Proliferation of Mast Cells in Normal and Dystrophic Mice.* (28313)

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The maintenance of a normal population of mast cells in mammals has been attributed to transformations of undifferentiated mesenchymal cells or other connective tissue cells into mast cells(1). However, Hunt and Hunt(2) who presented evidence of mitosis in mast cells, refuted the previous belief that those cells did not proliferate and established the possibility of replenishment of mast cells through mitotic activity. Speirs *et al.*(3) found no *in vitro* synthesis of DNA in mast cells of mice. Padawer(4) in an *in vitro* study of rat mast cells exposed to tritiated thymidine observed labeled nuclei, but concluded that, although DNA synthesis might be occurring, "mast cells are an end product that once fully differentiated do not undergo additional mitosis." Allen(5) using radioautographic techniques described labeled nuclei and unlabeled mitotic figures in mast cells of rats and stated that mitosis was the primary means of cell production. Walker(6), from studies of mast cells and other connective tissue cells in the skin, foot pad and tongue of mice following a series of tritiated thymidine injections, found that 0.4% of the mast cell population was labeled, but the method used did not distinguish between DNA synthesis by mature mast cells and transformation of precursor cells. The observation by O'Steen and Hrachovy(7) of greatly increased numbers of mast cells in the skin of mice with hereditary muscular dystrophy as compared with normal animals of the same strain raised the question of accelerated mast cell production in the dystrophic mice. The present experiment was designed to investigate the way in which mast cell populations increase in both normal and dystrophic adult mice.

Materials and methods. Two dystrophic and 2 normal mice of the 129 strain were each given 50 μc of thymidine-H^3 and killed 2 hours later. One normal and 4 dystrophic mice were each given 4 doses of 50 μc thymidine-H^3 over a 25 hour period and killed 5 days later. Skin and tongue were radioautographed by the fluid emulsion dipping method(8) and stained with aldehyde-fuchsin after development. Some sections from the same tissues were used for mitotic counts and were stained with aldehyde-fuchsin-hematoxylin, or toluidin blue, or Feulgen-Bismark brown. Sections of skin and tongue were scanned throughout, using an oil immersion objective.

Results. Radioactive nuclei (Fig. 1) were found in 0.36% of the mast cells from dystrophic mice and in 0.15% from normal mice (Table I). The higher percentage in dystrophic mice was due entirely to one animal which had 19 radioactive mast cell nuclei. No consistent difference in frequency of radioactive nuclei was seen between animals killed 2 hours after injection and those killed 5 days after injection (Table I). The average number of mast cells per mm^2 was 18.1 for dystrophic mice and 13.8 for normal mice in the total sample of 19,523 cells (Tables I and II).

Mitotic counts were attempted utilizing 3 different staining techniques. The Feulgen-Bismark brown technique recommended by Meggers and Allen(9) was superior to the others for revealing nuclear details (Fig. 2). Four mitoses (Table II) were found with this technique in sections from the same piece of skin which had shown the high frequency of radioactive mast cell nuclei.

Discussion. In the adult mouse, mast cells are probably not produced by proliferation and transformation of precursor cells, since as many radioactive mast cells were seen at 2 hours as at 5 days after thymidine-H^3 injection. Earlier work, dealing with intervals up to 21 days, did not reveal any accumulation of mast cells at later time intervals (6). The greater concentration of mast cells

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TABLE I. Frequency of Radioactive Mast Cells in Radioautographs Stained with Aldehyde-Fuchsin.

No. of animals	Phenotype	Tissue	Nuclei non-radioactive	Nuclei radioactive	Area in mm²	Interval after H^3
2	normal	skin	1416	1	157	2 hr
2	dystrophic	"	1487	19	94	2 "
1	normal	"	1091	0	59	5 days
4	dystrophic	"	2855	4	160	5 "
2	normal	tongue	1463	4	102	2 hr
2	dystrophic	"	1685	0	65	2 "
1	normal	"	737	2	46	5 days
4	dystrophic	"	2228	7	124	5 "

TABLE II. Frequency of Mitotic Figures in Mast Cells from Non-Radioautographic Slides.

No. of animals	Phenotype	Tissue	Non-mitotic	Mitotic	Area	Stain
1	dystrophic	skin	2762	0	210	Ald.-F.-H.
1	"	"	2196	4	97	Bis. brown
2	"	"	1004	0	39	Tol. blue
1	normal	tongue	558	—	16	" "

in skin of dystrophic mice reported by O'Steen and Hrachovy(7), and confirmed here, is apparently not due to a continuously higher rate of mast cell production, but could be due to sporadic bursts of proliferative activity, since only one animal in the present experiment was found to possess an unusually large number of radioactive mast cells.

No radioautographic reactions were seen over the cytoplasm of the mast cells although such reactions after thymidine-H^3 administration have been reported(10). These reactions are apparently due to "fogging" of the emulsion(11) and can appear even in radioautographs of non-radioactive tissues (controls), so no reliable conclusions about cell proliferation can be made under such circumstances.

Mitoses in mast cells were rare. Nevertheless, their frequency was not disproportionate to the frequency of radioactive nuclei, since the latter should exceed the former because the synthetic phase lasts longer than visible mitosis.

