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Production and life span of cutaneous mast cells in young rats

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INTRODUCTION

Mast cells are ubiquitous components of the connective tissues of vertebrate animals and are especially abundant in the skin of rodents. Their structure and their biochemical and pharmacological properties are well understood and the cells are known to have important functions in some immune responses (see Selye, 1965) and in the reactions of the skin to noxious physical stimuli (Valtonen, Jänne & Siimes, 1964; Whitting, 1969; Kiernan, 1972, 1975) and chemical irritants (Chahl & Ladd, 1976; Arvier, Chahl & Ladd, 1977; Kiernan, 1977). However, very little is known of the origin and life history of the mast cell.

Mitotic figures are absent or very rare in mast cells (see Padawer, 1963; Selye, 1965; Baeckeland, 1969). It is therefore generally considered that these cells arise by differentiation of precursors which do not contain the characteristic cytoplasmic granules (Fawcett, 1955; Combs, 1966). In the rat, mast cells are first seen on the sixteenth day *post coitum* (Gamble & Stempak, 1961) in the dermis and subcutaneous connective tissue, appearing simultaneously in several parts of the body (Holmgren, 1946; Burton, 1967). The cells are never seen in organ cultures of skin removed on the fifteenth day *post coitum* (or earlier), indicating either that the precursors arise elsewhere in the body or that some systemic humoral influence is necessary for their maturation (Kiernan, 1974). The techniques of organ and tissue culture provide no useful clues as to the life span of the mast cell. Baeckeland (1969) found the cells in pieces of mesentery maintained *in vitro* for up to 3 weeks, but in the skin (Kiernan, 1974) they survived for only 4 days.

A few investigators have attempted to estimate the rate of turnover of mast cells by labelling them with [^{3}H]thymidine. In the skin and tongue, Walker (1961) found very few labelled mast cells when looked for at intervals of one to 21 days following the administration at four hourly intervals of six doses in adult mice. Only six animals were used in this study, however, and only 724 mast cells were counted: in all, three labelled mast cells were seen. Realistic calculations of the life span of the cell type could not be obtained from such data, though a tentative estimate of 9–18 months was made. Baeckeland (1969) gave single injections of [^{3}H]thymidine to three adult rats which were killed 4 hours later. He found only one labelled mast cell in 9000 counted in various tissues. In experiments with survival times ranging from 30 minutes to 7 days, Asboe-Hansen, Levi, Nielsen & Bentzon (1965) examined the uptake of [^{3}H]thymidine by mast cells in carcinogen-induced cutaneous tumours in mice. Of the cells 2 % were labelled 20 hours after the injection, and the numbers increased during the following 3 days. The results of this investigation supported the view that the mast cells in this abnormal tissue arose by division of agranular precursors, but gave no indication of the life span.

Blenkinsopp (1967*a*, *b*) infused [^{3}H]thymidine continuously into adult mice and rats. The average proportion of cutaneous mast cells found to be labelled in any 24 hours was 1·057 % in the mouse and 0·11 % in the rat. He concluded that these rates of production of new mast cells were too low to allow for any appreciable turnover, and suggested that the cells remained *in situ* until the animals died. In contrast, Pelc (1963) found that 1·2 % of the mast cells in the adult rat's seminal vesicle were labelled 24 hours after a single injection of [^{3}H]thymidine, and estimated that all the DNA of the mast cells was renewed every 21 days. In the mesenteries of three rats, Allen (1962) found that 5·7 % of the mast cells were labelled 30 minutes after a single injection. The labelled cells were considered to be still in the pre-mitotic state. Padawer (1963) stated that 1·7 % of the mast cells in the rat's peritoneal fluid were radioactive after one hour of incubation in a medium containing [^{3}H]thymidine.

Different investigators evidently disagree concerning the rate of production of mast cells as revealed by autoradiography. Furthermore, there has been no adequate sequential study to determine the rate of disappearance of mast cells labelled on a particular occasion; consequently the life span of the mast cell *in vivo* remains unknown.

In the present investigation, [^{3}H]thymidine has been administered to immature rats in whom, it may reasonably be presumed, the population of mast cells is rapidly increasing. The fate of the labelled cells in the skin has been followed by autoradiography for times ranging from one to 112 days.

MATERIALS AND METHODS

Experimental animals and collection of specimens

The experimental animals were twenty male albino Wistar rats aged 7 days with an average weight of 10 g. They were selected from five litters, and four of the young animals were allocated randomly to each of the lactating mothers. After weaning on the thirtieth day of life the rats were caged individually and fed a standard cube diet with water *ad libitum*.

