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Mitotic Activity of Mast Cells. (22887)

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Michels(1) in his review of mast cells comments "practically without exception investigators who have encountered mitotic dividing mast cells have stated that instances of them are extremely rare." This opinion has apparently not changed in recent years judging by comments made by Asboe-Hansen(2) at Josiah Macy, Jr. Foundation Conference on connective tissues. On the other hand mast cells are often seen in what appear to be isogenous groups as shown here in Fig. 7 and it has been assumed(3) that mitosis or amitosis has occurred. Direct evidence has been lacking, however. That cell division has been observed rarely in mast cells does not necessarily mean that it does not occur even with considerable frequency. Observations may have been made at the wrong time or under unfavorable conditions or the division figures overlooked. As usually stained with toluidine blue, nuclear material is either obscured by heavy granulation or is relatively colorless if the stain has been acidified. This latter procedure facilitates the identification of mast cells but greatly reduces the chances of seeing mitotic figures.

In the present study, a considerable number of mitotic figures were observed in mast cells of the rat following injection of the histamine liberating compound 48/80.

Materials and methods. A Long-Evans strain of male rats 100 days old or more was used. Animals were injected intraperitoneally with the histamine liberator, compound 48/80.* Rats in Group 1 were given 100 γ daily (concentration of 1000 γ/ml of saline) for 7 days and then were killed either on 8th day or in a few cases on 16th or 24th day. Those in Group 2 had 500 to 1000 γ daily for the same length of time and were killed on the 8th day. At time of death preparations of mesentery and subcutaneous films of connective tissue of the back were made by stretching the tissue over Bristol board rings with punched openings about 15 mm in diameter. Preparations were fixed in cold formol and absolute ethanol (10:90) and stained in 0.1% toluidine blue which in most cases was not acidified. After dehydration and clearing, the tissue was cut from the ring and mounted.

Observations. In Group 1 (with the smaller dosage) the 48/80 was sufficient to result in disappearance of the large-sized, heavily granulated mast cells usually present in the mesentery. There remained, however, a considerable although variable number of medium-sized cells having diameters of 14 to 24 μ with fewer granules. Among these cells mitotic figures could be readily observed (Fig. 2 to 5).

It is difficult to give an accurate quantitative count of the mast cells in division because the mitotic figures are relatively widely and unevenly spaced and are easily overlooked. In one preparation of mesentery, however, covering approximately 12 square mm. 6 mitotic figures were seen among a total of 364 mast cells. In another preparation 2 mitoses were seen in one high power field. In other preparations dividing cells were more widely spaced but could be found with careful searching.

* The compound 48/80 was kindly supplied by Dr. E. J. deBeer of Wellcome Research Laboratories.

