J. Cell Sci. **2**, 33–37 (1967)
Printed in Great Britain

MAST CELL PROLIFERATION IN ADULT RATS

W. K. BLENKINSOPP
Department of Pathology, St Mary's Hospital Medical School, London, W.2

SUMMARY

Mast cell proliferation was investigated in adult male rats using continuous infusions of tritiated thymidine to label deoxyribonucleic acid. The results indicate that the total number of mast cells increases as body weight increases (though at a lower rate) and that there is no mast cell turnover in the sense of discard and replacement. No evidence was found of mast cell formation from 'stem' cells.

INTRODUCTION

Labelled mast cells are found in animals killed within 2 h of an injection of tritiated thymidine (Allen, 1962; Walker & O'Steen, 1963; Asboe-Hansen & Levi, 1962), which indicates that they synthesize deoxyribonucleic acid (DNA). Mast cells have also been found in mitosis (Allen, 1962; Hunt & Hunt, 1957). However, some authors have suggested that mast cells form by differentiation from 'stem' cells (Watson & Kennedy, 1960; Padawer, 1961) or from precursors in the adventitia of blood vessels (Riley, 1953), although animals killed at various intervals after injection of [^{3}H]-thymidine have not shown an increase in the percentage of labelled mast cells with time (Walker, 1961). One difficulty in mast cell studies is the low rate of labelling after [^{3}H]thymidine injections; the present report describes the results of continuous infusion of [^{3}H]thymidine for periods up to 68 days in rats. This procedure labels all cells which synthesize DNA during the infusion.

MATERIAL AND METHODS

Twenty-three male adult rats were used. Twenty-one were of an inbred black-hooded *PVG/C* strain (nos. BH 1–21) and 2 were Sprague–Dawley rats (nos. SD 1 and 2). Three rats (nos. BH 8–10) were continuously infused with 0·5 μc/g body weight/day of [^{3}H]thymidine by cannulation of a tail vein (Little, Brecher, Bradley & Rose, 1962). All the other rats were given intraperitoneal infusions of 1 μc/g/day by the method of Foot (1963). The procedures were as follows:

(i) Four rats (nos. BH 1–4) were infused with [^{3}H]thymidine (specific activity 5·0 c/mmole) for 3 days; each rat was killed at the end of the infusion and the anterior third of the tongue was removed.

(ii) Three rats (nos. BH 5–7) were infused with [^{3}H]thymidine (specific activity 3·0 c/mmole) for 140, 187 and 235 h, respectively; each rat was killed at the end of its infusion and the anterior abdominal skin was removed.

(iii) Three rats (nos. BH 8–10) were infused with [^{3}H]thymidine (specific activity 1·0 c/mmole) for 68 days, followed by thoracic duct drainage for 10 days without infusion; the animals were then killed and the trachea and oesophagus were removed.

(iv) Two Sprague–Dawley rats (nos. SD 1–2) were infused with [^{3}H]thymidine (specific activity 3·0 c/mmole) for 6 days; each rat was killed at the end of the infusion and the anterior abdominal skin was removed.

(v) Eleven rats (nos. BH 11–21) were infused with [^{3}H]thymidine (specific activity 2·42 or 3·0 c/mmole) for 72 h and killed at intervals of 0–11 days after the end of the infusion, when the anterior abdominal skin was removed.

Autoradiographs were prepared from 5-μ transverse sections of the tissues using Ilford K5 nuclear emulsion. These were exposed for 4 weeks at 0–4 °C, developed, and stained with haematoxylin and neutral red differentiated in 70 % ethanol to identify the mast cells (Allen, 1960). Labelled and unlabelled mast cell nuclei were counted. Details of infusions and counts are given in Tables 1 and 2.

RESULTS

The total number of mast cells and the number labelled in the sections from each rat are given in Tables 1 and 2, and in Table 2 this is expressed as a percentage. In Table 1 the percentage labelling in 24 h is given; this has been calculated on a basis that appears to be valid for mice (Blenkinsopp, 1967): the assumptions have been made that the duration of DNA synthesis (S) is 8 h and G_2 (pre-mitotic gap)+M (duration of mitosis) is 2h. S, G_2 and M are not known for mast cells, but Cameron & Greulich (1963) have suggested that S may be a constant for somatic cells at body temperature, and the majority of the values for G_2+M in rodents' cells are in the range 1–3 h (Knowlton & Widner, 1950; Koburg & Maurer, 1962; Lajtha, 1963). Since the rate of appearance of labelled cells which have not yet divided is half the

