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Fluctuating levels of 5-hydroxytryptamine and histamine in neoplastic mast cells*

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RANDOM measurements showed that the levels of 5-hydroxytryptamine and histamine in neoplastic murine mast cells vary widely whether the cells are grown in culture or in the mouse.[1,2] These fluctuations were seen in cells derived from both the Dunn–Potter[1,2] and Furth tumors,[3] and were observed in descendants of the original tumor as well as in descendants of cloned, single cells. It seemed of interest to follow the levels of amines for a longer period of time to see if there was any obvious rhythmicity in these fluctuations.

Measurements of 5-hydroxytryptamine[4] and histamine[5] were made on descendants of the Dunn–Potter tumor, grown as ascitic tumors.[1–3] The levels of 5-hydroxytryptamine in these cells varied over the period of measurement (Fig. 1), ranging from 0·5 to 357 $\mu g/10^9$ cells in the X-2 line, from 0·1 to 608 $\mu g/10^9$ cells in X-1-C line, and from 0·2 to 2000 $\mu g/10^9$ cells in X-1-D. There was no clear rhythmicity in these fluctuations. Similarly, no rhythmicity was seen in the fluctuating histamine levels (Fig. 2), which varied in the X-2 line from 0·6 to 68·5 $\mu g/10^9$ cells, in X-1-C from 7·0 to 95 $\mu g/10^9$ cells, and in X-1-D from an undetectable level to 334 $\mu g/10^9$ cells. It is likely that the changing levels are due to fluctuations in the metabolism of the mast cells rather than of the mouse, since similar fluctuations were seen in cells grown in culture.[1]

The changing amine levels are probably reflective of changes in the capacities of the population of cells to take up and decarboxylate the amino acid precursors[1] of the amines, and perhaps also to the changing capacities to take up the preformed amines.[2,6] It is relevant that normal mast cells also showed[7] individual differences in their capacities to take up both the precursor amino acids and the amines.

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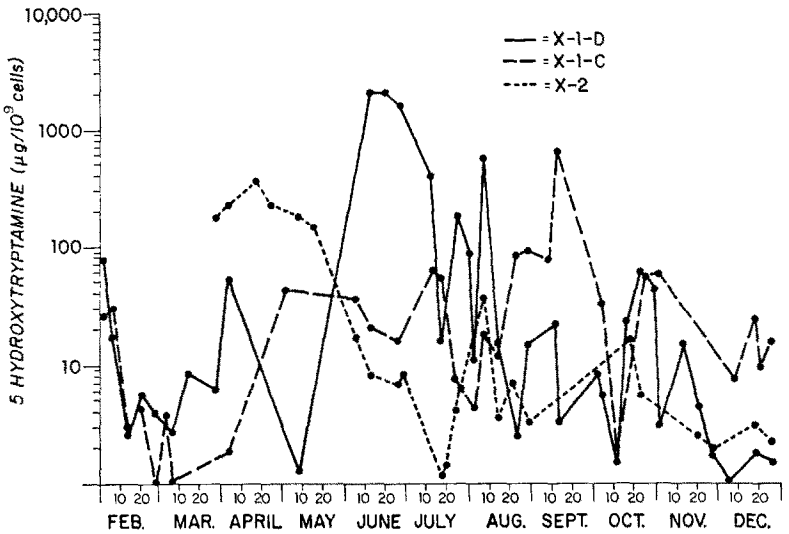

FIG. 1. Fluctuations in the levels of 5-hydroxytryptamine in three lines of neoplastic mast cells.

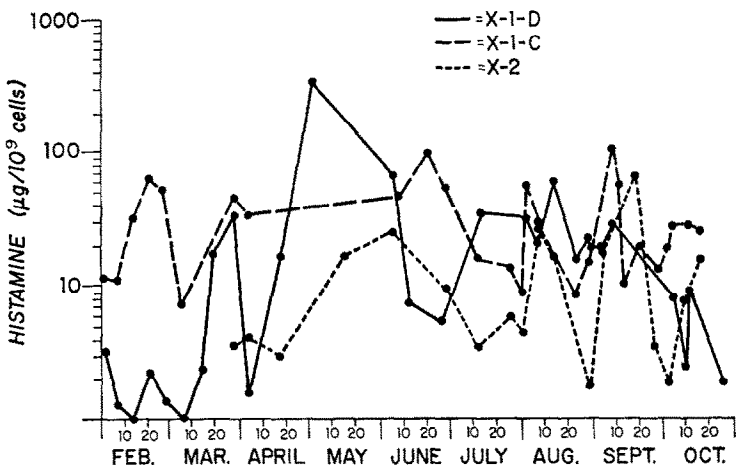

FIG. 2. Fluctuations in the levels of histamine in three lines of neoplastic mast cells.

The changing levels of amines in these cells do not necessarily imply that all cells in the population are synchronously changing in their content of amines. Rather, varying rates of proliferation of cells having varying contents of amines could account for the fluctuations. Supporting this suggestion are observations by fluorescence microscopy which revealed marked variation among individual neoplastic mast cells in their content of 5-hydroxytryptamine.[8,9]

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Histamine release by reserpine from rat peritoneal mast cells *in vitro**

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WHILE reserpine is well known to lower catecholamine and serotonin levels in many tissues,[1,2] its effects on histamine stores are less consistent. *In vivo* either no change or a slight to moderate decrease in the histamine content of various tissues has been reported after the administration of large doses of drugs.[3–6] Moran and Westerholm[7] found reserpine to have no effect on the histamine concentration of rat peritoneal mast cells *in vitro*, while Mannaioni *et al.*[8] more recently reported that reserpine released histamine from neoplastic murine mast cells. In this communication we describe conditions under which reserpine does release histamine from rat peritoneal mast cells *in vitro*.

The methods used for collection and preparation of mast cells are described in detail elsewhere.[9] Peritoneal cells were removed from Sprague–Dawley rats (200–300 g; Charles River Laboratories). When required, mast cells were purified partially by briefly centrifuging and resuspending the cells in fresh medium four to eight times. The incubation medium consisted of: NaCl, 154 mM; KCl, 2·7 mM; $CaCl_2$, 0·9 mM; KH_2PO_4, 2·7 mM; Na_2HPO_4, 4·0 mM; glucose, 0·1%; human serum albumin, 0·1%; the pH was 7·0. After incubation, cells and supernatants were separated and the histamine content of both fractions was measured fluorometrically.[10] The histamine content of unincubated cells was also determined; recoveries were always greater than 95 per cent. Mast cells were stained and counted as described by Bray and Van Arsdel.[11] Reserpine phosphate (Serpasil, lyophilized) was a gift of Dr. A. J. Plummer of Ciba Pharmaceutical Co.

Table 1 illustrates that the release of histamine from mast cells depends on the cellular composition of the incubation. The per cent of histamine released increased either when the number of mast cells in the medium was reduced or when mast cells formed a larger proportion of the total cell population. The lowest concentration of reserpine that released histamine under the present conditions was 1×10^{-5} M. In one experiment (mast cells = 48,000/ml) release after 2 hr was 18 and 27 per cent as compared with 10 and 12 per cent in the presence and absence of drug respectively; in another experiment (mast cells = 95,000/ml) the equivalent values after 4 hr were 55 and 57 per cent as compared with 10 and 15 per cent.

We verified that release was due to reserpine rather than to some contaminant by taking advantage of the relative insolubility of reserpine. Saturated solutions were prepared from two different amounts of drug (different by a factor of 3). The supernatants of these contained the same concentration of reserpine but, presumably, different concentrations of a hypothetical contaminant. Release was identical with both solutions.

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