5-Hydroxytryptamine in Mast Cells.* (22016)

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Two important physiologically potent tissue constituents have been associated with mast cells: heparin by Holmgren and Wilander(1) and histamine by Riley and West(2). In the present communication, we shall provide evidence that 5-hydroxytryptamine is present in the mast cells of at least one species, the rat.

Mast cells were prepared from the peritoneal cavity of rats by a modification of the method of Padawar and Gordon(3). The cells of the final concentrate appeared well preserved; they maintained their characteristic staining with toluidine blue. There was evidence of some shedding of peripheral granules. Total and differential counts were performed using the solution designed by Moore and James(4) for blood basophiles. Mast cells constituted from 87 to 92%, usually 90 to 92%, of the total population in the final preparations. The remaining cells were mesothelial, mononuclear cells and a few polymorphonuclear leukocytes. Estimates of the volume of cells were made using a red blood cell diluting pipette of about 1 ml capacity centrifuged at 1000 x g for 1.5 hours to constant volume. From 10 rats, 5 to 10 mm^3 of cells were obtained. The mean volume per cell was estimated in 4 determinations to be $1.26 \pm 0.13 \times 10^{-6}$ mm^3.

5-hydroxytryptamine was identified and estimated using the rat colon as described by Dalgleish, Toh, and Work(5). The volume of the bath was 16 ml. For extraction of 5-hydroxytryptamine from the cells the final concentrate in sucrose, containing 1 to 1.4 mm^3 of cells per ml, was diluted 1:20 with distilled water and frozen and thawed 3 times. Although this does not grossly fragment the cells, it does release their histamine and 5-hydroxytryptamine.

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Identification of 5-hydroxytryptamine in the frozen and thawed preparation was made as follows: 1) The material contracted the rat colon in a manner identical with synthetic 5-hydroxytryptamine(6). The capacity of the material in the extract to contract the colon strip was abolished by repeated application of relatively large amounts, 1 to 2 μg of 5-hydroxytryptamine to the strip, as with 5-hydroxytryptamine itself(6). Lysergic acid diethylamide, 10 μg in the 16 ml bath for 10 minutes, abolished the reaction to both extracted material and synthetic serotonin. Dibenamine hydrochloride, 100 μg in the bath, likewise abolished the effect of mast cell extract and reference standard(7). Final identification of the material was made by extraction and chromatography: 6.4 mm^3 of a preparation containing 92% mast cells was extracted 3 times with 95% acetone(6). The acetone was evaporated partly to dryness *in vacuo* at 32°C, applied to Whatman No. 1 filter paper and chromatogrammed using butanol-acetic acid-water (4:1:5)(5). The Rf value for the unknown was 0.44 and of synthetic standard was 0.46. Formaldehyde-bichromate spray and ultraviolet illumination were used for identification(8). The characteristic golden yellow fluorescence was observed.

The concentration of 5-hydroxytryptamine (as free base) was estimated by colon-assay to be 0.63 $\mu g/mm^3$ of cells. From the chromatogram it was estimated to be about 0.7 $\mu g/mm^3$. Evidence that the material is not present in the residual contaminating cells in the mast cell concentrate is as follows: In concentrating the exudate, the 5-hydroxytryptamine activity increases with increasing purity of mast cells. The skin and subcutaneous tissue of the rat contain material with identical activity on the rat colon strip and chromatographic mobility with the butanol-acetic acid-water solvent. The activity of this material

parallels the mast cell content in a manner similar to that previously shown for histamine(9).

The concentration of 5-hydroxytryptamine in the mast cells of the subcutaneous areolar tissue of the rat was computed from estimates of 5-hydroxytryptamine on homogenates of areolar tissue and measurements by the method of Chalkley(10) of the relative mast cell volume. By this means, a value of 0.72 μg 5-hydroxytryptamine per mm^3 of subcutaneous mast cells was obtained.

The evidence given demonstrates the presence of 5-hydroxytryptamine in the mast cells of the rat. We have found that this material has physiological activity when released by those agents which produce mast cell damage(11).

The presence and quantity of histamine and heparin in the isolated concentrated mast cells has been demonstrated using similar technics (12). The amount of histamine was estimated to be 10 μg/mm^3 and of heparin 30 μg/mm^3.

Definitive evidence of the presence of 5-hydroxytryptamine in mast cells along with histamine and heparin is important for several reasons: it emphasizes the significance of these cells as participants in the reaction to injury; it provides further evidence for a relationship between mast cells and blood platelets(13); finally, a connection is established between mast cells and the cells of the enterochromaffin system and with certain, as yet unidentified cells in the central nervous system since both of these, as well as the blood platelets, are now known to contain 5-hydroxytryptamine(14,6,15).

Summary. 5-Hydroxytryptamine as well as histamine and heparin was demonstrated to be present in purified mast cells from the peritoneal washings of rats. The material was identified by bioassay and chromatography. The concentration of 5-hydroxytryptamine found in isolated mast cells agreed with that found by indirect means in the mast cells of the subcutaneous areolar tissue of rats. Implications of this finding are briefly discussed.

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