Fate of Shed Mast Cell Granules.* (22445)

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Numerous investigations concerning the role of the mast cell in the physiology of the connective tissue have been stimulated following the observations that both heparin(1) and histamine(2) are concentrated in the cytoplasmic granules of this cell. More recently it has been reported that 5 hydroxy-tryptamine is also a constituent of mast cell granules(3). Ehrlich, who described the mast cell, observed that it appeared as an overnourished cell whose cytoplasm was well-filled with metachromatic granules and suggested that it may function as a storage cell of the connective tissue(4).

It has been reported that when loose connective tissue is exposed to any of various noxious stimuli, the mast cells therein release or shed their granules into the surrounding ground substance(5). In view of the proposed constituents of the mast cell(1-3), it has been of interest to ascertain the fate of these shed granules. In the following study it was observed that within 15 minutes to 1 hour after degranulation, metachromatic granules appeared within the cytoplasm of the surrounding fibroblasts. Further investigation showed that these fibroblastic granules were actually shed mast cell granules which had been taken into the cytoplasm of the fibroblasts.

Materials and methods. Injected materials. Materials for injection were suspended in sterile, pyrogen-free isotonic saline. 1. Mast cell granules were obtained by a method developed in this laboratory(6), which is as follows: sheets of loose subcutaneous connective tissue were stripped from the skins of freshly killed mice by applying the connective tissue surface of the skin to a freezing surface (stainless steel over dry ice) and then rapidly pulling the skin away, leaving the connective tissue as a frozen sheet. This tissue was then scraped into an isotonic sucrose solution and shaken over night with glass beads in a cold room (4°C). The mast cell granules were isolated from the mixture by differential centrifugation. Fig. 1 shows the nature of this granule preparation. 2. Radioactive mast cell granules were isolated from the connective tissue of 35 to 40 female CBA mice (12 to 16 months old) by the above technic. These animals had been injected intraperitoneally 48 hours previously with 0.1 cc of a carrier-free solution of $Na_2S^{35}O_4$‡ containing approximately 250 μc activity. Radioautographic analysis of tissue spreads at intervals after injection, obtained from similarly prepared mice, has shown that the highest relative concentration of radioactivity in the mast cells could be obtained approximately 48 hours after injection of the radioactive solution (Fig. 2). This is in agreement with the observations of Jorpes, *et al.*(7) as well as with those of Asboe-Hansen(8). Preparations of isolated granules (Fig. 3) were washed 2 times with isotonic sucrose to free the preparation from any excess radioactivity not bound to granules, then resuspended with isotonic saline for injection. *Tissue sampling.* CBA mice, 8 to 10 weeks old, were injected subcutaneously on the dorsal surface with 1.0 cc of air to form a bubble of air in the loose connective tissue (air pouch). The details of this method will be described elsewhere. Treatment and sampling of the tissue were carried out routinely in the following manner: test substances contained in a volume of 0.2 cc were injected into the pouch. After sacrifice of the animal at the appropriate interval following injection, the pouch was dissected free of the surrounding tissues and a section

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‡ $Na_2S^{35}O_4$ was obtained from Oak Ridge, Tenn.

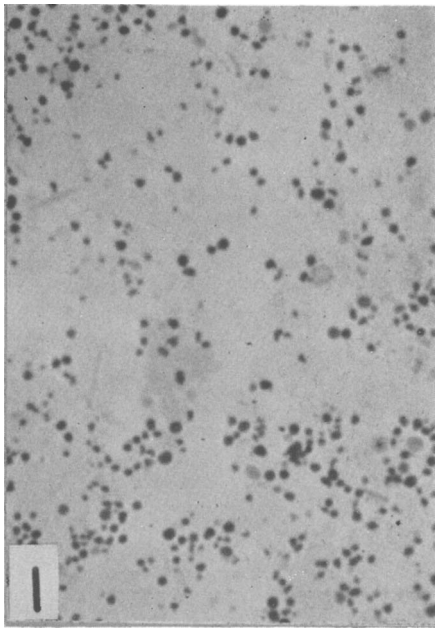


FIG. 1. Mast cell granules following extraction from mouse loose connective tissue. Stained with alcoholic solution of Azure A. (× 960)

