the LI is coupled with an increasing differentiation of the mammary gland. The moment when this differentiation begins and the LI decreases coincides with the appearance of rat chorionic mammatrophin (rCm) in the maternal circulation[11]. It is known that this hormone, among other things, stimulates in the mammary gland the formation of cytoplasm necessary for secretion[12]. This diminishing population of cells participating in the proliferation may indicate that a critical division indeed takes place. Another explanation may be that with increasing rCm concentration in the maternal blood the impulses to proliferation are overruled. The LI as a measure for proliferation can certainly be compromised during late pregnancy and lactation by DNA endoreduplication and polyploidy[12], but the importance of the latter processes cannot be investigated by light microscopy.

It is unlikely that labelling of the lactating mammary gland cell should be ascribed only to DNA endoreduplication, since mitoses are observed during the whole lactating period, though in very small quantities. It needs further investigation to establish whether undifferentiated cells still present (for which no morphological indications have been found[3]) are involved in this proliferation, or that differentiated cells divide again after dedifferentiation.

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Immunocytochemical localization of hyperglycemic hormone (HGH) in the neurosecretory system of the eyestalk of the crayfish *Astacus leptodactylus*

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Summary. Hyperglycemic hormone (HGH) from *Astacus leptodactylus* can be visualized by an immunocytochemical procedure using a specific antiserum against HGH and the peroxidase-anti-peroxidase (PAP) method. HGH containing cells are localized in the most distal portion of the x-organ in the medulla terminalis. Their cell diameter is about 47 ± 7 µm; the nucleus measures about 22 ± 2 µm. They form a subgroup of neurosecretory cell type 1, already described for the crayfish. A large amount of this HGH material is homogeneously stored in the sinus gland. A group of fibres in the x-organ – sinus gland – tractus can be followed, due to their positive reaction with the PAP-reagent.

Studies of hormonal regulation in Crustacea have all pointed to the important role played by the neurosecretory system in the eyestalk[1-3]. From many light microscopic[4] as well as electron microscopic studies[5,6], it appears that various neurosecretory cell groups, mostly located in the x-organ(s), send axons into the sinus gland, a neurohemal structure where axon terminals store the neurohormones and then release them into the blood. Despite these findings, it was not possible to correlate a specific neurosecretory substance with a typical neurosecretory cell group or neurosecretory granule type. We have therefore started an investigation with the ultimate goal to localize the hyperglycemic hormone (HGH) in the neurosecretory system of the eyestalk of the crayfish *Astacus leptodactylus*, and to obtain a cellular basis for an integration of our biochemical, histochemical and ultrastructural results[5,7-9].

Material and methods. Eyestalks were cut off from normally fed, adult male crayfish of the species *Astacus leptodactylus*, which were in stage C of their molting cycle. They were fixed in Bouin-Hollande fluid, containing 10% of a saturated aqueous solution of sublimate. The fixed material was dehydrated and cleared according to the conventional histological procedure and embedded in paraplast. Serial sections (7 µm) were deparaffinized, washed in Lugol and a hyposulfite solution, rinsed in distilled water and equilibrated in 0.05 M Tris-HCl buffered saline pH 7.6. The immunocytochemical staining procedure was as follows and based on the method of Sternberger et al.[10]: 1. Normal goat serum (dilution 1:5): 10 min, wash; 2. anti HGH serum (dilution 1:25): 45 min, wash; 3. goat-anti-rabbit IgG serum (dilution 1:10): 20 min, wash; 4. PAP (peroxidase-anti-peroxidase) complex (dilution 1:25): 20 min, wash; 5. 200 ml 0.05 M Tris-HCl (pH 7.6) containing 0.005% H_2O_2 and 100 mg 4-Cl-1-naphthol: 5 min, wash.

After staining, the sections were mounted in Gurr's water-mounting medium. The specificity of the immunocytochemical staining method was tested by successively substituting one of the aforementioned steps by buffer and by incubation in the substrate solution without any immune reagent. These controls were carried out on sections which were adjacent to the sections, stained with the complete immuno-enzyme cytochemical procedure.

The method for production and the characteristics of the rabbit antiserum against HGH will be described elsewhere[9].