Table 1. *Mast cells in rats infused with* [^{3}*H*]*thymidine for various periods and killed at the end of the infusion*

Rat no.	Rat weight (g)	Infusion time	Mast cell nuclei: Labelled	Mast cell nuclei: Total	Calculated percentage labelled in 24 h
BH 1	180	72 h	7	1358	0·165
BH 2	180	72 h	4	1350	0·095
BH 3	210	72 h	6	1814	0·106
BH 4	210	72 h	4	1495	0·086
BH 5	320	140 h	8	1001	0·134
BH 6	300	187 h	7	1166	0·076
BH 7	314	235 h	23	1341	0·173
BH 8	330	68 days	14	256	0·081
BH 9	330	68 days	14	192	0·108
BH 10	330	68 days	11	209	0·077
SD 1	200	6 days	8	824	0·158
SD 2	200	6 days	16	996	0·262

Table 2. *Mast cells in rats killed at various intervals after a 3-day infusion of* [^3H]*thymidine*

Rat no.	Rat weight (g)	Time between end of infusion and sacrifice (days)	Mast cell nuclei		
			Labelled	Total	Percentage labelled
BH 11	315	0	4	605	0·66
BH 12	155	1	2	598	0·33
BH 13	315	2	0	545	0·00
BH 14	300	3	1	630	0·16
BH 15	280	4	0	632	0·00
BH 16	292	5	7	450	1·55
BH 17	322	6	4	462	0·87
BH 18	287	7	4	498	0·80
BH 19	265	8	1	259	0·39
BH 20	300	10	3	643	0·47
BH 21	215	11	3	441	0·68

rate of appearance of labelled cells which are dividing, the cells labelled during the period $S+G_2+M$ = 10 h can be considered to occupy a 5-h period at the rate of labelling of dividing cells. It is thus possible to extrapolate the linear rate of labelling of dividing cells back to 0%, as this point is G_2+M-5 h after the beginning of the infusion; that is, 3 h *before* the beginning of the infusion. The 'effective' length of an infusion is thus 3 h longer than the length of the infusion. As the infusions were for 3–68 days, even a large error in the suggested values for S and (G_2+M) would result in only a small error in the effective labelling time.

The mean percentage of mast cell nuclei labelled in 24 h and the standard deviation of the mean are given in Table 3.

Table 3. *Summary of mast cell labelling in the 12 rats listed in Table 1*

Rat nos.	Mean percentage of mast cell nuclei labelled in 24 h (calculated)	Standard deviation
BH 1–4	0·11	0·036
BH 5–7	0·13	0·049
BH 8–10	0·09	0·018
SD 1–2	0·21	0·075

DISCUSSION

Allen (1962) found 5·7% of 4500 mast cell nuclei were labelled in mesenteric spreads of 30-day (57 g) rats given one injection of [^{3}H]thymidine and killed at ½ h. Walker (1961) gave 6 injections of [^{3}H]thymidine at 4-h intervals to adult mice, which were killed at 1–21 days. He counted a total of 727 mast cells in the skin, foot-pad, and tongue, of which only 3 were labelled. Walker & O'Steen (1963) gave normal and

dystrophic adult mice either a single injection of [^{3}H]thymidine or 4 injections in 25 h and killed the animals 2 h or 5 days later. Seven of 4707 mast cells were labelled in the skin and tongue of 6 normal mice, and 30 of 8255 were labelled in 12 dystrophic mice (19 of the 30 were in the skin of one animal). Asboe-Hansen & Levi (1962) found that 'roughly 1%' of mast cell nuclei were labelled in carcinogen-induced skin papillomas in 6-week mice ½–20 h after one injection of [^{3}H]thymidine. Pelc (1963) described 1·2% 'high level' nuclear labelling in mast cells after one injection of [^{3}H]thymidine in adult mice.

This previous work has shown that mast cells can synthesize DNA, and division by mitosis is also established (Allen, 1962). The proportion of labelled cells has been very small, except for Allen's young rats (Allen, 1962), and Allen (1961) has suggested that in rats aged 10–120 days the rate of proliferation of mast cells is directly related to age.

In the present study the mean percentage labelling in 24 h was 0·11% in male adult black-hooded rats. If no cells are lost this represents an increase in the total number of mast cells of 0·11/2 = 0·055% per day. The mean increase in body weight in these rats was 0·4% per day. The Sprague–Dawley male adult rats grew more quickly than the black-hooded rats: at 200 g their mean increase in body weight was 1·7% per day. The increase in the total number of mast cells was 0·21/2 = 0·105% per day.

