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

S. NORN, *Department of Pharmacology, University of Copenhagen, Denmark*

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.

(1) Female albino rats actively sensitized to horse serum (donors) (Norn, 1965) were treated during the first week of the sensitization period with a daily subcutaneous injection of one of the substances listed in Table 1. The rats were killed by bleeding from the carotid arteries 3 weeks after the first treatment with horse serum. From each rat a suspension of peritoneal cells was incubated *in vitro* with horse serum and the quantity of histamine liberated was determined as a percentage of the total content (Norn, 1967). Serum (2 ml.) from each rat diluted with 6 ml. of modified Tyrode solution (Norn, 1965) was injected in two intraperitoneal doses of 4 ml., 2 hr apart, into each non-sensitized rat (recipient). The histamine release in peritoneal cell suspensions from these rats was determined in a similar way 48 hr after the passive sensitization.

TABLE 1. *Influence of antirheumatic agents on histamine release from actively and passively sensitized rat peritoneal mast cells*

Pre-treatment of donors for:	% Histamine release by Donors		Recipients	
	Control	Test	Control	Test
1 *week; daily dose*				
Hydrocortisone 43 mg/kg	57*	34*	50†	18†
21 mg/kg	52	45	60†	25†
Sodium aurothiosulphate 21 mg/kg	52	44	60	56
Phenylbutazone 86 mg/kg	57	49	50	40
3 *weeks; daily dose*				
Hydrocortisone 12 mg/kg	61	58		
Sodium aurothiosulphate 17 mg/kg	69	69		
Phenylbutazone 67 mg/kg	61	69		

Each group comprised eight–twelve rats. * $P<0.05$ by t test. † $P<0.01$.

(2) Corresponding rats (donors) were treated during 3 weeks before the active sensitization with a daily subcutaneous injection of an anti-rheumatic agent (see Table 1). The corresponding histamine release from these rats is given in the Table.

Pre-treatment of rats with hydrocortisone given at the beginning of the sensitization period inhibited the histamine release in peritoneal cell suspensions from these rats as well as from rats passively sensitized with their serum. This indicates an inhibition of the antibody production. Inhibition was not obtained with sodium aurothiosulphate or phenylbutazone. In contrast, none of the three antirheumatic substances changed the histamine release from actively sensitized rats when given before the sensitization period.

REFERENCES

NORN, S. (1965). Influence of antirheumatic agents on the release of histamine from rat peritoneal mast cells after an antigen–antibody reaction. *Acta pharmac. tox.*, **22**, 369–378.

NORN, S. (1967). Release of histamine from sensitized rat peritoneal cells by specific and unspecific antigens. *Acta pharmac. tox.*, **25**, 456–460.

Vascular and metabolic effects of histamine and compound 48/80 in subcutaneous adipose tissue

B. B. FREDHOLM*, H. C. MENG, B. ÖBERG and S. ROSELL, *Department of Pharmacology, Karolinska Institute, Stockholm, Sweden*

Adipose tissue has been found to contain histamine and 5-hydroxytryptamine (5-HT). The amount of these amines in adipose tissue decreased after treatment with compound 48/80, a potent mast cell degranulating agent. It was concluded that histamine and 5-HT were probably stored in mast cells (Bieck, Stock & Westermann, 1967).