On the seventh day *post partum* each young rat received four subcutaneous injections (over the rump) of 10 μCi of [^{3}H]thymidine, with a specific activity of 18 Ci/m-mole. The injections were given at 07.00, 10.00, 13.00 and 16.00 hours. Exogenous thymidine is available for incorporation into DNA for one hour (Cronkite, Bond, Fliedner & Rubini, 1959; Nygaard & Potter, 1959; Messier & Leblond, 1960). Since the synthetic (S) phase of the cell cycle has an average duration of 7 hours in mammals (Stanners & Till, 1960; Cameron & Greulich, 1963; Bertalanffy, 1964), at least one hour of incorporation of [^{3}H]thymidine would have occurred in any S-phases arising during the 22 hours of exposure to the isotope.

Animals were killed, in groups of three, by overdosage with ether vapour, at 10.00 hours on days 1, 2, 7, 21 (only one animal), 28, 56, 84 and 112 after injection. The pinna of the left ear, and a piece of skin from the back were removed and fixed by immersion in Carnoy's fluid. Additional specimens of dorsal skin were obtained by biopsy under ether anaesthesia from some of the rats on days 1, 2, 7, 14, 21, 28, 35, 42, 49, 56 and 84 after injection. The site of each biopsy was closed with a single suture of braided nylon. When more than one specimen of dorsal skin was taken from an animal, the chosen sites were widely separated (being located over the scapulae and over the dorsal parts of the iliac crests). At least

2 weeks were allowed to elapse between consecutive biopsies, or between biopsy and necropsy, and no more than four biopsies were taken from any one rat.

Morphometric observations

Area

The growth in area of the regions of skin studied was followed in three young rats similar to those used in the autoradiographic investigation. Their ears were measured daily (to the nearest 0·5 mm) and their areas calculated by assuming the pinna to be a semicircle (age 7–9 days) or a circle (age 10–120 days). The area of the pinna did not increase linearly with age, so a mean value was calculated for each of the ages at which animals in the autoradiographic series were killed.

The distances between the acromion processes and between the hip joints, and the length of a line in the sagittal plane joining the lines of the two lateral measurements, were also determined. The area of the skin of the back was calculated as that of a trapezium. This area was found to increase linearly with age (correlation coefficient, $r = 1.00$) throughout the period of observation, in accordance with the regression equation: $y = 88x + 51$ (y = area in mm^2; x = age in days). Values for the area of the back were calculated from this equation for each of the ages at which specimens for autoradiography were taken.

Volume

It was found that the histological processing resulted in negligible areal shrinkage of the pinna and the skin of the back. The thickness of the skin, however, was reduced to 85 % of that of the fresh tissue. Measurements of thickness determined from the autoradiographic preparations were therefore multiplied by 1·176 in order to obtain values appropriate to the living animals.

In the pinna, the thickness of the skin varied considerably within individual specimens, so it was necessary to calculate a mean value. Stained sections of all the pinnae used for autoradiography were projected onto weighed and measured sheets of paper at known magnifications from $\times 20$ to $\times 45$. Each of these sections passed through the middle part of the pinna, from its base to its apex. It was assumed that the variable thickness of the auricular skin would be adequately sampled in these representative sections. The outlines of the dermis of both sides of the pinnae were then drawn, care being taken to exclude the auricular cartilage and muscles. The length of each section was measured and the area of the sectioned skin was determined by cutting out and weighing the drawing. The mean thickness of the auricular skin was calculated for each specimen by dividing the area by the length. The volume of the skin was calculated for each animal by multiplying this thickness (corrected for shrinkage) by the mean area of the pinna (determined as described above).

The sections of dorsal skin had simple rectangular outlines. The mean of three measurements of the thickness of each specimen used for autoradiography was therefore corrected for shrinkage and multiplied by the area of the back to obtain the volume of the skin of the back of each animal.

Histological and autoradiographic procedures

The specimens were fixed in Carnoy for 6 hours, dehydrated in ethanol, cleared in terpineol and embedded in paraffin wax. Vertical sections, 4 μm thick, were mounted on albuminized slides.

The sections were stained with Alcian blue, pH 2·5, as described by Pearse (1968), washed thoroughly in water and allowed to dry. The slides were then coated with Kodak NTB2 nuclear track emulsion, dried and placed in dark boxes containing desiccant for 3 weeks at 4 °C. The autoradiographs were developed in Kodak Dektol (2 m) and fixed in Kodak general-purpose hardening fixer (10 m), following the instructions of Kopriwa & Leblond (1962). After fixation and washing, the sections were counterstained with alum–brazilin (made exactly as Mayer's haemalum, but with brazilin instead of haematoxylin), which coloured the nuclei red. The slides were then washed, dehydrated, cleared and mounted in DPX.

