immunoreactive components, one peak eluting close to the exclusion volume and another peak coeluting with (125 I) sCT.

The calcium level in the hemolymph of *Palaemon serratus* showed a maximum in the premolt stage and a minimum at the post-molt stage B (fig. 3). The calcitonin-like material in hemolymph, on the other hand, attained a maximum during stage B of the molt cycle and a minimum at stage C.

No variations of the calcium concentration in hemolymph at different times (0, 15, 30, 60 min) were observed after administration of synthetic salmon calcitonin (100 ng/animal) at the stage D1 or D2.

Discussion. The results demonstrate the presence in the hemolymph of *Palaemon serratus* of immunoreactive salmon calcitonin-like molecules and confirm preliminary results obtained by immunocytochemistry[13]. On gel filtration analysis of hemolymph, two major immunoreactive peaks were detected, one with an apparent mol.wt similar to salmon calcitonin, and a larger mol.wt component. In human plasma, several immunoreactive components with mol.wt larger than those of human calcitonin (1–32) have been recognized[14]. The large immunoreactive form in hemolymph may represent aggregates or the small forms could be breakdown products of the larger form.

Variations of the concentration of the calcitonin-like molecules in hemolymph during the molt cycle were of particular interest. Our findings suggest that calcitonin-like peptides play a role in invertebrates in relation to the molt cycle. The fall in hemolymph calcium levels may be the consequence of the molecules. However, we have been unable to reduce hemolymph calcium levels by the exogenous administration of synthetic salmon calcitonin. In another invertebrate, *Ciona intestinalis,* human calcitonin-like molecules are present[6], and therefore the ancestral calcitonin gene is considered to be of the human type. Duplication of this ancestral gene[15] may give rise to the salmon, eel or chicken calcitonins[16–18] which seem to have appeared later in evolution. Our findings in *Palaemon serratus* of salmon calcitonin-like molecules suggests that in addition to a human calcitonin-like gene, a second salmon calcitonin-like gene may exist in invertebrates. We therefore propose that duplication of an ancestral calcitonin gene gave rise to both the human and teleostean genes at an early stage of evolution.

In conclusion, *Palaemon serratus* is an important animal model for the study of possible roles of calcitonin-like molecules present in invertebrates.

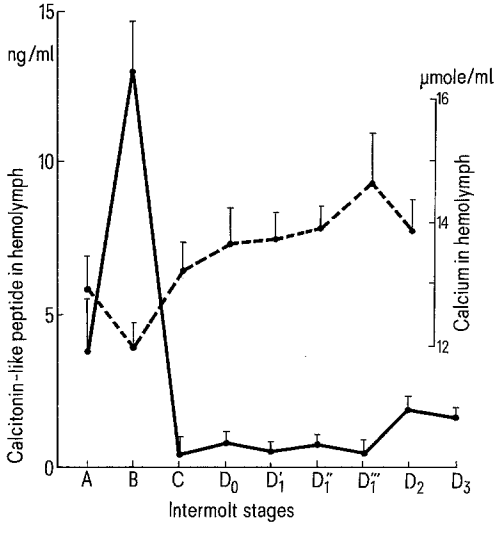

Figure 3. Levels of immunoreactive calcitonin (—) and calcium (– – – –) in the hemolymph during the intermolt cycle of *Palaemon serratus*. Measurement in triplicate of pooled (at least 10 shrimps) hemolymph samples.

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Vasoactive intestinal polypeptide stimulates prolactin release in vivo in the ring dove *(Streptopelia risoria)*

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Summary. I.v. administration of vasoactive intestinal polypeptide (VIP) to ring doves significantly elevated the plasma concentration of prolactin after 10 min in a dose-related manner. The plasma prolactin concentration of nonbreeding doves with low basal levels was increased by a similar amount as in brooding doves whose initially high concentration of plasma prolactin had been reduced by nest deprivation prior to treatment.

Key words. Prolactin release, stimulation of; vasoactive intestinal polypeptide; ring doves; nest deprivation.

Vasoactive intestinal polypeptyide (VIP), originally isolated from porcine duodenum[1], is also present in the median eminence of Man[2] and in the hypothalamus[3] and hypophysial portal blood of rats[4,5]. Because VIP stimulates prolactin release in Man[6,7] and rats[8] in vivo and from rat pituitary cells in vitro[9] it is considered a putative mammalian prolactin releasing factor (PRF). Under normal physiological conditions it does not affect the plasma concentration of other anterior pituitary hormones[10].

A serotonergic mechanism can induce prolatin release in rats[11] and also stimulates the release of VIP[12,13]. It has, therefore, been suggested that in rats serotonergic induced prolactin stimulation acts through the release of VIP. In support of this, the prolactin response to serotonin was blocked by anti-VIP serum[14].

