that they, as a group, showed yet a third distinctive pattern of sensitivity to these agents. At the same time it was observed that the reductases from trypanosomes of African origin (*T. brucei*, *T. rhodesiense*, *T. equiperdum*, *T. congolense*, and *T. vivax*) had closely similar drug sensitivity profiles, clearly distinguishable in certain respects from those of the reductases from two species of non-African origin (*T. lewisi* and *T. cruzi*). Thus, for example, the ID50 of 2,4-diamino-5-(3',4',5'-trimethoxybenzyl) pyrimidine (trimethoprin) for the reductases of the seven species was: *T. brucei*, 5×10^{-7} M, *T. rhodesiense*, 2.5×10^{-7} M; *T. equiperdum*, 1×10^{-6} M; *T. congolense*, 5×10^{-7} M; *T. vivax*, 7×10^{-7} M; *T. cruzi*, 1×10^{-5} M and *T. lewisi*, 1.5×10^{-5} M.

No significant differences have yet been found in the properties of dihydrofolate reductases from bloodstream and culture forms of either *T. cruzi* or *T. rhodesiense*.

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Evidence for the storage of oxytocin with neurophysin-I and of vasopressin with neurophysin-II in separate neurosecretory granules

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Two of the soluble, high molecular weight constituents of neurosecretory granules isolated from bovine pituitary posterior lobes (Dean & Hope, 1967) are known as neurophysin-I and neurophysin-II: each of these proteins can bind oxytocin and

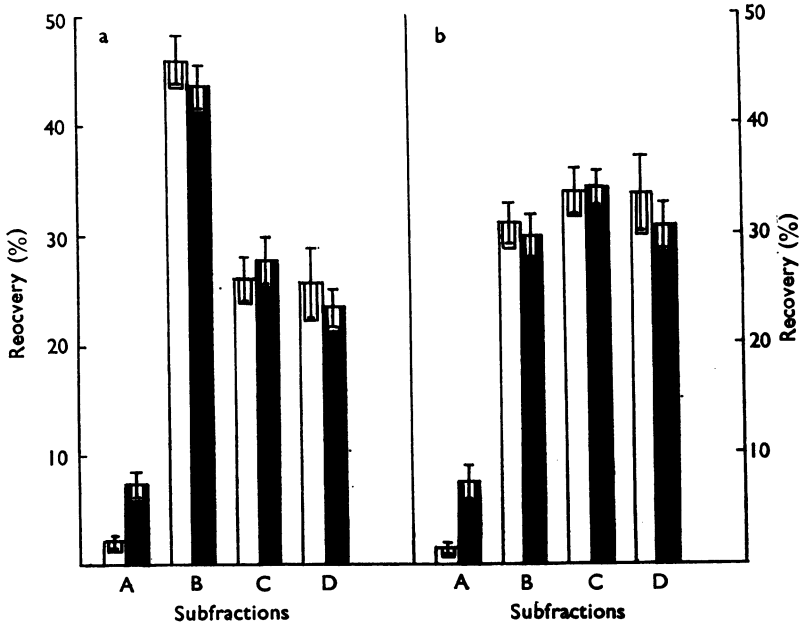

FIG. 1. Distribution of oxytocic and pressor activities and of neurophysin-I and -II in subfractions of neurosecretory granules taken from a density gradient. Column height represents the percentage of material recovered (mean±S.E.M., $n=7$). (a) □, Oxytocin; ■, neurophysin-I; (b) □, vasopressin; ■, neurophysin-II.

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.

This work was supported by a research grant from the Medical Research Council.

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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.