Mast Cells and Intercellular Mucopolysaccharides in Rous Fowl Sarcoma I

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The intercellular ground substance in the connective tissue of Rous fowl sarcoma is known to be extremely viscous, with a high content of hyaluronic acid (4, 6, 7). In histological sections, this substance is found to be highly metachromatic upon staining with toluidine blue. Loomis and Pratt (5) demonstrated a metachromatic ground substance which yielded to a 2-hour incubation with hyaluronidase. Claude (2) reported that extracts of Rous sarcoma are sensitive to the action of leech hyaluronidase, which reduces their viscosity. In general, the mucinous substance is said to be nonsulfuric. By chemical methods, Harris et al. (3), however, found that, in addition to large amounts of hyaluronate, the mucinous substance contains a little sulfuric polysaccharide, which they believed to be heparin because it possessed antithrombic and hyaluronidase-inhibiting activity. Finding the stromal areas very poor in mast cells, they considered the heparin or the heparin-like material to be located in the ground substance. As hyaluronic acid and heparin are believed to be formed by the mast cells of the connective tissue, the occurrence of these cells at the time of earliest appearance of the metachromatic substance was studied in some detail in this laboratory.

EXPERIMENTAL

Ten Brown-Leghorn fowls, 2 months of age, were given injections of lyophilized Rous sarcoma virus into the pectoral muscles. On the 4th-6th day, the animals were sacrificed. Muscle specimens were removed, one part was fixed in 4 per cent lead subacetate solution, the other in methyl alcohol. All preparations were stained with 1 per cent aqueous toluidine blue solution and 1 per cent toluidine blue in 40 per cent alcohol. Microscopic examination revealed incipient development of tumor tissue and a connective tissue ground substance giving intense metachromasia with aqueous toluidine blue. In metachromatic areas, particularly in their periphery bordering on healthy muscle, an accumulation of mast cells of varying granularity could be observed, the cells being least granulated in areas showing most marked metachromasia. The mast cells showed distinctly, particularly when stained in the alcoholic solution of toluidine blue, because alcohol eliminates, wholly or partially, the metachromasia of the surrounding intercellular substance (Fig. 1). The accumulation of mast cells was observed during the first days after metachromasia appeared; thereafter, the number of cells decreased. In more advanced growth marked metachromasia was still

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Fig. 1.—Scattered mast cells in the periphery (B) of sarcoma cell area (A). Staining: toluidine blue, 1 per cent in 40 per cent alcohol. Magnification X80.

Fig. 2.—Phase-photomicrograph of muscular tissue invaded by Rous sarcoma cells. Microscope focused on the tissue. Magnification. X520.

Fig. 3.—Stripping-film autoradiograph of same field. Microscope focused on the emulsion. Note blackening of the film by the S<sup>2</sup>-containing sarcoma cells. Scattered black grains over the sarcoma cell area; rows of black grains indicating sarcolemmic membranes.
present, whereas the number of identifiable mast cells was small. On the other hand, among the numerous sarcoma cells were many with a nongranulated, metachromatic cytoplasm; these cells were rounded or spindle-shaped, fibroblast-like.

After the injection of virus, 48 hours before the animals were sacrificed, radioactive sulfur (S\(^{35}\)) was injected into the femoral muscles as labeled sodium sulfate in doses of 4 mc/kg. The methyl alcohol-fixed specimens were embedded and sectioned in the usual way.

Autoradiographs were made with the stripping-film technic of Pele. After an exposure time of 14 days, the films were developed, and the blackening examined by light-field and dark-field microscopy. The autoradiographs revealed an uptake of S\(^{35}\) by the mast cells as well as the sarcoma cell area and the metachromatic amorphous ground substance (Figs. 2, 3). The different cells could be identified after the preparations were stained through the film with an aqueous solution of toluidine blue.

**DISCUSSION**

During the period initiating the mucopolysaccharide formation in the Rous sarcoma, i.e., on the 4th–6th day, an accumulation of mast cells was observed. This is in accordance with findings in other conditions in which new formation of hyaluronic acid takes place (1). Substances containing radioactive sulfate after previous distant injection of S\(^{35}\), i.e., in the tissues mainly sulfuric mucopolysaccharides, appear to occur in mast cells as well as in areas without granulated mast cells but with sarcoma cells and metachromatic ground substance. This latter blackening seems to be due to S\(^{35}\) of intracellular as well as extracellular sulfo-mucopolysaccharides. These may be a product of the nongranulated cells of the sarcoma cell area.

**SUMMARY**

On the 4th–6th day following injection of Rous sarcoma virus into the pectoral muscles of 2-month-old Brown-Leghorn fowls, histological preparations showed accumulation of mast cells and incipient formation of a metachromatic substance.

After systemic injection of radioactive sulfur as S\(^{35}\)-labeled sulfate, autoradiographs revealed blackening of a stripping film in direct contact with the mast cells as well as the sarcoma. Some cells of the latter contained a metachromatic cytoplasm, and its intercellular ground substance stained metachromatically with toluidine blue. This intercellular material may be elaborated by the nongranulated cells of the sarcoma cell mass.

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