In birds the control of prolactin secretion primarily involves a stimulatory serotonergic mechanism[15]. However, serotonin does not stimulate prolactin release from the chicken pituitary in vitro if it is incubated without the presence of the hypothalamus[16] and must thus affect its action through an intermediate. Recent research has shown that VIP can directly stimulate the release of pituitary prolatin in the turkey[17] and chicken[18,19]. Furthermore, a VIP-like substance has been identified immunohistochemically in the brain of the chicken[18].

Unlike other avian species so far studied, the concentration of plasma prolactin in the ring dove *(Streptopelia risoria)* is not high at the onset of incubation but increases later to reach a peak at hatch[20]. This is possibly because in columbiformes, prolactin specifically stimulates the crop sac to produce 'crop-milk' which is fed to the squabs[21].

The purpose of this study was to investigate the ability of VIP to stimulate prolactin release in vivo in nonbreeding and brooding ring doves.

Materials and methods. Adult ring doves, aged between 18 and 24 months, were selected from a closed colony that has been housed at the Department of Psychology, Edinburgh, since 1962. The birds were given food (wheat, 50%; maize, 25%; millet, 25%) grit and water ad libitum and kept on a 14 h of light, 10 h of darkness photoperiod. In both experiments the b.wt of a bird was assumed to be 150 g.

Experiment 1. On the day prior to treatment 22 nonbreeding doves of both sexes were moved to individual white wooden cages (30 × 41 × 47 cm). The birds were divided randomly into four groups and given a single i.v. injection of either 30 µg/kg b.wt, 75 µg/kg, or 100 µg/kg of VIP (Cambridge Research Biochemicals Ltd, Cambridge) in 0.1 ml saline or of saline alone. Blood samples (approximately 0.5 ml) were taken just prior to injection and 10 and 40 min after injection. The samples were centrifuged and the plasma was stored at −20 °C.

Experiment 2. 12 breeding pairs of ring doves were housed in white wooden cages (60 × 41 × 47 cm). Each cage was equipped with a nest bowl and straw. After each pair had nested and the eggs had hatched, the nest and newly hatched squabs (between 1 and 3 days old) were removed. 60 min after the nest was removed the doves were given a single i.v. injection of either 75 µg/kg b.wt of VIP in 0.1 ml saline or of 0.1 ml saline alone. Blood samples (approximately 0.5 ml) were taken prior to nest removal, 1 h later immediately before injection and then 10 and 40 min after injection. The samples were treated in a similar way to those in experiment 1.

Plasma prolactin was measured by a heterologous radioimmunoassay[22]. This used an antiserum which is raised against human prolactin (AGP4; kindly supplied by A. S. McNeilly) and which binds iodinated ovine prolactin (NIAMDD-OPRL-15). A micro-modification of the assay procedure reported by Ebling, Goldsmith and Follett[23] was used. Each sample was assayed in duplicate at a volume of 40 µl. To avoid interassay variation all plasma samples were assayed in a single assay. Paired and unpaired Student's t-tests were used where appropriate.

Results and discussion. Experiment 1. The results for experiment 1 are shown in figure 1. Plasma prolactin concentration increased significantly 10 min after injection in nonbreeding doves given VIP compared to plasma levels in nonbreeding doves injected with saline (30 µg/kg b.wt, $p < 0.05$; 75 µg/kg b.wt, $p < 0.01$; 100 µg/kg b.wt, $p < 0.025$). At this time the levels of plasma prolactin were not significantly different between birds injected with 75 µg/kg and 100 µg/kg b.wt of VIP, however birds injected with these high concentrations of VIP had significantly greater levels of prolactin than the birds injected with 30 µg/kg b.wt ($p < 0.05$). 40 min after injection with 30 µg/kg and 75 µg/kg b.wt of VIP, prolactin levels were not significantly different from those in saline treated birds, however the prolactin levels in birds treated with 100 µg/kg b.wt VIP remained significantly higher ($p < 0.005$).