Summary. Using thymidine-H^3 incorporation as a criterion of cell division, mast cells in adult mice show a very low rate of proliferation which may increase sporadically in dystrophic mice. No evidence was found for a precursor cell. A few mitotic figures were identified.

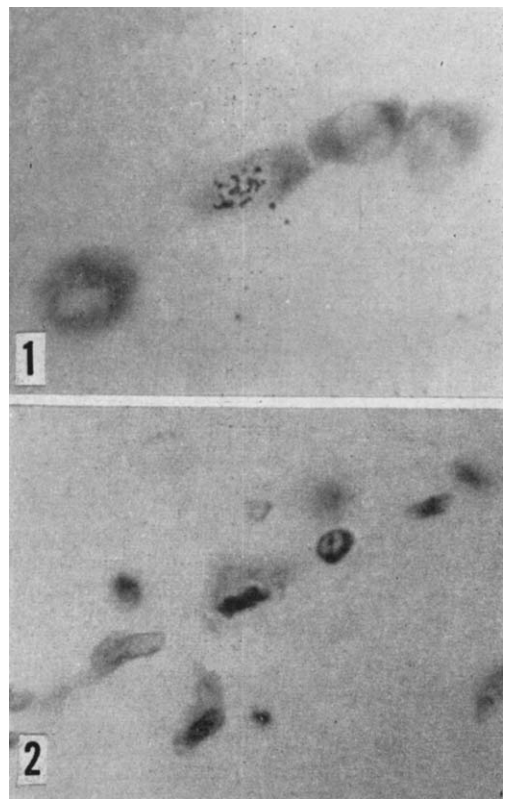

FIG. 1. Radioautograph of 4 mast cells, one of which shows silver grains over the unstained nucleus. Mast cell cytoplasm stained with aldehyde-fuchsin.

FIG. 2. Mast cell at metaphase. Feulgen-Bismark brown stain. In both figures, the tissue was from a dystrophic mouse killed 2 hr after injection of thymidine-H^3. × 1100.

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1. Kelsall, M. A., Crabb, E. D., *Lymphocytes and mast cells,* Williams and Wilkins Co., Baltimore, 1959.
2. Hunt, T. E., Hunt, E. A., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 166.
3. Speirs, R. S., Osada, Y., Jansen, V., *ibid.,* 1961, v106, 251.
4. Padawer, J., *Angiology,* 1961, v12, 538.
5. Allen, A. M., *Lab. Invest.,* 1962, v11, 188.
6. Walker, B. E., *Nature,* 1961, v192, 980.
7. O'Steen, W. K., Hrachovy, J. J., *Tex. Rep. Biol. and Med.,* 1962, v20, 70.
8. Walker, B. E., *ibid.,* 1959, v17, 375.
9. Meggers, D. E., Allen, A. M., *Stain Technol.,* 1962, v37, 221.
10. Asboe-Hansen, G., Levi, H., *Acta Path. et Microbiol. Scand.,* 1962, v56, 241.
11. Goldman, A. S., Walker, B. E., *Lab. Invest.* 1962, v11, 808.

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Quantitative Extraction of Pontamine Blue from Skin; Its Application for Measurement of Increased Capillary Permeability.* (28314)

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The capillary wall is a barrier freely permeable to water, electrolytes and small molecules but only slightly permeable to proteins. The term "increased capillary permeability" therefore refers to an alteration in the capillary wall leading to an accelerated rate of passage of plasma proteins into the extravascular tissues. This phenomenon is one of the cardinal features of acute inflammation. It has been accepted that some vital dyes, such as trypan blue or pontamine sky blue, given intravenously, become bound to plasma proteins, particularly to albumins; therefore their accumulation in inflamed skin sites indicates exudation of plasma proteins. However, evaluation of experimental results in such tests often lacks precision(1), although there have been attempts to estimate quantitatively the amounts of accumulated dye in the inflamed or treated skin sites for instance, in terms of "mean lesion diameter"(2) or by comparing the color intensity with a series of comparator cards(3). The present paper describes a method of quantitative extraction and estimation of pontamine blue in the skin.

Materials and methods. Adult male albino rabbits were used.

(a) Solutions of pontamine blue at graded concentrations in physiological saline were injected intradermally in the depilated skin of the flanks. The volume injected was 0.1 ml. The skin sites were excised 30 minutes after injections, immediately after killing the animals. After removing the fat and muscle, the skin was cut into several pieces with scissors. The skin pieces were immediately placed into 10 volumes of a mixture of absolute ethanol and dioxane (1:1) at about 20 mm Hg of vacuum at room temperature. When bubbling stopped, after 20 to 30 min., the solvent was removed by centrifugation and the skin pieces were placed into an incubator at 37°C. After incubation of 15-20 minutes, the skin pieces became rubbery in consistency, and could be minced into fine pieces with scissors.

The minced skin was placed into 10 volumes of dioxane for one hour at room temperature, with constant stirring. After removal of the dioxane by centrifugation, the skin pieces were placed into 10 volumes of a mixture of chloroform and methanol (2:1) for 30 minutes at room temperature, with constant stirring. At this point ¼ volume of absolute ethyl ether was added and 10 minutes later the supernatant was removed by centrifugation.

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