Examination of autoradiographs and calculation of results

The preparations were examined at a magnification of $\times 806$. The area of the field was 0·0222 mm^2. The autoradiographic background was light, so there was never any difficulty in identifying labelled nuclei. All the dermal mast cells with nuclei of full size present in the plane of section were counted and classified as labelled or not labelled with tritium, in all the regions of the dermis that could be included completely in the field of the microscope. The number of fields examined within each section was also noted. For each specimen, six to eight widely separated sections were used for this quantitative procedure.

From the results obtained it was possible to calculate for each specimen the number of mast cells per mm^2 of sectioned dermis and the percentage of these cells whose nuclei were radioactive. The number (M) of mast cells (labelled plus unlabelled) per cubic millimetre was calculated as:

$$M = 0{\cdot}5 \times \frac{1000}{t} \times m$$

(t = thickness of section, in this case 4 μm; m = number of mast cells per mm^2 of sectioned tissue). The factor of $\times 0{\cdot}5$ was applied as a correction factor for cells present in more than one of the hypothetical sections composing the millimetre cube. This factor is appropriate when the thickness of the section is approximately equal to the diameter of the unit counted (Escobar, Sampedro & Dow, 1968; Konigsmark, 1970). Most cutaneous mast cells in the rat have nuclei 4 μm in diameter in histological preparations of the type used in this study (Lampe & Kiernan, 1977). The number of mast cells per mm^3 of living tissue was determined by correcting for shrinkage.

The total number of mast cells in the skin of the pinna and back was estimated by multiplying the number per mm^3 by the volume of the skin in the region. The total numbers of labelled mast cells were obtained by multiplying the total numbers by the proportions of labelled mast cell nuclei found in the autoradiographs.

Other calculations will be explained as the results are presented. Statistical methodology followed Zar (1974).

RESULTS

Qualitative observations

Mast cells were easily recognized because of their cytoplasmic granules, which are stained by Alcian blue. The nuclear chromatin of all cells assumed the red colour of the alum–brazilin lake used as a counterstain. Otherwise, the latter dye produced only a faint pink coloration in the cytoplasm of epidermal cells and muscle

Table 1. *Mast cells in the skin of the back*

([^{3}H]thymidine administered at age 7 days.)

Age (days)	Total number of mast cells × 10^{-3} (mean ± S.D.)	Number of mast cells per mm^3 of dermis (mean ± S.D.)	Percentage of labelled mast cells (mean ± S.D.)	Number of specimens	Total number of mast cells counted
8	5903 ± 734	9953 ± 1231	7·5 ± 3·7	4	1068
9	5762 ± 1605	7158 ± 1993	9·5 ± 2·4	4	959
14	6658 ± 1701	6194 ± 1117	1·9 ± 0·3	4	2370
21	15410 ± 5261	8821 ± 3013	1·2 ± 0·7	4	2402
29	24770 ± 7533	13737 ± 3280	0·6 ± 0·4	4	2510
35	34560 ± 3712	8724 ± 914	0·3 ± 0·2	4	2619
42	20090 ± 3053	5230 ± 792	0	4	1306
49	33850 ± 7372	6073 ± 1324	0	4	1730
56	34000 ± 6403	5495 ± 1042	0	4	1337
63	38590 ± 9370	4893 ± 1189	0	4	1282
91	48400 ± 11900	5720 ± 1405	0	3	1030
119	56570	3181	0	1	228

Table 2. *Mast cells in the skin of the external ear*

([^{3}H]thymidine administered at age 7 days.)

Age (days)	Total number of mast cells × 10^{-3} (mean ± S.D.)	Number of mast cells per mm^3 of dermis (mean ± S.D.)	Percentage of labelled mast cells (mean ± S.D.)	Number of animals	Total number of mast cells counted
8	164·9 ± 25·20	16667 ± 2629	12·3 ± 5·4	3	914
9	220·6 ± 16·30	12708 ± 937	16·2 ± 2·0	3	1119
14	269·0 ± 66·0	7458 ± 1830	3·8 ± 2·4	3	1740
28	1867	24750	0·4	1	1101
35	1588 ± 245·0	22333 ± 3454	0·6 ± 0·4	3	2117
63	1901 ± 427·0	18375 ± 4128	0·1 ± 0·1	3	2567
91	1546 ± 254·0	16688 ± 2739	0·1 ± 0·1	2	1088
119	2841 ± 492·0	14063 ± 795	0	2	1687

fibres. This combination of colours made it easy to determine which mast cells had their nuclei largely contained within the section.

Mitotic figures were seen occasionally in the epidermis and dermis of the younger rats, but never in mast cells.