of the enclosed connective tissue bubble rapidly removed, spread on a glass microscope slide, air-dried, and stained with May-Gruenwald Giemsa in the usual manner(9). Similar preparations were stained with an alcoholic solution of Azure A for comparison. *Radioautographic technics.* Contact radioautograms of connective tissue spreads were prepared by apposition to 10 μ thick NTB, nuclear track plates (Eastman Kodak Co., Rochester, N. Y.). The radioautogram was superimposed upon the corresponding connective tissue spread and examined microscopically. A more detailed examination of the radioautogram and tissue spread was made by taking photomicrographs from corresponding areas of tissue spread and radioautogram (Fig. 2 and 9). Detailed radioautograms of the injection material (radioactive granules spread on glass microscope slides) were prepared with Eastman Kodak's permeable base stripping film. The technic used is a modification of a method described by Arnold and Jee(10). Due to the extensive washings of the tissue preparation, the granules were swollen (Fig. 3) and had a reduced affinity for the stain. Control preparations reacted

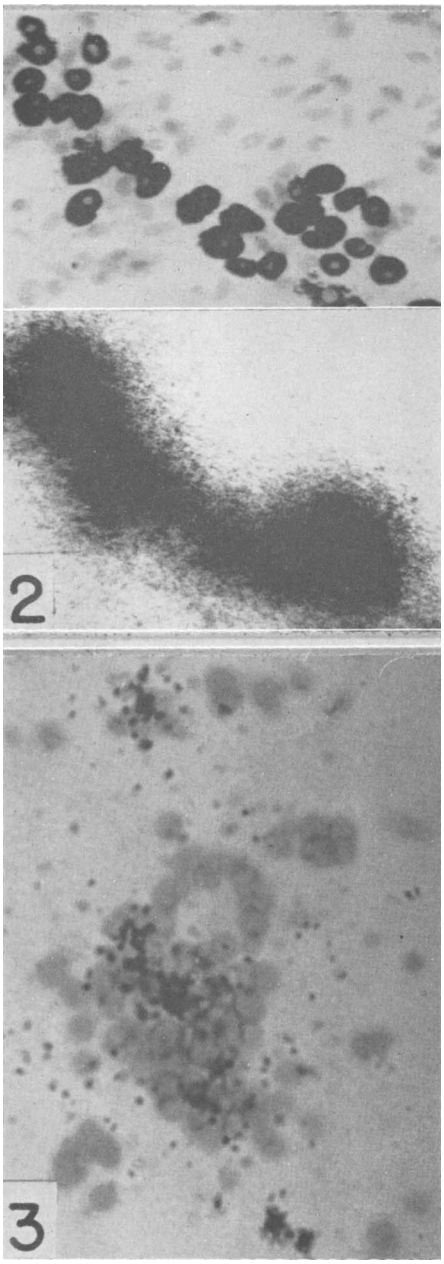
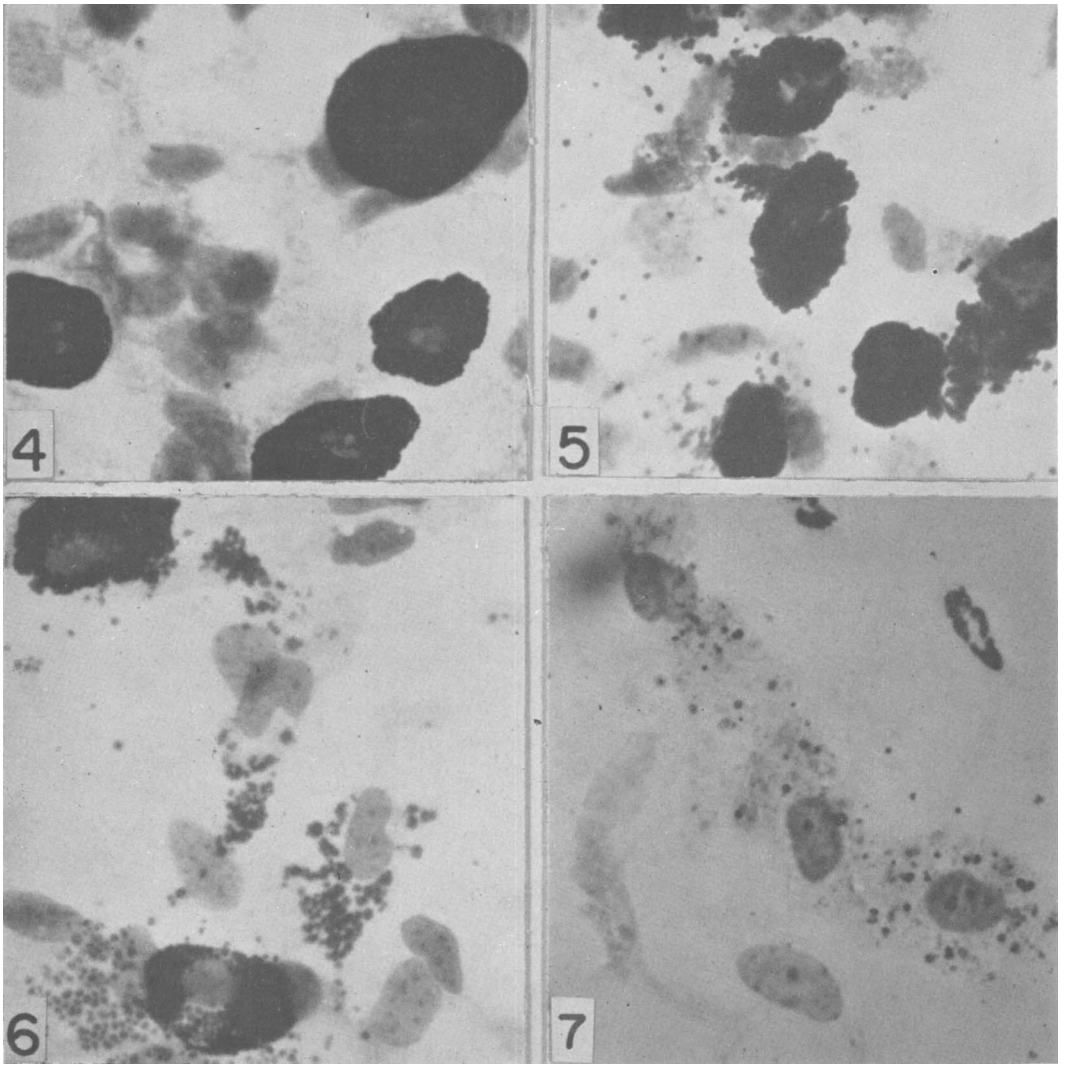


