arginine vasopressin (Hollenberg & Hope, 1968). The presence of two polypeptide hormones and two hormone-binding proteins led us to suggest that one of the proteins is normally associated with oxytocin and the other with vasopressin.

Differences in the distribution of oxytocin and vasopressin have been observed after density gradient centrifugation of bovine neurosecretory granules (La Bella, Reiffenstein & Beaulieu, 1963; Dean & Hope, 1968). We have now studied the distribution of the two neurophysins in density gradients under similar conditions. A granular fraction (II and III) prepared from homogenates of bovine pituitary posterior lobes as described by Dean & Hope (1968) was resuspended in 0.3 M-sucrose (6 ml.) and layered over a density gradient, linear between 1.35 M and 1.55 M-sucrose. The gradients were centrifuged at 138,000 *g*, for 5 hr, and cut into four subfractions, A (clear supernatant) B and C (bands of particulate material) and D (pellet). Extracts of the subfractions in 0.1 N-HCl were assayed for oxytocic and pressor activities, dialysed against gel buffer and centrifuged at 38,000 *g* for 1 hr. The clear supernatants were concentrated *in vacuo* and the final volumes adjusted to 0.5 ml. with water. Aliquots of between 20 and 100 μl. were placed in starch gels for electrophoresis as described by Dean, Hollenberg & Hope (1967). The protein bands were stained in 0.05% nigrosine and the amount of dye taken up was measured by transmission densitometry in a Vitatron densitometer. Neurophysin-II was used as a standard; 25, 50 and 75 μg were run simultaneously in each gel.

The results, set out in Fig. 1 as a histogram, indicate that neurophysin-I and oxytocin are stored together in neurosecretory granules which are different from those in which neurophysin-II and arginine vasopressin are stored.

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The total hormone-binding capacity of the neurophysins and the oxytocin and vasopressin content of the posterior pituitary

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The bovine pituitary posterior lobe contains two principal polypeptide hormones, oxytocin and vasopressin, and in addition two major hormone-binding proteins, neurophysin-I and II (Dean & Hope, 1968). Dean, Hope & Kažić (1968) have shown that oxytocin is stored together with neurophysin-I in one population of neurosecretory granules and vasopressin is stored in a second population of granules containing neurophysin-II. Evidence has been presented which shows that *in vitro* both neurophysins possess three hormone-binding sites per molecule of protein (Hollenberg & Hope, 1968). Neurophysin-I forms a non-crystalline complex with oxytocin containing three molecules of hormone per molecule of protein and neurophysin-II forms a crystalline complex containing two molecules of vasopressin per molecule of protein.

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