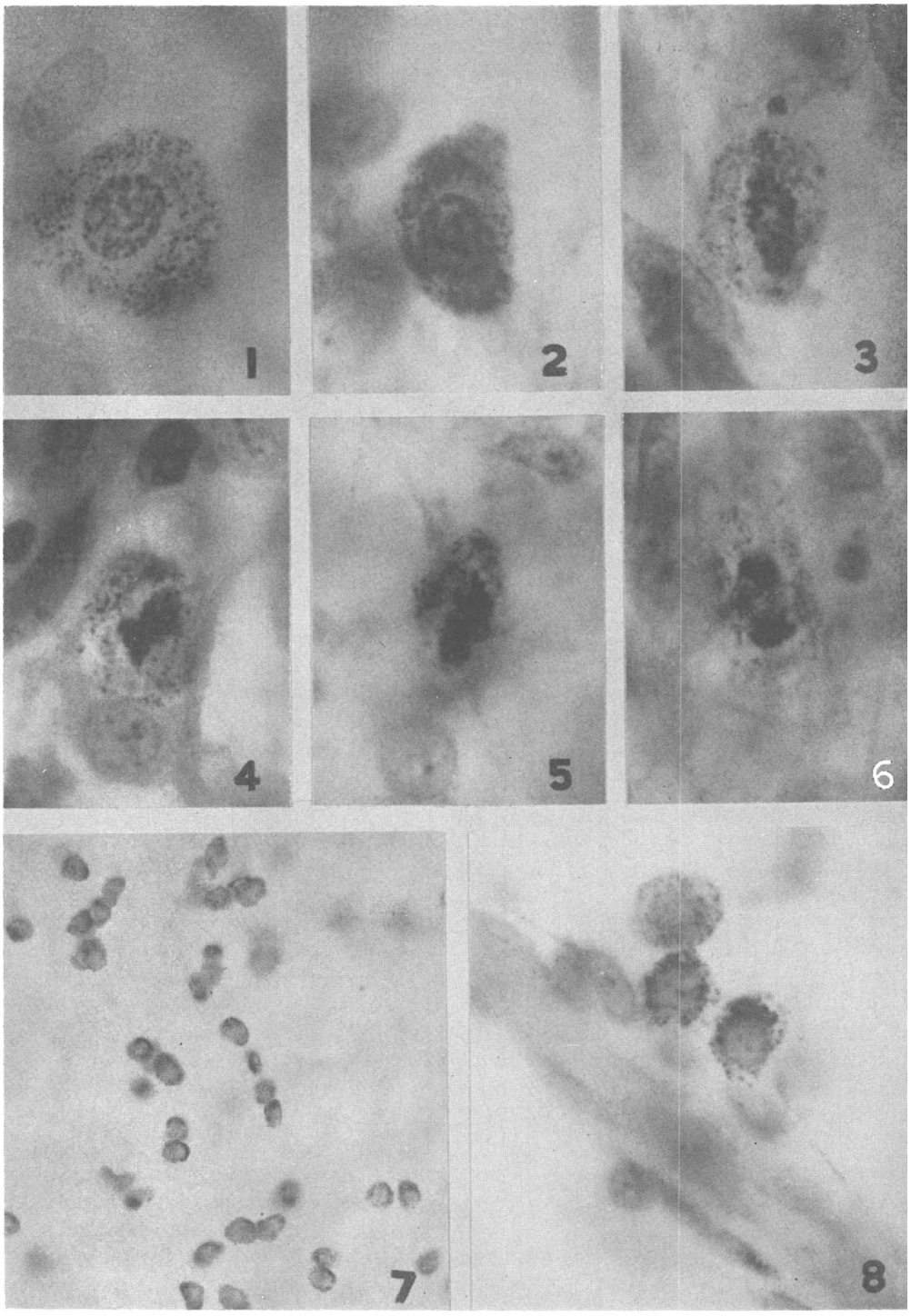

FIG. 1. Mast cell in prophase in subcutaneous connective tissue. ×1400.
FIG. 2-5. Mitotic figures in mast cells of mesentery. ×1400.
FIG. 6. Mast cell in telophase in subcutaneous connective tissue. ×1400.
FIG. 7. Isogenous groups of mast cells in subcutaneous connective tissue. ×380.
FIG. 8. Mast cells along capillary of omentum; rounded ones above vessel, elongated one below vessel. ×1400.

In animals receiving heavier doses of 48/80 no mast cells remained in the mesentery presumably because of their disintegration. In subcutaneous connective tissue, however, they were affected only moderately and while the larger 20 to 30 μ, heavily granulated cells were no longer present as such, there were considerable numbers of smaller ones 12 to 15 μ in diameter. In one preparation a total of 8 mitoses was seen (Fig. 1 and 6). In some regions many isogenous groups of 2, 3 or more cells occur (Fig. 7). These cells, having an average diameter of 10 μ, are believed to have divided recently and have not yet had time to increase in size. Some mitoses were also found among these cells but the nuclear material was stained so poorly as a rule that mitotic figures could not often be identified with certainty.

In animals that were killed 9 days after the smaller doses of 48/80, the mast cells of the mesentery had again become heavily granulated and by the 17th day most had reached a diameter ranging from 16 to 20 μ.

Discussion. In previous investigations(4, 5) the conclusion was reached that cells produce a mitotic inhibiting substance which when diminished sufficiently allows mitosis to occur. The diminution may occur after strong secretory activity as well as after death of some of the cells in the area. In the case of mast cells the 48/80 causes a diminution of the product of mast cells, histamine, from the vicinity of the cells(6). Fawcett(3) has shown that a moderate amount of 48/80 results in a release of granules followed by rapid cellular recovery. The released granules, which may include the mitosis inhibiting substance as well as histamine, are rapidly removed from the region of recovering cells by phagocytic activity of macrophages(3) or fibroblasts(7) or both, and consequently conditions are then realized which will activate cell division.

