^{22}Na was removed by washing at 0° C in buffered salt solution. The ^{22}Na present in the cells after washing was determined by liquid scintillation spectrometry. In a study of the ^{22}Na uptake during very short incubation periods, the incubations were stopped by adding 9 ml. of ice-cold salt solution to the 1 ml. incubation mixtures and cooling to 0° C.

The results of time course studies show that at 37° C ^{22}Na uptake and histamine release both begin after a latent period of about 5 sec but, whereas histamine release is complete after approximately 30 sec, ^{22}Na uptake is not maximal until after 10 min. Assuming that ^{22}Na and ^{23}Na are taken up similarly, we have calculated that the quantity of ^{23}Na taken up during the period of histamine release was quite insufficient to replace the amount of histamine released if an ion-exchange mechanism is involved. We conclude from these observations that intracellular exchange of sodium for histamine does not play a significant part in the release of histamine induced by compound 48/80.

When the incubations were carried out at 0° C or 46° C, ^{22}Na uptake by mast cells treated with compound 48/80 was not significantly greater than that in untreated cells ; after incubation at 37° C the treated cells contained more than five times the amount in control cells. Uvnäs & Thon (1961) reported that histamine release induced by compound 48/80 was optimal at 37° C and did not take place at 0° C or above 44° C.

The large increase in ^{22}Na uptake by mast cells following exposure to compound 48/80 can be prevented by pre-treating the cells with N-ethylmaleimide or ninhydrin, which also inhibit the response of mast cells to this releaser. We found some evidence for the existence of a sodium pump mechanism which could be inhibited by ouabain, ninhydrin or N-ethylmaleimide.

It is concluded that the increased sodium uptake by mast cells exposed to compound 48/80 is secondary to the degranulation process. Whether or not this sodium uptake is involved in the restoration of the mast cell discharge mechanism is not yet known.

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Site of ionic binding of sodium and histamine in mast cell granules

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Previous studies in our laboratory have demonstrated the cation exchange properties of isolated mast cell granules. When suspended on a sodium-containing medium, the histamine in the granules is exchanged for sodium and vice versa. The pH dependence of the binding mechanism in the granules indicates a binding of the cations to weak acid groups—for example, carboxyl groups.

The granules of rat peritoneal mast cells are composed mainly of a water-insoluble protein–heparin complex. This complex dissolves in salt solutions—in M KCl, for example—and can then be electrophoretically or chromatographically separated into its heparin and protein components. The protein part consists of a basic polypeptide with a molecular weight around 4,500.

The aim of the present investigation was to localize the cation binding carboxyl groups in the granules. Did they belong to the heparin or to the protein part of the granule complex or to both?

Histamine-containing granules were isolated by differential centrifugation of water-lysed rat peritoneal mast cells. The granules were depleted of their histamine by suspension in 10 mM NaCl. Sodium was then removed by washing the granules three times in slightly acid deionized water.

The capacity of the granules to bind sodium and histamine was determined by re-suspending them in sodium- and histamine-containing media with an admixture of ^{22}Na and ^{14}C-histamine respectively. The maximal uptake of the two cations reached equi-molar levels corresponding to about 650 mμ-equiv/mg granules (dry weight). The curves showing the binding capacity of sodium and histamine at different pHs suggested that the ionic binding sites were carboxyl groups.

Titration with HCl of the granule complex dissolved in M-KCl indicates (after correction for the carboxyl groups present in an amount of heparin corresponding to 30% of the granule complex) the presence of 600–700 carboxyl groups in the protein part of the heparin-protein complex. The carboxyl groups of the protein therefore seem adequate to account for the ionic binding capacity of the granules.

In previous model studies we have investigated the sodium and amine binding capacity of a protamine–heparin complex. Qualitatively this complex behaved exactly as mast cell granules in its sodium and histamine binding properties. Quantitative uptake and titration studies led us to propose a gross structure of the complex with the terminal COO^- groups of the protamine as cation binding sites. Tentatively, we would like to propose a similar arrangement of the protein–heparin complex of the granules, with the cationic binding sites localized to the terminal ends of the polypeptide chains. It is true that the polypeptides contain dicarboxylic acids (glutamic and aspartic acids), but considering the high isoelectric point of the protein (9–10) the non-terminal carboxyl groups might very well be amidated and thus not available as ionic binding sites.

The present observations on the ionic binding of histamine in the granules confirm our previous reports that histamine is stored in the granules in an ionic linkage easily broken on exposure of the granules to cations. According to our proposal the histamine release from mast cells—for example, that due to compound 48/80—is a two-step procedure, the first being the degranulation with an active extrusion of histamine-containing granules, the second an extracellular cationic exchange between histamine in the granules and sodium in the extracellular fluid.

Influence of anti-rheumatic agents on histamine release from actively and passively sensitized rat peritoneal mast cells

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When peritoneal mast cells from sensitized rats are incubated *in vitro* with a specific antigen, histamine is liberated (Norn, 1965). In the present study it was investigated whether pre-treatment of the rats with various anti-rheumatic agents as hydrocortisone, sodium aurothiosulphate or phenylbutazone would inhibit the histamine release when given (1) at the beginning or (2) previous to the sensitization period.