The present work was undertaken to determine the molar ratios of the two hormones and of the two proteins in fresh bovine pituitary posterior lobe tissue in order to establish the number of hormone-binding sites that are occupied *in vivo*. Glands from six animals were homogenized individually in 0.1 N-HCl (Dean, Hollenberg & Hope, 1967). The extracts were assayed for oxytocic and pressor activities, dialysed against gel buffer (Ferguson & Wallace, 1961) and concentrated to a volume of 0.5–1.5 ml. *in vacuo*; aliquots of 10 and 20 μl. were placed in starch gels for electrophoresis as described by Dean, Hollenberg & Hope (1967). The gels were stained in nigrosine (0.05%) and the amount of neurophysin in each band was determined by transmission densitometry. Each gel contained standards of 25, 50 and 75 μg of either neurophysin-I or neurophysin-II together with three unknowns. Hormone and protein estimations were carried out eight times for each gland.

The glands contained 561 ± 36 i.u. of oxytocic activity, 454 ± 11 i.u. of pressor activity, 11.08 ± 0.31 mg of neurophysin-I and 8.94 ± 0.11 mg of neurophysin-II per gram of fresh tissue. On the basis of the results described by Dean, Hope & Kažić (1968) and taking the molecular weights of neurophysin-I and II as 20,075 and 19,757 respectively, the oxytocic activity of oxytocin as 546 i.u./mg and the pressor activity of arginine vasopressin as 430 i.u./mg., the number of molecules of oxytocin bound per molecule of neurophysin-I was 2 (2.08) and of vasopressin per molecule of neurophysin-II was 2 (2.26). Thus each molecule of neurophysin-II is associated with the same number of molecules of vasopressin in the gland as was found in the crystalline complex. Although neurophysin-I is capable of forming solid complexes containing three molecules of oxytocin per molecule of protein, this neurophysin in the gland is associated with only two molecules of oxytocin. The significance of the third binding site present in each neurophysin remains to be established.

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Dissociation of histamine release and ^{22}Na uptake in rat mast cells exposed to compound 48/80 *in vitro*

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The relationship between ^{22}Na uptake by, and histamine release from, rat mast cells exposed to compound 48/80 *in vitro* has been investigated in order to elucidate the mode of action of this releaser.

Isolated mast cells were incubated in a buffered salt solution (pH 7) containing ^{22}Na (~5 μc/ml.) at 37° C. After incubation, the cells were centrifuged and the extracellular

^{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.