Cytochemical Studies of Normal and Tumor Mast Cells in Tissue and *in Vitro*  

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Since the first description of spontaneous mast cell tumors (mastocytomas) of dogs (2), subsequent studies have been concerned with the morphology and behavior of the neoplastic mast cells in tissue culture (11). Chemical preparations of these tumors have demonstrated considerable quantities of an anti-coagulating substance that is presumably heparin (10). The present study demonstrates the presence of lipids, cytochrome oxidase, and acid and alkaline phosphatases in normal and tumor mast cells and of tumor mast cells grown in tissue culture for several weeks.

**METHODS**

Normal tissue mast cells were examined in sections of rectum and in whole mounts of mesentery obtained from healthy dogs. The tumor mast cells were obtained from a solitary benign mastocytoma located in the subcutaneous tissue of a dog. Imprints were made of the fresh tumor and stained with Wright-Giemsa. Tumor fragments were planted in 1 drop of chicken blood plasma, 2 drops of dog serum and 1 drop of chick embryo extract for cultivation *in vitro*. The cultures were fixed *in toto* and not removed from the cover glass, thus avoiding disturbance of the delicate new cells radiating into the culture medium.

The lipids were studied in tissues fixed in formol-calcium-cadmium, stained with Sudan IV and Sudan Black B according to the procedure of Baker (1). The Smith-Dietrich test for phospholipids was also used on all tissues. The M-Nadi reagent for "stabile" cytochrome oxidase was applied to fresh and formalin-fixed tissue (7). Alkaline phosphatase was demonstrated by Gomori's method (5) and acid phosphatase by Wolf, Kabat and Newman's modification (12) of Gomori's method (6).

**RESULTS**

Table I summarizes the enzyme and lipid content found in uncultured normal and tumor mast cells, and in tumor mast cells cultivated *in vitro*. The phosphatases were present in the form of cytoplasmic granules; however, it was difficult to ascertain whether or not these corresponded to the mast granules. Some cultured cells contained a paranuclear granule-free area (11). When stained for acid phosphatase this region revealed a delicate black reticulum (Fig. 4) which is suggestive of the Golgi apparatus revealed in other tissue cells (3, 4) when they are stained for phosphatases.

**DISCUSSION**

The observations in Table I reveal that both normal and tumor mast cells contain acid and alkaline phosphatases in their cytoplasm (Figs. 1-4). In tumor mast cells, however, both enzymes were found in the nucleus as well (Figs. 2, 4). In normal mast cells of the rat, Noback and Montagna (9) demonstrated alkaline phosphatase only in the cytoplasm. The cultured tumor cells evidenced phosphatase content identical with the original tumor cells, indicating that the former maintain their phosphatase constituents despite the changes in cell morphology which occur in tissue culture.

Controversy exists concerning the lipid character...
FIG. 1.—Normal mast cells from the interstitial tissue of the anal sacs of dog. Alkaline phosphatase reaction restricted to the cytoplasm. Counterstained with paracarmine. Mag. X 970.

FIG. 2.—Tumor mast cell cultured for 4 weeks in vitro with strong alkaline phosphatase reaction in the nucleus and in the cytoplasm. Mag. X 970.

FIG. 3.—Normal mast cell from the interstitial tissue of the anal sacs of dog demonstrating acid phosphatase reaction confined to the cytoplasm. Counterstained with paracarmine. Mag. X 970.

FIG. 4.—Tumor mast cell cultured for 4 weeks in vitro showing strong acid phosphatase reaction in both the nucleus and cytoplasm. The structure adjacent to the nucleus is probably the Golgi apparatus. Mag. X 970.

of the mast granules of normal mast cells (8). In our material, Sudan black B revealed lipid granules in all categories of mast cells examined (Fig. 5). Sudan IV, however, gave negative results. The distribution of the lipid droplets appeared to coincide both qualitatively and quantitatively with the distribution of the mast granules. Excellent staining of the lipid granules were obtained despite previous immersion of the tissues in alcohol, acetone, chloroform, and other lipid solvents at 60° C. for 48 hours. When mast cells fixed in Baker's formol-calcium-cadmium are stained by the Smith-Dietrich method, vaguely distinguishable black granules can be seen in the cytoplasm. These facts give pre-
FIG. 5.—Normal mast cell (inset) from interstitial tissue of the anal sacs of dog depicting lipid granules. Sudan black. Mag. \( \times 440 \).

The larger cell with tenuous processes is a cultured tumor mast cell with lipid granules. Sudan black. Mag. \( \times 440 \).

FIG. 6.—Imprint preparation of tumor mast cells stained with Wright-Giemsa. Mag. \( \times 970 \). This cell type in vitro resembles morphologically those depicted in Figs. 2, 4, 5, 7 and 8.

FIG. 7.—Living unstained tumor mast cells cultured 6 weeks in vitro. Mag. \( \times 440 \).

FIG. 8.—Tumor mast cells cultured 6 weeks in vitro; with large cytoplasmic granules. Iron hematoxylin. Mag. \( \times 970 \).

Opinions differ concerning the presence or absence of oxidase in the mast cell granules (8). In our material, “stabile” cytochrome oxidase was demonstrated in the mast granules of uncultured normal and tumor cells, and of cultivated cells in vitro.

SUMMARY

Histochmical studies of normal and tumor mast cells, and of tumor mast cells cultivated in vitro
reveal that the mast cells in all these categories contain lipids, cytochrome oxidase, and acid and alkaline phosphatases in their cytoplasm. The tumor cells, in addition, contain alkaline and acid phosphatases in their nuclei.

REFERENCES

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