The dosage per kg b.wt of VIP used and the magnitude and time of response obtained is in good agreement with that observed in the bantam hen[18]. In this earlier study,[18] doses of VIP below that

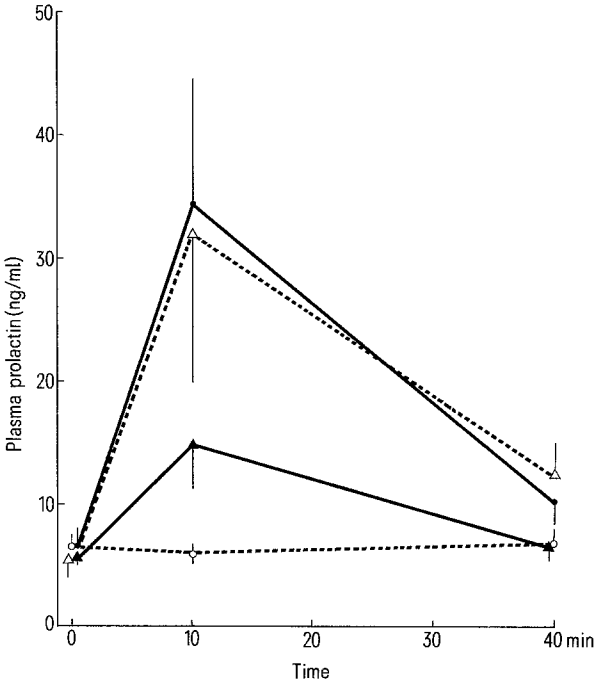

Figure 1. Changes in the concentration of plasma prolactin in sexually quiescent birds given an i.v. injection of either 0.1 ml saline (○, n = 6), 30 µg/kg b.wt (▲, n = 6), 75 µg/kg (●, n = 5) or 100 µg/kg (△, n = 5) VIP.

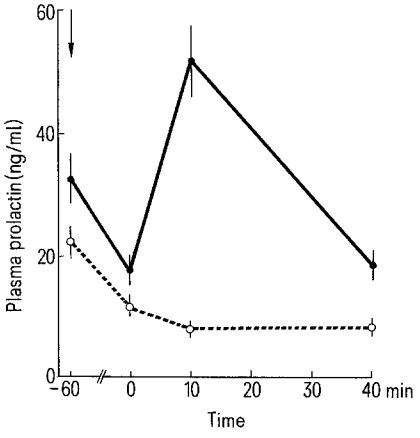

Figure 2. Changes in the concentration of plasma prolactin in brooding doves following nest deprivation (↓) and given either an i.v. injection of 0.1 ml saline (○, n = 6 pairs) or 75 µg/kg b.wt VIP (●, n = 6 pairs) at time 0.

of 25 µg per kg b.wt failed to cause a significant change in the concentration of plasma prolactin.

Experiment 2. The results for experiment 2 are shown in figure 2. As there was no difference in plasma prolactin concentration between the sexes the results for each sex have been combined. 1 h after the nests were removed the levels of plasma prolactin had fallen significantly ($p < 0.001$). 10 min after injection with 75 µg/kg b.wt of VIP, prolactin levels were significantly higher than those in saline-treated birds ($p < 0.001$). After 40 min, however, the prolactin levels in the VIP treated birds fell ($p < 0.001$), but they remained significantly higher than those in the saline treated birds ($p < 0.001$).

The levels of plasma prolactin reached 20 min after injection with 75 µg/kg VIP were not significantly different between the brooding (experiment 2) and the nonbreeding doves (experiment 1).

These results show that VIP can stimulate prolactin release in vivo in the ring dove in a dose-dependant manner. The dosages of VIP, the time course of response and the levels of plasma prolactin reached are in agreement with observations made in the chicken[19].

Other peptides in birds have been shown to stimulate the release of prolactin for example thyrotropin releasing hormone (TRH) and substance P in the chicken[24] and dermorphin in the pigeon[25] and therefore, VIP is probably not the only avian PRF. However, in the ring dove the plasma concentrations of thyroxine and growth hormone do not increase during the breeding cycle[26,27] which suggests that TRH is not the PRF responsible for the increased levels of prolactin during late incubation and brooding.

Studies on other avian species in contrast to the ring dove, have shown that plasma prolactin levels increase significantly around the onset of incubation, e.g. bantam[28], turkey[29], mallard[30] and canary.[31]. Further, it is considered that the maintenance of the increased concentration of prolactin is dependent upon the physical act of nesting[32–34]. That the presence of the nest is necessary for the maintenance of high levels of plasma prolactin in the brooding dove is shown by the observation that nest removal caused a sharp decline in prolactin concentration. However, in the ring dove, incubation of the eggs at the start of incubation is ineffective in stimulating prolactin release[20]. Therefore, it is of interest in this study that nonbreeding doves were sensitive to the prolactin releasing action of VIP and suggests that prolactin levels are low during the early stages of incubation because prolactin secretion is either actively inhibited or hypothalamic PRF is not being secreted. Though birds differ from mammalian species in that the control of prolactin secretion is primarily stimulatory[15], there is evidence that dopamine can inhibit release[35]. Treatment of doves with pimozide, however, a dopamine receptor blocker, failed to alter the profile of plasma prolactin at the onset of incubation (personal observation).

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