As the mast cells in rats appear to increase in number at a rate much slower than the rate of increase in body weight, probably a small proportion of them divide and the others survive as long as the animals. It is unlikely that they have a turnover (which implies an orderly process of discard and replacement). Similar conclusions were reached in a study of mast cells in mice (Blenkinsopp, 1967).

Since some authors (Watson & Kennedy, 1960; Padawer, 1961; Riley, 1953) have suggested that mast cells form by differentiation from precursor cells, a series of rats (nos. BH 11–21) given 3-day infusions of [^{3}H]thymidine were killed at 0–11 days after the end of infusion. The results (in Table 2) show no evidence of an influx of labelled mast cells except perhaps at 5 days. However, the daily percentage labelling is similar in the black-hooded rats given infusions for more than 5 days and less than 5 days. It is therefore probable that in normal adult rats the total number of mast cells increases only as a result of mitosis of mast cells.

I am grateful to Dr K. A. Porter, who provided material from the three rats given 68-day infusions, to Miss J. Gock Chew for technical assistance, and to St Mary's Hospital Research Fund for financial assistance.

REFERENCES

ALLEN, A. M. (1960). Two methods for coloring mast cells of mammalian tissues. *Am. J. clin. Path.* **33**, 461–469.

ALLEN, A. M. (1961). The occurrence and demonstration of spontaneous and experimentally-induced mitosis in mast cells of the rat. *Diss. Abstr.* **21**, 3598.

ALLEN, A. M. (1962). Deoxyribonucleic acid synthesis and mitosis in mast cells of the rat. *Lab. Invest.* **11**, 188–191.

ASBOE-HANSEN, G. & LEVI, H. (1962). Mitotic division of tissue mast cells as indicated by the uptake of tritiated thymidine. *Acta path. microbiol. scand.* **56**, 241–244.

BLENKINSOPP, W. K. (1967). Mast cell proliferation in adult mice. *Nature, Lond.* (In the press.)

CAMERON, I. L. & GREULICH, R. C. (1963). Evidence for an essentially constant duration of DNA synthesis in renewing epithelia of the adult mouse. *J. Cell Biol.* **18**, 31–40.

FOOT, E. C. (1963). Eosinophil turnover in the rat. *Nature, Lond.* **198**, 297–298.

HUNT, T. E. & HUNT, E. A. (1957). Mitotic activity of mast cells. *Proc. Soc. exp. Biol. Med.* **94**, 166–169.

KNOWLTON, N. P. & WIDNER, W. R. (1950). The use of X-rays to determine the mitotic and intermitotic time of various mouse tissues. *Cancer Res.* **10**, 59–63.

KOBURG, E. & MAURER, W. (1962). Autoradiographische Untersuchung mit [^{3}H]Thymidin über die Dauer der Deoxyribonukleinsäuresynthese und ihren zeitlichen Verlauf bei den Darmepithelien und anderen Zelltypen der Maus. *Biochim. biophys. Acta* **61**, 229–242.

LAJTHA, L. G. (1963). The use of radiation in studies of cell proliferation. In *Cell Proliferation: A Guinness Symposium held at University of Dublin Trinity College* (ed. L. F. Lamerton & R. J. M. Fry), pp. 80–91. Oxford: Blackwell.

LITTLE, J. R., BRECHER, G., BRADLEY, T. R. & ROSE, S. (1962). Determination of lymphocyte turnover by continuous infusion of H^3-thymidine. *Blood* **19**, 236–242.

PADAWER, J. (1961). Autoradiography of mast cells incubated with tritiated histidine, cytidine or thymidine. *Angiology* **12**, 538–545.

PELC, S. R. (1963). On the question of renewal of differentiated cells. *Expl Cell Res.* **29**, 194–198.

RILEY, J. F. (1953). The relationship of the tissue mast cells to the blood vessels in the rat. *J. Path. Bact.* **65**, 461–469.

WALKER, B. E. (1961). Mast cell turnover in adult mice. *Nature, Lond.* **192**, 980–981.

WALKER, B. E. & O'STEEN, W. K. (1963). Proliferation of mast cells in normal and dystrophic mice. *Proc. Soc. exp. Biol. Med.* **113**, 183–185.

WATSON, W. C. & KENNEDY, J. S. (1960). Regeneration of the tissue mast cell: an autoradiographic study. *Br. J. exp. Path.* **41**, 385–388.

(*Received* 16 *August* 1966)