Accumulations of developed silver grains were present over many nuclei, including those of mast cells, in animals killed up to one week after administration of [^{3}H]-thymidine. At later times they were less numerous, though occasional labelled cells in the dermis and in striated muscle were noticed even after 112 days. Cells considered to be labelled showed 15–25 silver grains over the nucleus and immediately adjacent cytoplasm. The great majority of unlabelled cells had no grains over their nuclei, though up to three were sometimes present. Nuclei with 5–10 silver grains over them were also seen, especially 1–3 weeks after injection of [^{3}H]thymidine. These were considered to represent a second generation of the cells originally labelled. A few were mast cells, but in the quantitative assessment they were scored as 'unlabelled' because they would not have been synthesizing DNA at the time the [^{3}H]thymidine was administered. Such second-generation mast cells were found from 1 to 8 weeks after injection of the nucleotide, but were rare, only three or four being present in any one section.

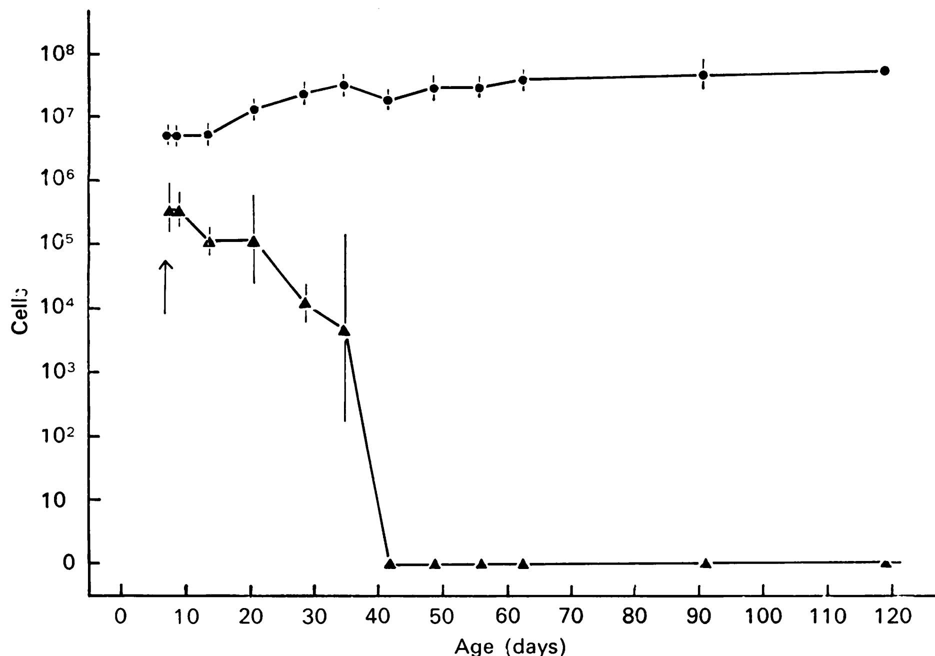

Fig. 1. Mast cells in the skin of the back. The upper line (—●—) shows the trend of the total population and the lower line (—▲—) the numbers of mast cells labelled with [^{3}H]-thymidine. Dots represent means and vertical bars standard deviations, both statistics having been calculated from logarithmically transformed data. Dots without bars indicate either single observations or equality of all numbers at a particular age. The arrow indicates the administration of [^{3}H]thymidine at age 7 days.