FIG. 2. Loose connective tissue spread (upper) from mouse 48 hr after inj. of $Na_2S^{35}O_4$, and contact radioautogram (lower) of accumulated radioactivity in the mast cells. Note conformity of mast cell distribution with darkened area of film. (× 500)

FIG. 3. Strip film radioautogram of mast cell granules isolated from subcut. loose connective tissue of mice inj. with $Na_2S^{35}O_4$ 48 hr previously. The granules are somewhat swollen and weakly staining following radioautographic procedures. However, note that the radioactivity (black dots) is concentrated with the granules. (× 1350)

to the stain as those in Fig. 1. However, it is apparent that the radioactivity was bound to the mast cell granules.

Results. Following the local injection of various substances (*e.g.*, histamine, gelatin, agar-agar, protamine, saline, etc.), the mast cells, which are normally quite intact and filled with granules (Fig. 4) can be seen to degranulate and shed their granules into the surrounding ground substance (Fig. 5). The number of mast cells exhibiting this reaction is generally related to the nature of the material injected; that is, the injection of air or saline will stimulate a small reaction, whereas



Figs. 4-7. Loose subcutaneous connective tissue spreads. Stained with May-Gruenwald Giemsa. (× 850)

FIG. 4. Mast cells prior to exposure to noxious stimuli.

FIG. 5. Non-specific degranulation of mast cells in response to stimuli (0.1 mg histamine).

FIG. 6. Fibroblastic uptake of shed mast cell granules by cells proximal to degranulating mast cells. Note the concentration of granules in the poorly staining cytoplasm of fibroblasts at center of microphotograph.

FIG. 7. Fibroblastic digestion of ingested mast cell granules after degranulation of mast cells 2 hr earlier. The cytoplasm exhibits varied degrees of metachromasia as the granules undergo dissolution.