Mast cells may be affected to varying degrees by various stimulating substances such as 48/80 so that there may be a range from mild response to complete disintegration of mature cells in a region such as the mesentery. With mild stimuli the cells are believed to pass through a secretory phase without division. With a stronger stimulus as given in the present experiments the cells are more nearly exhausted and cell division occurs. With still stronger doses disintegration of the cells occurs and replacement then may follow by differentiation from stem cells.

The development of mast cells from stem cells is comparable to heteroplastic hemopoiesis. In the lymphatic milk spots of omentum or in their vicinity, there are many small mast cells only 5 to 8 μ in diameter (Fig. 8) having many of the characteristics of medium-sized lymphocytes. Also along the small blood vessels are irregularly shaped, small mast cells such as Riley(8) described. These cells, which are much smaller than fibroblasts of the vicinity, are believed to have differentiated from mesenchymal cells. Their number appears to have increased after 48/80 but this is difficult to prove because they vary so much in frequency in control animals. They do not release their granules even with the largest doses of 48/80.

Fawcett(3) in describing pairs of mast cells after moderate doses of 48/80 considers that they have appeared probably due to cell division, but he does not offer an opinion or evidence to support the statements by others that division occurs either by mitosis or amitosis. Except for the occurrence of what appear to be isogenous groups there is little or no evidence that amitosis of mast cells occurs *in vivo*.

The percentage of mast cells undergoing division at any one time probably varies greatly just as it does in other types of cells, and the number depends on mitotic stimulating factors. In the one instance in which a quantitative count was attempted, 1.6% of the mast cells were found in division. In organs such as liver, kidney and adrenal gland, Stevens and Leblond(9) state that only 0.1% of the cells are in division at any one time. Thus on a comparative basis the occurrence of mitosis in mast cells of mature animals is no more infrequent than it is among other cells where the occurrence of mitosis is unquestioned.

Summary. 1) Mitotic activity occurs in

adult mast cells of the mesentery of rats after moderate intraperitoneal doses of the histamine liberator, compound 48/80. After larger doses, which cause disintegration of mesenteric mast cells, mitoses are found in mast cells of the subcutaneous tissue. 2) Replacement of cells after disintegration appears to be by heteroplastic differentiation of lymphocytes or undifferentiated mesenchymal cells or both.

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Co^{60} Vitamin B_{12} Binding Capacity of Normal Human Serum.* (22888)

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Recent studies(1-4) of plasma concentration and transport mechanisms of vit. B_{12} have included microbiological measurements of its serum *in vitro* binding capacity. Results have varied widely. The present investigations are concerned with estimation of Co^{60} vit. B_{12} serum binding capacity.

Methods. Sera were obtained from 40 normal persons. The Co^{60} vit. B_{12} had a specific activity of 0.786 mc/mg. Its physiological activity was proved when 3 μg given intravenously to a patient with pernicious anemia in relapse induced a moderate reticulocyte response and clinical improvement. To 1 ml of serum was added an aliquot of Co^{60} vit. B_{12} ranging from 1 to 500 μg. This was done either by adding the chosen amount of vitamin directly, or by making an initial concentrated solution in serum, then rediluting with more serum at once. These methods gave equal results. Specimens were prepared in duplicate, one to serve as a standard. Each was pipetted into a Visking bag. Samples were incubated at room temperature for 2 hours, then subjected to constant agitation and exhaustive dialysis in cold, running tap water for 48 hours. Standards were placed in 4 ml vials at once; samples were placed in similar vials at conclusion of dialysis. Each vial was filled with concentrated sulfuric acid, dissolving the bag and allowing complete mixing within the vial. Counting was done in a well-type scintillation counter. "Bound" vit. B_{12} was calculated as activity retained in the bag after dialysis. Parallel studies were made with $Co^{60}Cl_2$ and sera from 14 normal subjects. The specific activity and cobalt content of the $Co^{60}Cl_2$ solution were equal to that of the radioactive vit. B_{12}. Continuing dialysis for 72 or 96 hours did not decrease residual activity further than the 48-hour period used. Incubation of the serum-B_{12} mixture for more than 2 hours prior to dialysis did not alter amount of vit. B_{12} bound. Dialysis for 48 hours in a reservoir of normal saline, 6% dextran in normal saline, or human plasma 50x the volume of the samples and changed at 24 hours gave the same results as exhaustive dialysis in tap water. Co^{60} B_{12} in saline dialyzed for 48 hours in tap water showed residual activity within the Visking bag of less than 2% at any concentra-

*Co^{60} Vit. B_{12} was obtained from Merck & Co, Rahway, N. J.