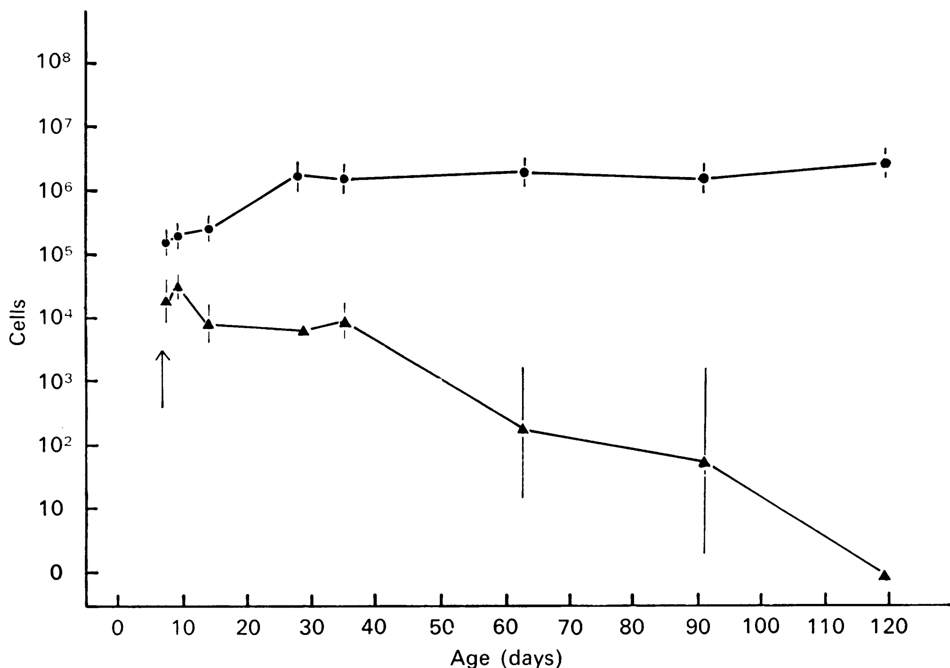

Fig. 2. Mast cells in the skin of the pinna of the ear. Data displayed as in Fig. 1.

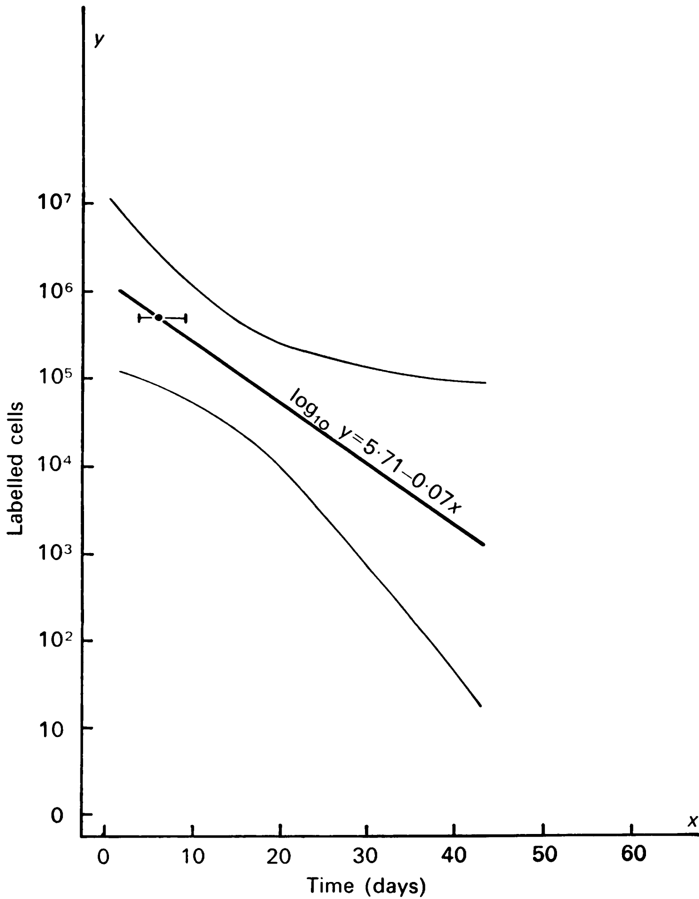

Fig. 3. Least-squares regression line showing the decline in the numbers of labelled mast cells (y) in the skin of the back with time (x) after injection. The thin, curved lines enclose 95 % confidence limits for predicted values of y. The dot shows the time at which 50 % of the labelled cells have disappeared, and the ends of the horizontal bar indicate equivalent times traced along the upper and lower levels cf the 95 % confidence bands. The regression equations were calculated from logarithmically transformed data.

Numbers of labelled and unlabelled mast cells

The total numbers of mast cells in the skin of the back, the numbers per mm^3 of dermis, and the proportions labelled by [^{3}H]thymidine are presented in Table 1. Equivalent data for the external ear are given in Table 2. In both regions the numbers of mast cells increased with age. The number per mm^3, however, was reasonably constant throughout the period of observation, though in the pinna it was lower at 14 days than at other ages, and there was a gradual decline with age in the back skin.

The standard deviations of the numbers of cells increased with the means. Logarithmic transformation of all the numerical data was therefore required in order to obtain among the various groups of observations the homoscedacity necessary for the use of parametric statistical tests. The equation for the transformation was: $x' = \log_{10}(x+1)$, where x represents each datum and x' the transformed datum. This transformation of data is explained more fully by Zar (1974,

