Autoradiography of Mast Cells in Experimental Skin Tumors of Mice Injected with Radioactive Sulfur (S\textsuperscript{35})

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Mast cells are present in the organism wherever there is connective tissue. They are large, with cytoplasmic granules which stain metachromatically with certain basic dyes, such as toluidine blue. Recent research on connective tissue has clarified the chemistry and thus the function of these cells.

INTRODUCTION

In 1937, Holmgren and Wilander (10) maintained that mast cells in connective tissue contain a polysulfuric acid ester giving a heparin effect and that it is their function to secrete the anticoagulative substance into the blood. This contention is supported by the finding that tissue which is rich in mast cells—e.g., the hepatic capsule of the cow—has more pronounced anticoagulative properties than other tissue with a lower content of mast cells—such as the hepatic capsule of the sheep. Moreover, these authors based their view on Lison’s (12) opinion that metachromatic staining with toluidine blue is due to the presence of high molecular polysulfuric acid esters. Heparin is one such substance, a sulfic mucopolysaccharide. Subsequent workers, however, have shown that other substances, such as monosulfuric acid esters and the nonsulfuric but closely related mucopolysaccharide hyaluronic acid (which makes up a considerable part of the connective tissue ground substance) stain metachromatically with toluidine blue and similar dyes (1–5, 7, 14, 16, 18). Like heparin, hyaluronic acid is a polymer which, on hydrolysis, gives glucosamine and glucuronic acid; but unlike heparin it does not contain sulfur. The mucopolysaccharide in the cornea, a monosulfuric acid ester of hyaluronic acid, stains metachromatically.

In 1948, Jorpes, Werner, and Åberg (11) found that, besides staining metachromatically like the acid mucopolysaccharides, mast-cell granules stain with leucofuchsin following oxidation with periodic acid by the method of McManus (13). They pointed out that heparin and chondroitin sulfuric acid do not stain this way, whereas hyaluronic acid and mucoin monosulfuric acid are stained distinctly. Jorpes and co-workers concluded that the compound present in the mast-cell granules, although not heparin proper, may be its precursor.

Asboe-Hansen (2–5) was unable to demonstrate a definite relation between the number of mast cells in the tissue and its content of sulfomucopolysaccharides, whereas he found a correlation between the number of mast cells and the quantity of hyaluronic acid in the tissue, as determined by chemical and histochemical methods. The author also observed signs of a new formation of hyaluronic acid from mast cells following injection of hyaluronidase into the tissue (in vivo experiments). Apparently, under the influence of hormonal action, the mast cells secrete the mesenchymal mucopolysaccharide, hyaluronic acid, perhaps by way of a sulfic precursor resembling heparin.

It is known (6, 17) that when a number of high molecular nonsulfuric polysaccharides, including hyaluronic acid, are sulfonated they may acquire anticoagulative properties.

From chemical, histochemical, and histophysiological investigations it seems justifiable to infer that the mast cells contain a sulfuric mucopolysaccharide closely related to heparin and hyaluronic acid, though not identical with either, however. The mast cells seem to give off a hyaluronidase-sensitive mucopolysaccharide to the connective-tissue ground substance, which does not appear to possess anticoagulative properties.

The chemical changes in the substance of the granules possibly occurring before, during, or after its transfer from the cell to the connective tissue ground substance are unknown and the subject of intense study in various laboratories.
MATERIALS AND METHODS

Autoradiographic investigation.—Histochemical methods cannot offer quantitative information, and chemical analysis of tissue reveals nothing concerning the location of sulfur in the tissue. The author therefore used autoradiography to demonstrate the localization of sulfur-containing compounds in connective tissue, their relative quantities and distribution.

