., liver and spleen (Figure, block 2), and to less extent, thymic parenchyma, the population of mast cells is characterized by an initial high count followed by a rapid decline to virtual absence by the 30th day of life and thereafter. In the liver, mast cells during the initial 14 days almost invariably lie free in the sinusoids of the haemopoietic foci. The hepatic count becomes minimal by the 18th day, but then steadily rises (Figure; see below). In the spleen of neonatal rats, mast cells occur mainly in the red pulp and frequently in the lumen of associated small blood vessels. Beyond the 30th day, a few mast cells appear in the capsule. Of the 10–20% of thymic mast cells not associated with interlobular stroma (see above), rather more occur in medulla than in cortex. But even in the cortex, the mast cells are closely associated with fibres of reticulin. In thymic parenchyma, the number of mast cells becomes maximal by the 6th day and progressively declines thereafter.

In the 3rd group (Figure, block 3), comprising the *connective tissue* of heart, lung, gastric wall and liver, mast cells are practically absent at birth, but rapidly and

[1] J. L. D. acknowledges Scholarships from the National Health and Medical Research Council of Australia while working on this project for the degree of B. Sc. (Med.), the University of New South Wales, and subsequently from the National Heart Foundation of Australia.
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progressively increase in number throughout the initial 30 postnatal days. Thereafter, their number remains fairly steady. In the heart, mast cells are associated mostly with intermuscular stroma, and in the lung with interstitial tissue of alveolar walls, with peribronchial connective tissue and pleura. Those in the stomach are mainly confined to the submucosa. Reference has already been made to the numerical increase in the liver of mast cells beyond the 8th postnatal day (Figure). These new cells are largely confined to the portal tracts, particularly the bundles of collagen surrounding the bile ducts. They are conspicuously absent from the vicinity of the portal vein.

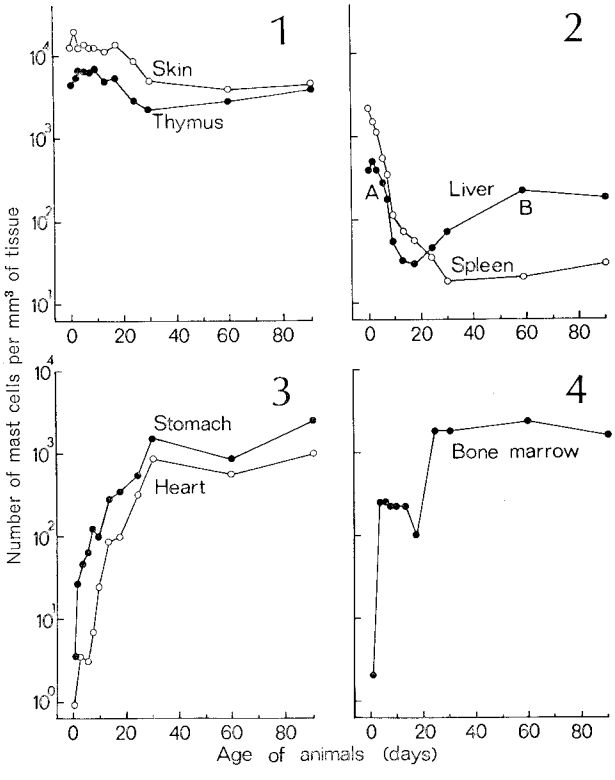
The 4th group consists only of bone marrow (Figure, block 4). Red marrow is scanty in the tibia of rats until the animals are older than 2 days. When examined at $3^1/_2$ days, the marrow contains a moderate number of mast cells. The number remains steady for the next fortnight, but then increases sharply between the 18th and 24th days, though changing little thereafter. The pattern for bone marrow therefore resembles that of the connective tissues of the 3rd group, except that a noticeable increase in mast cells is delayed until the 3rd week, rather than being evident from the outset (Figure).

In thick films of mixed venous and arterial blood prepared from each animal when sacrificed, mast cells have been identified at every test interval up to 30 days after birth. This result is consistent with earlier reports[14–16] of mast cells being identifiable in the peripheral blood of mature foetal or adult rats. In the present work, mast cells have been most frequent in peripheral blood from the 2nd to 10th days inclusive, though their number has never exceeded 25 cells per thick film. Between days 18 and 30, occasional mast cells still occur in peripheral blood, though not for all test animals.

Our results therefore indicate that in the newborn rat a population of mast cells has been established in the extramedullary haemopoietic tissues of the liver and spleen, as well as in association with connective tissues in the skin and thymus. The number of mast cells in haemopoietic foci in liver and spleen declines rapidly during the initial 3 weeks of life, coinciding with the termination of myelopoiesis in these organs, whereas that in skin and thymus falls only slightly to a level which is maintained during the succeeding 2 months.

The decrease in numbers of mast cells in haemopoietic tissues of the liver and spleen is matched by a corresponding increase in bone marrow, particularly during the 4th postnatal week. On the other hand, mast cells are noticeably sparse in the connective tissues of the heart, lung, pleura and stomach (and probably other organs) of the new-born rat, but rapidly and progressively increase in number throughout the initial 30 days of life.

The presence at birth of substantial numbers of mast cells in extramedullary, and subsequently in medullary foci of haemopoiesis, suggests that the process of mastopoiesis may be analogous to that of granulopoiesis[17] in haemopoietic tissues. Mast cells in connective tissues might arise from circulating precursors derived from haemopoietic tissues, or develop from local mesenchymal cells. The former possibility gains support from the diphasic pattern of distribution of mast cells in the liver – the cells being initially confined to haemopoietic foci, and appearing in the stroma of the portal tracts only toward the end of the 3rd week. Although mast cells are relatively numerous at birth in skin and thymus, the cells in both organs are particularly associated with connective tissues. Apart from the possible analogy of mastopoiesis to granulopoiesis, the accumulation of mast cells in association with connective tissues occurs earlier in skin and thymus than in other organs such as heart, lung, stomach and portal tract of liver[18]. Whether these cells arise from circulating haemopoietic precursors, from local mesenchymal cells, or from thymocytes, is being further investigated.



Changes of numbers of mast cells with increasing age in various organs of rats aged $^1/_2$ to 90 days. In the response-line for liver in block 2, A corresponds to mast cells in haemopoietic foci, B to mast cells mainly in portal tract stroma. To avoid confusion in identifying closely positioned response-lines, that for lung has been omitted from block 3. It is similar to, and intermediate in position between the response-lines for stomach and heart.

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