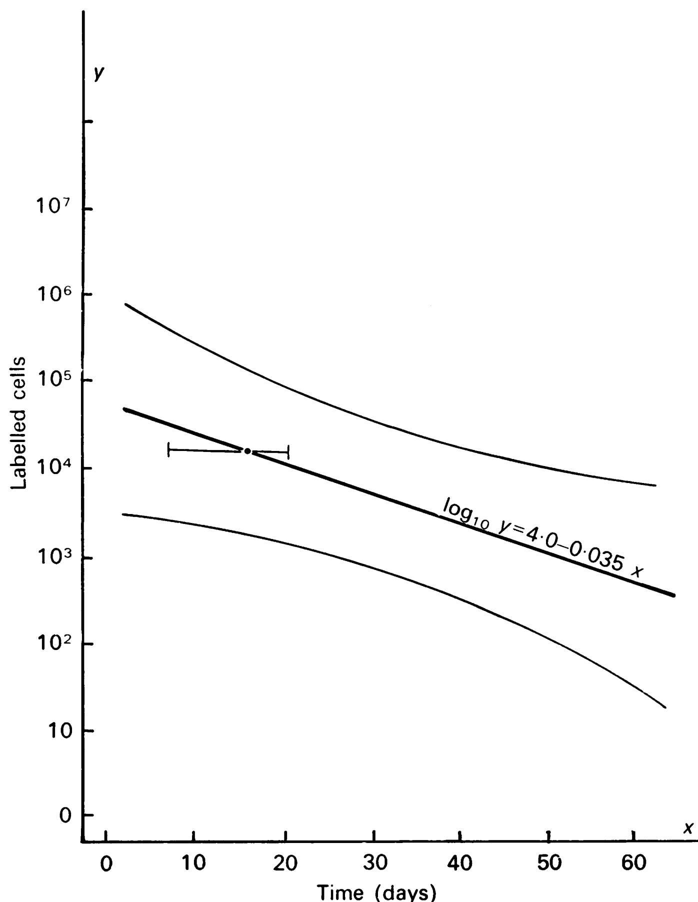

Fig. 4. Least-squares regression line showing the decline in the numbers of [^{3}H]thymidine-labelled mast cells in the pinna, with 95 % confidence bands, and estimated half-life, as explained in legend to Fig. 3.

p. 184). The changes with time in the total numbers of mast cells and in the numbers of labelled mast cells are displayed in Figures 1 and 2, which relate to the back and pinna respectively. These graphs are based on the logarithmically transformed data.

The total populations of mast cells at different ages are shown by the upper lines in Figures 1 and 2, while the lower lines indicate the changes in the numbers of labelled mast cells in the two regions. The maximum number of labelled cells was attained in the back 24 hours, and in the ear 48 hours, after the first injection of [^{3}H]thymidine. Thereafter, the logarithms of the numbers of labelled cells declined to unity in an approximately linear fashion. The extent of variation in the numbers of cells is indicated by the bars showing the standard deviations of the logarithmically transformed data. There was much more variation among the numbers of labelled cells than among the total numbers.

In order to determine the average life span of the mast cells least-squares regression equations were calculated for the numbers of labelled mast cells during the periods of linear decline (1–28 days in the back; 2–84 days in the ear). These regression lines are shown in Figures 3 and 4. From these graphs it is possible to estimate

the times, after administration of [^{3}H]thymidine, by which the numbers of labelled cells had fallen to half their highest values. Taking into account the 95 % confidence limits shown on the graphs, the calculated half-lives are 4–9 days in the back and 7–20 days in the pinna of the ear. In both cases there is a 95 % probability that the true half-lives lie within the ranges indicated.

DISCUSSION

Identification and staining of mast cells

In this investigation mast cells were identified by staining with Alcian blue at pH 2·5. It has been claimed that mature mast cells have no affinity for this dye, but can be recognized by their acquisition of a pink colour in a staining method using Alcian blue and safranine (Combs, Lagunoff & Benditt, 1965; Yong, Watkins & Wilhelm, 1975). Investigators using the Alcian blue – safranine method have variously stated that mast cells arise from mesenchymal cells already present in connective tissues (Combs *et al.* 1965; Combs, 1966) and that they are derived from precursors in lymphoid and haemopoietic tissue which are distributed by the blood after differentiation has occurred (Csaba & Hodinka, 1970; Csaba & Forgacs, 1971). However, the use of the two dyes in combination has been shown to have no histochemical significance and to give inconsistent results when applied to mast cells (Tas, 1977). In the skin of the adult rat it has been shown, in a quantitative histochemical study, that Alcian blue at pH 1·0 or 2·5 stains all mast cells (Lampe & Kiernan, 1977). The tinctorial properties are attributable to the presence of heparin in the cytoplasmic granules (Jaques, 1961; Chiu & Lagunoff, 1971). The name 'heparin' includes a variety of sulphated glycosaminoglycuronans, some of which are synthesized by cells other than mast cells, though not in the form of stainable granules (Dietrich & Montes de Oca, 1970; Jaques, 1975). Alcian blue was used at pH 2·5 rather than at pH 1·0 since treatment with the more acidic solution might have hydrolysed DNA with consequent loss of radioactive purine residues. The mast cells were stained before applying the emulsion to the slides because it had been found in pilot studies that the stainability of mast cells could be suppressd by prior treatment with developing and fixing solutions.

