# STUDIES ON MECHANISM OF HISTAMINE BINDING IN MAMMALIAN TISSUES

*By*

M.K. BAGCHI, K.C. GUPTA AND G.S. SINGH

*From the Department of Pharmacology, Maulana Azad Medical College, New Delhi*

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The properties of naturally occuring mast cell grannules obtained from the rat and the mouse have been found to resemble biologically and chromatographically, those of histamine heparin complex prepared by suitable mixture of both and subsequent precipitation. Trace amounts of 5-HT in addition to histamine and heparin have been detected in mast cell suspension from both species studied.

Sanyal and West (1956, 1959) described the formation of a complex of histamine and heparin *in vitro* and demonstrated the similarity of properties of the complex with naturally occuring mast cell grannules. The present studies were undertaken in order to ascertain if the natural mast cell grannules in rats and mice also exist in the form of a complex or otherwise.

## METHODS

*Collection of mast cell grannules.*—Healthy albino rats and mice of either sex were employed for collection of peritoneal mast cells. The animals were anaesthetised with ether inhalations, and injected intraperitoneally with 3-5ml of normal saline depending on size. The abdominal wall was massaged with glass rods with uniform firm pressure. The fluid from the abdomen was then collected and used for experiments. It is known that there is a considerable number of mast cells in the mesentery of these animals and it is expected massaging would liberate some of them (Padawer and Gordon, 1955).

*Histamine heparin complex.*—Histamine heparin complex was prepared by mixing solutions of 20 mg of heparin with 1 mg of histamine acid phosphate and then precipitated with alcohol to give a final concentration of 70 percent (Sanyal and West, 1956).

*Chromatographic procedures.*—Standard procedures were adopted for chromatography. The chief solvent systems used were butanol-acetic acid or 70 percent ethanol adjusted to pH 6.0. Whatman No. 1 paper was used for all studies. Histamine was developed by Pauly's reagent; Ehrlich's reagent was

used for developing 5-hydroxytryptamine (5-HT) and heparin was developed by toluidine blue. Presence of adrenaline was tested by potassium ferricyanide.

*Biological procedures.*—Tests for histamine were made on atropinized guinea-pig ileum and 5-HT was assayed on atropinized oestrous rat uterus.

*Staining reactions.*—The naturally occuring mast cells collected as above or smears of histamine heparin complex were stained with toluidine blue or by Pauly's reagent, and compared with subcutaneous or mesenteric mast cell spreads from rats or mice. Pauly's reagent failed to stain and it's use was later on abandoned.

## RESULTS

### A. Experiments with histamine-heparin complex.

(i) *Chromatographic procedures.*—The formation of a histamine-heparin complex in mixture of the two substances was confirmed. The mixture as such (prior to addition of alcohol) was spotted and run in butanol - acetic acid solvent, as well in 70 percent ethanol adjusted to pH 6.0. In both solvents heparin was at Rf=0.0, but histamine spot had travelled up (Rf=0.2 for the former and Rf=0 6 for the latter solvent). The mixture yielded one spot corresponding to the heparin and a faint one corresponding to free histamine, thus proving quite a bit of histamine is held by heparin.

In another experiment histamine-heparin complex was precipitated and then redissolved in saline before running chromatograms as above. Again most of the histamine was seen to be at Rf=0 with heparin, and a faint spot corresponding to free histamine also being obtained.

Chromatograms were run of mixtures of heparin with either adrenaline, 5-HT ,5-HTP, but heparin was not seen to form any complex with any one of these substances.

(ii) *Staining procedures.*—A smear of the histamine-heparin complex on staining showed metachromasia resembling naturally occuring mast cell granules.

### B Experiments with peritoneal mast cells

(i) *Chromatographic procedures.*—The peritoneal mast cells were treated with small amount of distilled water to rupture them and liberate the granules (Archer, 1961). Spots were prepared on filter paper and developed. Granules obtained from the rat showed on development colour reactions of histamine and heparin. When spots were run in butanol acetic acid solvent, in three

experiments, traces of histamine could not be detected on developing. However, in two experiments, heparin as well as histamine were seen at Rf=0.0. No histamine spot of the peritoneal fluid corresponding to free histamine (Rf=0.2) could be seen. This would suggest that histamine in the natural state in the rat is in combination with heparin. Neitner in spots, nor in running chromatograms the presence of 5-HT or adrenaline could be detected.

(ii) *Biological procedures.*—The peritoneal fluid suspensions prapared as above obtained from both rats and mice caused a contraction of the atropinized guinea-pig ileum, the contractions matching with histamine on doubling, and being blocked by mepyramine ($10^{-7}$g/ml). The peritoneal fluid did not show presence of 5-HT (as tested in oestrous rat uterus) in four experiments with rats, and one with mouse, threshold sensitivity being 0.04—0.06 $\mu$g/ml. With more sensitive test preparations (sensitivity 0.01—0.02 $\mu$g / 1 ml), contractions were obtained in two experiments in rats and three in mice mast cell suspensions. In each case, the reaction could be blocked by 2-bromolysergic acid diethylamide ($10^{-6}$g/ml).

(iii) *Staining procedures.*—The smear of peritoneal mast cell suspensions morphologically resembled those in tissue spreads, but were mixed with leucocytes.

### C. Other Studies

Histamine is usually extracted from tissues with 10 per cent trichloracetic acid (Parratt & West, 1957). In order to see if similarly extracted histamine was in free form or in the nature of a complex, spots of the extract were run in butanol-acetic acid solvent. Histamine spots were at Rf=0.2, corresponding to free histamine and none was seen at Rf=0. As such it seemed probable that the histamine extracted was in free form.

## DISCUSSION

The experimental results indicate that histamine in the peritoneal mast cells of either rats or mice is likely to be in a complex formation with heparin. The chromatographic and staining properties of the natural mast cell granules obtained from peritoneal fluids and the synthetic granules prepared by mixture of histamine and heparin *in vitro* appear to be similar. However trichloracetic acid treatment ruptures the bondage and extracts histamine in the free state, it being assumed that most of the histamine comes from mast cells. Such an assumption is however based on demonstrated facts (Riley and West, 1955). A controversy has usually raged about the presence of 5-HT in mast cells. Benditt and his co-workers (1955) associated 5-HT with mast cells of rats,

but Parratt and West (1957) produced a number of evidences to show lack of association of 5-HT and mast cells in many species including the rat though it was conceded that trace amounts of 5-HT may be present in mast cells of the rat and the mice.

Amino acid decarboxylase enzymes have been detected in mast cells (Benditt, 1958; Hagen and Lee, 1958) and 5-HT has been demonstrated in mast cell tumors of the mouse (Sjoerdesma, Waalkes and Weissbach, 1958). The present demonstration of trace amounts of 5-HT in mast cell suspensions from both rats and mice in physiological conditions would indicate that at least mast cells in the peritoneal cavity may contain 5-HT in addition to histamine and heparin in these two species.

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