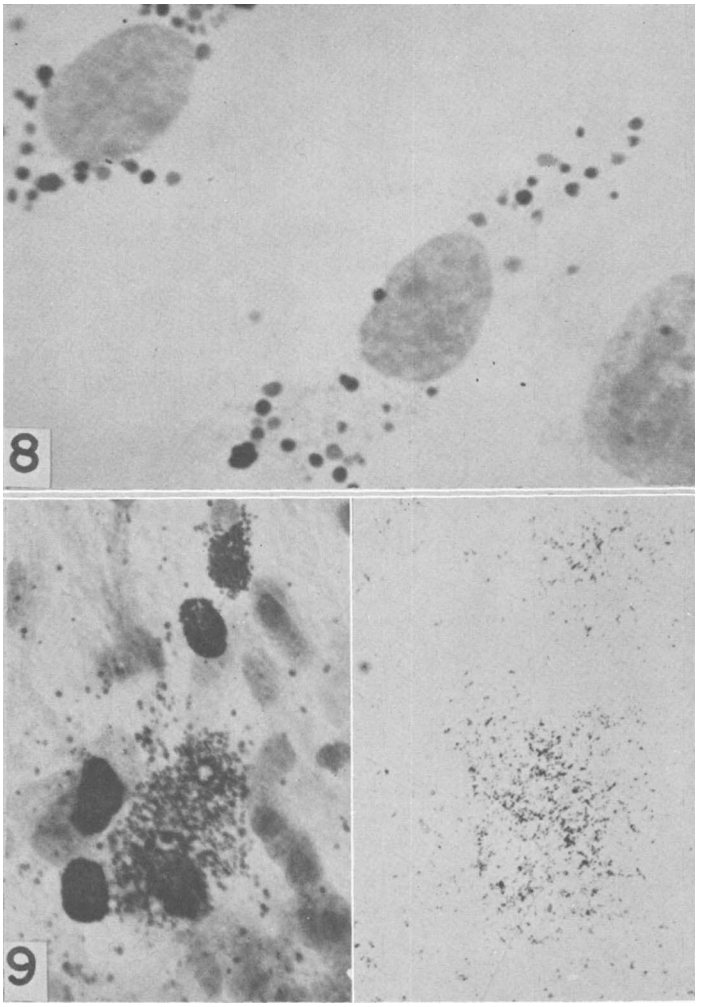


FIG. 8. Loose subcut. connective tissue spread from mouse inj. subcut. 2 hr previously with preparation of isolated mast cell granules. Observe that granules are confined within cytoplasm of cell. Stained with May-Gruenwald Giemsa. (× 1250)

FIG. 9. Loose connective tissue spread (left) and contact radioautogram (right) from mouse 1 hr following inj. of preparation of isolated S^{35}-labeled mast cell granules. Inj. granules at center show retention of radioactivity, whereas little to no radioactivity is associated with mast cells to left and above center. Note presence of "newly granulating" cell at upper border in comparison to relative lack of activity associated with nearby mast cell. Stained with May-Gruenwald Giemsa. (× 500)

agar-agar or histamine provokes a rather extensive amount of degranulation. Injection of a mild antigen (horse serum) into the connective tissue of sensitized animals produces a rapid and marked degranulation of the exposed mast cells, whereas the same antigen injected into a non-sensitized animal elicits a comparatively small response(11). It has therefore appeared unlikely to us that degranulation is an artifact, as recently suggested by Devitt(12). In a series of experimental observations in this laboratory over a period of years, it has become apparent that degranulation is followed by the appearance of granules in the fibroblasts of the local area (Fig. 6). This is evident within 15 minutes to 1 hour after degranulation has been initiated. During the next 1 to 2 hours, the presence of granules in fibroblasts tends to decrease and many of the fibroblasts have a

diffuse metachromasia in the cytoplasm and/or contain granules in various stages of dissolution (Fig. 7). This suggests that many of the fibroblasts are able to digest this material.

Isolated Granules. To confirm these observations, mast cell granules isolated from mouse connective tissue relatively free from other cellular elements (Fig. 1) were resuspended in isotonic saline and aliquots injected into mice subcutaneously. At intervals of 15, 30, and 60 minutes, 2, 3, 4, 5 and 6 hours, tissues were obtained from at least 4 animals and prepared for microscopic observation. Only those tissue areas containing patches of injected granules and relatively free of mast cells were examined, and it was observed that, within 15 minutes after injection, metachromatic material as well as granules appeared in the cytoplasm of fibroblasts. The granule uptake appeared to reach its maximum within the first to second hour following injection. By the fourth hour the cytoplasmic granules had largely disappeared. Fig. 8 shows an area of a tissue sample 2 hours after granule injection in which the cytoplasm of the fibroblasts contains typical mast cell granules. The time necessary for granule digestion is probably much less than our observations would suggest, since in any one cell there is usually evidence of granule breakdown coexistent with normal appearing granules.