Development and differentiation of mast cells

Labelled mast cells were numerous in the skin of the back and external ear 24 hours after administration of [^{3}H]thymidine, but in the ear the largest numbers of such cells were seen after 48 hours. It is likely, therefore, that mitosis of the precursor cell is followed by a variable interval of time, up to 2 days, during which the daughter cells differentiate into recognizable mast cells. This differentiation entails the production of heparin-containing cytoplasmic granules. The first mast cells to appear in intrauterine life have most of their granules located on one side of the nucleus (Arvy, 1957; Kiernan, 1974), but in the labelled cells seen in this study the granules were always uniformly distributed in the cytoplasm.

The proportions of mast cells found to be labelled in the first 2 weeks after administration of [^{3}H]thymidine were about twice as high in the pinna as in the dorsal skin (see Tables 1, 2). Mast cells were also more abundant in the external ear. It is apparent that the rate of production of mast cells is higher in the pinna than in the back, though the cells mostly have shorter life spans in the latter region. Thus the higher population of mast cells (per unit volume of dermis) in the external

ear may result from a combination of more frequent mitosis of the precursors with longer survival of the differentiated cells.

There is other experimental evidence to indicate that the production of cytoplasmic granules probably does not occur immediately after mitosis of the precursor cells. Thiede *et al.* (1971) found that mast cells in the hilar regions of regional lymph nodes in rats were killed (with disintegration of the nuclei as well as the cytoplasm) by intradermal injection of an anti-macrophage serum. Small mononuclear cells, each containing a few cytoplasmic granules, appeared 3–4 days later. The granules increased in number for a further 2 days, by which time the cells were recognizable as fully differentiated mast cells.

The identity of the precursor which divides and then differentiates into mast cells is still obscure. Thiede *et al.* (1971) considered that the stem-cell was a mononuclear leukocyte, but it has also been suggested that the precursors are mesodermal elements such as reticulum cells and unidentified cells in vascular adventitiae (Fawcett, 1955; Riley, 1959; Combs, 1966). Evidence for the adventitial origin of mast cells has been obtained mainly from the study of their 'regeneration' in rats recovering from treatment with compound 48/80. This is an agent which degranulates the cells so that they 'disappear'. However, autoradiographic studies with [^{35}S]sulphate, a metabolic precursor of heparin, indicate that mast cells are not killed by compound 48/80, and that they begin to synthesize heparin immediately after they have discharged all their granules (Watson & Kennedy, 1960). In the experimentally injured cornea of the rat, vascularization precedes the appearance of mast cells alongside the newly formed vessels (Smith, 1961). This observation supports the idea that the precursor cells are derived either from the vessel wall or from the blood.

Observations of fetal skin maintained *in vitro* also lend some support to the idea that precursors circulate in the blood and differentiate into mast cells after they have emigrated from blood vessels. Alternatively, a circulating humoral factor may stimulate the differentiation of primitive cells already present in the connective tissues (Kiernan, 1974). Cells with metachromatic granules have been found to form in cell cultures derived from lymphoid and haemopoietic tissues, especially when the nutrient media contain exogenous heparin (Csaba, Toro & Mold, 1962; Ginsburg & Sachs, 1963; Cantino & Comoglio, 1967; Ginsburg & Lagunoff, 1967). There is an element of uncertainty, however, in equating these cells with the mast cells that occur *in vivo*, owing to the unnatural physical and chemical environments of the cultures used in these experiments.

Life span and fate of mast cells

Owing to considerable variation in the numbers of labelled mast cells, the estimates of the life span cannot be very accurate. Indeed, it seems probable that different mast cells live for different lengths of time. From the data of Figures 3 and 4, half-lives of 4–9 days (in the skin of the back) and 7–20 days (in the pinna) were determined. The longest times after which any labelled mast cells were seen were 28 days in the back and 84 days in the external ear. Evidently the time elapsing between mitosis and the eventual disappearance of the cell is, on average, about twice as long in the auricular skin as in the skin of the back. Of the mast cells formed as a result of mitoses on the seventh day of extrauterine life, none lasted longer than 12 weeks.