In order to obtain connective tissue containing mast cells in large numbers, experimental skin tumors (papillomas), containing cells presumably at the maximum of their functional capacity, were induced in mice of the strain St/Eh by a single painting with 9,10-dimethyl-1,2-benzanthracene, a carcinogenic hydrocarbon. In the dermal connective tissue of the painted areas the tumor-bearing mice revealed a marked accumulation of mast cells, most of which are large and richly granulated (8, 9). Nine of these tumor-bearing mice received one intraperitoneal injection of \( 5^{35}S \), 8 \( \mu \)c/gm of body weight with 0.1 mg. \( Na_2SO_4 \) as carrier in 0.2 ml. of sterile water. After 48 hours the mice were killed; the tumor and a piece of normal skin from a symmetrical site were excised weight with 0.1 mg. \( Na_2SO_4 \) as carrier in 0.2 ml. of sterile water. After 48 hours the mice were killed; the tumor and a piece of normal skin from a symmetrical site were excised and divided into three portions, which were fixed in 70 per cent alcohol, 10 per cent formalin, or 4 per cent basic lead acetate, respectively, while one portion was freeze-dried. The tissue was imbedded in paraffin and cut in sections 7 \( \mu \) thick. After the sections were deparaffinized and dried, autoradiographs were made applying the stripping film technic developed by Pele (15). The films were exposed for 17 days. Some unstained preparations and the superimposed autoradiographs were studied in the phase contrast microscope; others were stained, after exposure, with 1 per cent aqueous solution of toluidine blue for 15 minutes and then inspected microscopically.

To check the autoradiographic method as applied here, a number of controls were made on mice which had not been injected with \( 5^{35}S \). Tissue sections from tumor and from normal skin did not cause blackening of the stripping film after several days' exposure. It can therefore be concluded that the results obtained by autoradiography after injection of \( 5^{35}S \) are independent of whether the specimens were fixed or whether the experimental period (48 hours) is too long or too short, whether or not the sulfuric acid content of individual mast cells varies—these are problems which require extensive investigations. Such studies are in progress.

RESULTS AND CONCLUSIONS

Forty-eight hours after injection of radioactive sulfur, monitoring with a Geiger counter revealed a pronounced accumulation of radiosulfur in the tumor.

After 17 days' exposure, a stripping film showed marked blackening over the mast cells in the connective tissue of the skin tumor. In addition, there was a less intense, diffuse blackening over the connective tissue ground substance. In autoradiographs of normal, unpainted skin, only a slight blackening could be observed.

A few mast cells in the connective tissue of the unpainted skin and in muscle gave no distinct blackening, and in some of them the content of radiosulfur could not be demonstrated with certainty.

It cannot be ascertained whether the diffuse blackening of the film, produced by the connective tissue in the tumor, is due to radiosulfur leached out from the mast cells in the course of the histological fixation or during the stripping procedure, or whether it is caused by an increased content of sulfur in the connective tissue ground substance of the tumor. The autoradiographs were very similar, independent of whether the specimens were fixed in one of the three different solutions or freeze-dried. Furthermore, the blackening caused by mast cells appears to be very much the same in all preparations. This is noteworthy in view of the fact that in histological preparations mast cells appear larger and more regular in freeze-dried specimens and after fixation with basic lead acetate than after fixation in alcohol.

Whether the ability of mast cells to take up radiosulfur depends on their age and metabolic state, whether the experimental period (48 hours) is too long or too short, whether or not the sulfuric acid content of individual mast cells varies—these are problems which require extensive investigations. Such studies are in progress.

SUMMARY

Previous histochemical and chemical studies indicated that the granules of the connective tissue

![Fig. 1.—Autoradiograph from section of experimental skin tumor; unstained preparation; dark-field microscopy. Above, the hyperplastic epithelium with deep extensions into the corium. In the connective tissue the mast cells appear as bright discs, indicating a high radiosulfur content; diffuse blackening over the ground substance. Mag. \( \times 800 \).](image1)

![Fig. 2.—Unpainted skin from a site symmetrical to the skin area in Fig. 1; unstained; dark-field microscopy; radiosulfur content low. Hairs above. Mag. \( \times 350 \).](image2)

![Fig. 3.—Unstained preparation; connective tissue area of Fig. 1. Eleven mast cells have caused intense blackening of the stripping film. Mag. \( \times 1800 \).](image3)

![Fig. 4.—Stained preparation. Staining: toluidine blue, 1 per cent aqueous solution, for 15 minutes. A vessel with two mast cells in its immediate surroundings. Intense blackening of the film over the sites of the mast cells. Mag. \( \times 1850 \).](image4)
mast cells contain a sulfuric mucopolysaccharide. This substance is closely related to heparin and hyaluronic acid without being identical with either.

Stripping-film autoradiography of connective tissue in experimental skin tumors in mice injected intraperitoneally with S^{35}, with sodium sulfate as carrier, showed that the majority of the mast cells take up sulfur. This uptake manifests itself as a blackening of a stripping film.

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REFERENCES

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