FLUORESCENCE MICROSCOPY OF THE MAST CELLS OF THE CONNECTIVE TISSUE

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I. P. Shlykov

Department of Histology (Head, Corresponding Member AMN SSSR Prof. A. A. Voitkevich)
Voronezhskii Medical Institute
Presented by Active Member AMN SSSR V. V. Parin
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At the present time mast cells are thought to play an important part in the formation of the ground substance of connective tissue. They liberate heparin, histamine, and ribonucleic acid, and appear to transport many enzymes [2, 3, 6, 7]. The mast cells (heparinocytes) have been studied in a number of physiological and pathological conditions, particularly in relation to hormonal changes [1]. The heparinocytes may be demonstrated by various methods which include staining with azure-II-eosin, toluidine blue, aldehyde-fuchsin, Schiff's reagent, etc.

We have studied the heparinocytes in the endocrine organs (intact and hypophysectomized animals), and have used the method of fluorescence microscopy.

EXPERIMENTAL METHOD

The work was carried out on the thyroid glands and the adrenals of young rats weighing from 85-140 g. The material was fixed for 1-3 days in 10% formalin, or for 3-10 h (according to the size of the specimen) in Carnoy. After the gland had been washed in water (if fixed in formalin) it was dehydrated in alcohols of increasing strengths, and embedded in paraffin in the usual way. Sections 5-7μ thick were cleared twice in xylol (3-5 min in each case), and then passed through alcohols of descending strength, and then placed in water. After the sections had been washed for 5 min in distilled water a fluorochrome stain consisting of acridine orange or coriphosphine was applied.

We used the following weak aqueous solutions of the fluorochromes: acridine orange 1:10,000, coriphosphine 1:5,000. The solutions were prepared from distilled water or from physiological saline, and they were kept in dark bottles. The best results were obtained from fresh fluorochrome solutions. The sections were left in the fluorochrome stains for 1-2 min. They were then rapidly rinsed in distilled water, the excess removed with filter paper or gauze, and they were then placed in sugar syrup prepared by the method of Shalumovich [5].

Good results were obtained when the sections were stained after being cut on a freezing microtome; this method greatly reduced the time taken by the histological treatment. The fixed material was frozen with carbon dioxide, and sections cut. The frozen sections were transferred to distilled water, and then into the fluorochrome solution. The fluorochromed sections were washed with distilled water, spread out on microscope slides, and then mounted in physiological saline or sugar syrup. They were then examined under the MUF-3M fluorescence microscope.

EXPERIMENTAL RESULTS

In the thyroid gland of the control and the experimental group of animals the mast cells showed up clearly in the capsule of the organ and in the inter-follicular connective tissue. Quite frequently isolated mast cells or small groups of 2-4 cells were found. They were of an irregular round or oval shape; some also were elongated and flattened by compression between 2 follicles.

The mast cells showed a characteristic fluorescence which distinguished them from the other thyroid cells. The cytoplasm showed up as a bright orange (or reddish) hue. The nuclei of the heparinocytes, which are usually large, gave a marked yellow-green (or pale green) fluorescence. At high magnification with an immersion objective numerous large granules giving an orange fluorescence were found in the cytoplasm of the mast cells (Fig. 1).

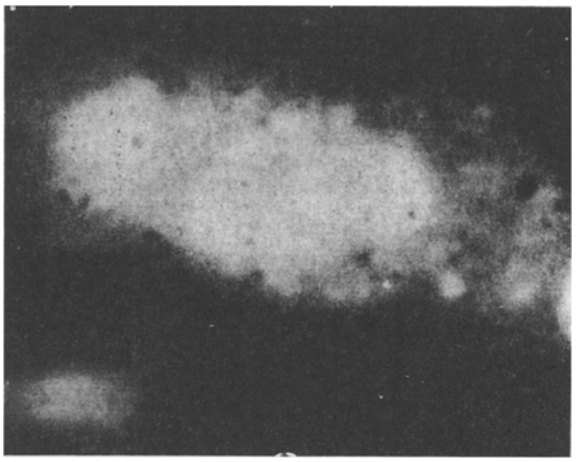

Fig. 1. Two mast cells in the interfollicular connective tissue of the rat thyroid gland. Micrograph. Stain acridine orange. Ocular 10x, objective 90x.

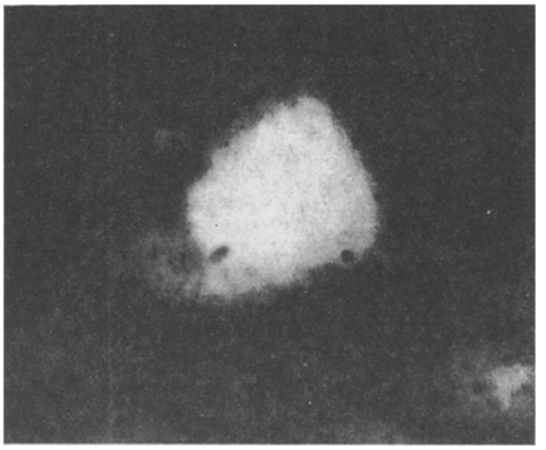

Fig. 2. Mast cell in capsule of rat adrenal. Micrograph. Stain acridine orange. Ocular 10x, objective 90x.

Under the fluorescence microscope the mast cells can be distinguished from other elements of the connective tissue and of the thyroid cells by the brightness and color of the luminescence. The protoplasm of the thyroid epithelium gives a weak yellow-orange fluorescence, while the cytoplasm of the connective tissue cells, especially the fibroblasts (fibrocytes) gives a yellow or weak yellow-orange color, and the protoplasm of the mast cells gives off a bright orange (reddish) light. The nuclei of the heparinocytes fluoresce a more intense yellow-green then do nuclei of the other connective tissue cells.

In the adrenal, just as in the thyroid gland occasional mast cells or small groups of mast cells were gathered around small blood vessels. They lay in the capsule of the adrenal gland (Fig. 2), and in the connective tissue surrounding it. No heparinocytes were found in the parenchyma of the organ itself, or in the cortex, or medulla. When acridine orange was used as a stain a characteristic luminosity of the mast cells could be seen under the microscope; the cytoplasm gave an orange and the nuclei a yellow-green fluorescence. Examination of preparations stained with coriphosphine revealed that the cytoplasma had not an orange but a brick-red luminescence.

The different luminescence of the histological structures is due to their different chemical compositions. In work with pure preparations of nucleic acids and nucleoproteins it has been shown [4] that the green luminescence of nuclei stained with acridine orange is due to nuclear nucleoprotein (DNA); the orange fluorescence of the cytoplasm indicates the presence of ribonucleic acid (RNA). The orange light from the cytoplasm and the green fluorescence of the nuclei of the mast cells are both due to nucleic acids.

Thus, with the help of the luminescence microscope mast cells can be rapidly demonstrated and their nucleic acids revealed. The bright reddish light from the heparinocytes distinguishes them from other cellular elements, and the method may be used in order to make a count.

SUMMARY

Fluorescence microscopy was used to study the mast cells in the thyroid and adrenal glands of white rats. A method for detection of the cells and staining with acridine orange and coriphosphine is described. Orange fluorescence of the cytoplasm of the mast cells was caused by ribonucleoproteins, but the green fluorescence of the nuclei was produced by desoxyribonucleic acid.

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