This finding does not agree with the conclusions of Walker (1961) and Blenkin-

sopp (1967*a*, *b*) who deduced from comparable autoradiographic studies that there was no turnover of mast cells in the adult animal. Blenkinsopp's (1967*b*) observation that only 0·11 % of the rat's cutaneous mast cells incorporated [^{3}H]thymidine every 24 hours indicates that mast cells produced in later life live for much longer than those which develop in infancy. A very long life span was also suggested by Padawer (1974), who labelled the granules of the peritoneal mast cells of 30 days old rats with colloidal thorium dioxide. He found that 90 % of the peritoneal mast cells contained this electron-dense tracer after one week, 39 % after 2 months, and 1 % after 10 months. No allowance was made, however, for the possibility that the colloidal thorium dioxide may have been expelled by exocytosis of the granules, dispersed in the peritoneal fluid, and eventually taken up by other mast cells. Future studies of the incorporation of [^{3}H]thymidine into mast cells should involve the administration of the nucleotide to animals of various ages.

Any explanation of the different rates of production and life spans of mast cells in different regions of the integument is necessarily speculative. Mast cells are known to discharge their granules not only in pathological circumstances but also in response to acute noxious stimuli such as physical injury (Kiernan, 1972, 1975) and chemical irritation (Kiernan, 1977). The numbers of the cells in the skin have been shown to fall following exposure to cold (Héroux, 1961) and to increase after repeated exposures to ultraviolet radiation (Valtonen, 1961). Mast cells are also more abundant around healing wounds than in comparable areas of normal skin (Wichmann, 1955; Whitting, 1969). The pinnae of the ears, by virtue of their position and structure, may well be more susceptible to adverse environmental influences than the skin of the back. Consequently, the more rapid production, and greater longevity, of the auricular mast cells may indicate the provision of a more active defensive mechanism. This idea accords with Selye's (1965) 'emergency kit hypothesis' in regard to the role of the mast cell in the body. If his contention is correct, it is to be expected that mast cells in the skin of the feet and tail of the rat would be produced even more rapidly, and have even longer lives, than those in the pinna. Conversely, the mast cells in internal organs should be produced more slowly than in the skin, and should have generally shorter life spans.

The mode of demise of the mast cell is likewise problematical. If the cells simply die *in situ*, some mast cells with pyknotic or fragmented nuclei should always be present in normal tissues, especially in young rats, but such cells have never been described. Alternatively, each mast cell may discharge all its granules on a certain occasion and then not synthesize any more, in which case the cell would no longer be recognizable. In the normal skin of the adult rat's external ear (fixed by perfusion of a buffered formaldehyde–glutaraldehyde mixture) about 35 % of the dermal mast cells can be seen to be discharging at least one granule (Lampe & Kiernan, 1976). The percentage is higher after fixation in Carnoy (Kiernan, 1971), but it is known that structural disturbances can result from the rehydration and staining of mast cells following alcoholic fixation (Padawer, 1965). If all the apparently degranulating cells were to cease to be identifiable as mast cells, the rate of replacement necessary to maintain a stable population would have to be considerably higher than that found in the present and other autoradiographic studies. It is possible, however, that most of the mast cells discharge only a few granules at a time, while a few empty their cytoplasm completely. Isolated peritoneal mast cells can be induced to secrete histamine without any detectable release of granules (Diamant & Krüger, 1968), though exocytosis of these organelles probably always

accompanies the liberation of heparin into the extracellular space (Uvnäs, 1974). The regional and age-related variations in the turnover of mast cells may reflect different rates of usage of heparin and other components of the cytoplasmic granules in response to local environmental stresses.

SUMMARY

Tritiated thymidine was injected into young rats (age 7 days) in such a way as to be incorporated into the nuclei of all cells that were synthesizing DNA during a period of 22 hours. Specimens of the skin of the back and the pinna of the ear were taken at intervals from one to 112 days after injection of the [^{3}H]thymidine. The mast cells were stained with Alcian blue, and autoradiographs were prepared. The nuclei were counterstained with alum–brazilin. Making due allowance for growth of the animals, and for shrinkage due to histological preparation, the total numbers of mast cells in the dorsal skin and in the pinna were determined. The numbers of mast cells containing [^{3}H]thymidine were calculated from the proportions of those cells found to have radioactive nuclei. Using these data, the rates of appearance of labelled mast cells, and of decline in their numbers with time were determined for both regions of skin. No mitotic figures were seen in any mast cells.

It is concluded that mast cells arise by the division of agranular precursor cells of unknown identity. The characteristic cytoplasmic granules appear to be produced by the daughter cells during the 24–48 hours following premitotic replication of DNA in the precursors. The differentiated cells have half-lives of 4–9 days in the skin of the back and 7–20 days in the external ear. All the labelled mast cells had disappeared after 28 days in the back and after 84 days in the ear. Comparison of these findings with the results of other investigators suggests that the mast cells produced early in life have much shorter life spans than do most of the mast cells present in adult rats.

The longer life span found in the pinna may account for the greater density of the cells there than in the back. This regional difference may reflect the greater need for mast cells in a region which is more susceptible to adverse environmental influences.

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