Radioactive Mast Cell Granules. To test these observations further, mice were injected subcutaneously with aliquots of an S^{35}-labeled granule preparation. Samples of loose connective tissue were removed from the mice 1 hour after the injection with radioactive granules and prepared for contact radioautography. In Fig. 9 the labeled granules can be seen in the tissue with a distribution of radioactivity in fair agreement with granule distribution. It should be noted that the intact mast cells show no evidence of accumulation of radioactivity, while there is an apparent formation of a "new" mast cell resulting from the fibroblastic uptake of radioactive granules.

Discussion. Connective tissue is a primary site of inflammation and serves as a mediator between the parenchymal cells and the blood-vascular system throughout the body. Probably the earliest obvious change occurring in the loose connective tissue following stimulation by any of a number of noxious agents is the degranulation of the local mast cells. It has been demonstrated that, following degranulation, these granules can be taken into the cytoplasm of the local fibroblasts, where they are digested. In essence, this phenomenon is a turnover of mucopolysaccharide in the connective tissue. It is probable that this is but a single aspect of a more general fibroblastic participation in the catabolism of ground substance mucopolysaccharides.

This fibroblastic function, unlike classical phagocytosis, is not generalized with respect to the particle. When connective tissue is exposed to an inoculum of young staphylococci, it shows a selective fibroblastic uptake of granules from degranulated mast cells in the tissue, rather than of the bacteria. Since this cellular phenomenon has been demonstrated principally with the heparin-containing mast cell granules or polysaccharides of large molecular weight of (or resembling those of) tissue origin, it has been termed "micellophagosis"(13) and implies the fibroblastic uptake of polysaccharides or polysaccharide-bound substances.

In view of the biologically active nature of some of the proposed constituents of mast cell granules (*i.e.*, heparin(1), histamine(2), and 5 hydroxytryptamine(3)), this fibroblastic function readily suggests itself as a protective mechanism for the maintenance of connective tissue function. Tissue injury initiates the release of a factor or factors (*e.g.*, histamine, etc.), which can induce the shedding of mast cell granules. The intracellular granules may serve to fix low concentrations of noxious amines in a manner similar to their binding (vital staining) with the amine, toluidin blue. Larger concentrations of the amines may injure the mast cell and thereby stimulate the release of granules into the ground substance, where the acidic polysaccharide (heparin) constituent of the granules may continue to bind additional amines before ingestion by local fibroblasts. The release of histamine from mast cell granules by the phenyl alkyl-

amine, 48/80(14), and the binding of heparin by this substance(15) suggest that this granule constituent may exchange histamine for various other amines, thereby forming complexes with the latter substances. In any event, ingestion and subsequent detoxification of this material by fibroblasts would tend to reduce the stimuli for the inflammatory response. Under physiological circumstances, noxious amines arising as the result of metabolism and/or cell death could be dealt with in these manners and the production of inflammatory lesions avoided(16).

Summary. It has been observed that following mild trauma to the skin of mice, degranulation of mast cells occurs in the subcutaneous connective tissue and is followed by the appearance of metachromatic granules within the cytoplasm of surrounding fibroblasts. Investigation of this phenomenon by subcutaneous injection of isolated mast cell granules showed that fibroblasts began to ingest the granules within 15 minutes after injection. Granulation appeared to be maximal within one to two hours and digestion of granules was apparent within four hours. These observations on the fibroblastic uptake of mast cell granules were confirmed by using S^{35}-labeled mast cell granules for injection. It is suggested that this phenomenon represents one aspect of local detoxification in the tissues.

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Effect of Total Body X-Irradiation on the Parakeet.* (22446)

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The biological effect of total body x-irradiation on vertebrates has been investigated chiefly in mammals; a few data have also been reported on fishes, amphibians, and reptiles(1). Although birds retain many characteristics of reptiles, they differ in being homeothermal and having a metabolic rate akin to that of mammals. Nevertheless, relatively few radiation studies have been conducted on birds, and most of these have employed the chicken(2-5). To provide a broader base for comparing the response of birds with other classes of vertebrates we have

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