Results and discussion. Previous structural studies on the crayfish eyestalk reveal that the neurosecretory system in that structure is characterized by a group of neurosecretory cells, the x-organ, located in the medulla terminalis (MT) and a neurohemal organ, the sinus gland (SG), situated dorso-laterally at the transition of the medulla externa (ME) and the medulla interna (MI). Durand[11] described 2

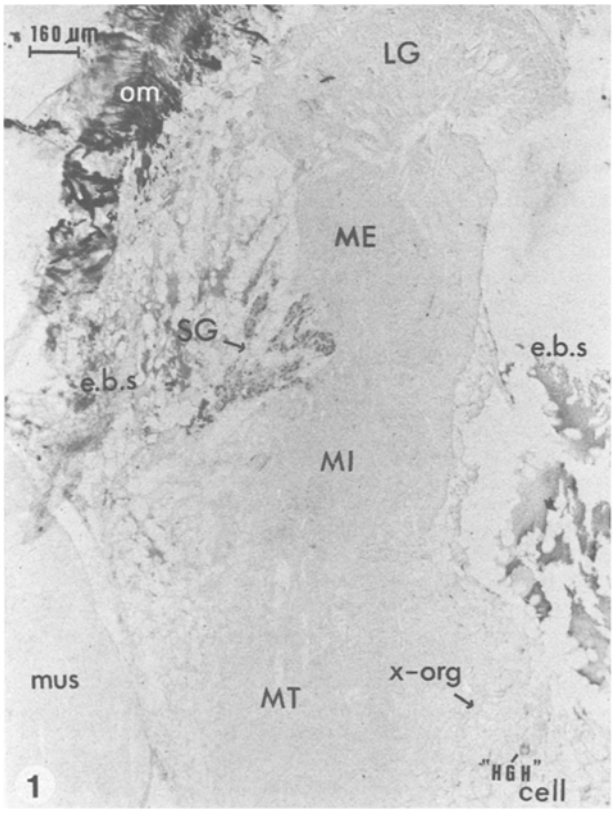
neurosecretory cell types in the x-organ of *Orconectes virilis* after staining with the aldehyde fuchsin method (type 1: 60 µm and type 2: 30 µm). In an electron microscopic investigation of the eyestalk of the same crayfish species, Shivers[12] could confirm the existence of 2 cell types in the x-organ. Type 1 has dense, membrane-limited granules (diameter 100–170 nm). These granules correspond to the major granule type of the sinus gland. Cell type 2 is characterized by electron-dense granules, 150–210 nm in diameter. Andrew et al.[6] re-evaluated the fine structure of the eyestalk of *Orconectes virilis,* based on a combination of iontophoresis and electron microscopy. They indicated that only eyestalk cell bodies in the region of the x-organ contribute axons to the sinus gland.

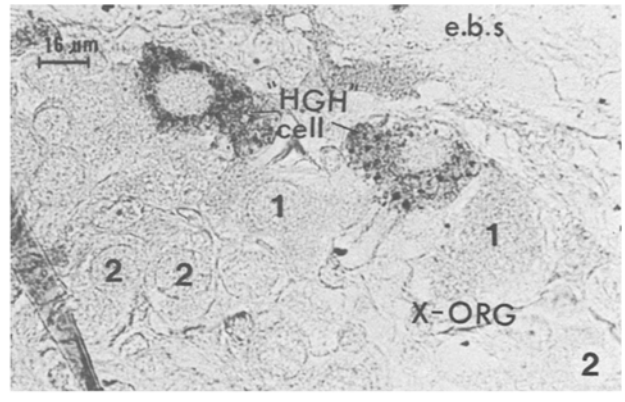
Our light microscopic work on the eyestalk of *Astacus leptodactylus* confirms these results. On the basis of their location, size, outline and staining with the aldehyde fuchsin-trichrome staining procedure, the x-organ in the MT is characterized by 2 'neurosecretory' cell types. Type 1 has a large cell body (mean diameter: 49 µm; SE = 7; n = 24), possesses a granular cytoplasm and contains a large nucleus (mean diameter: 20 µm; SE = 2; n = 24). Type 2 is smaller. The cell body has a mean diameter of 22 µm (SE = 2; n = 10), the cytoplasm is much more aggregated and the nucleus is about 17 µm (SE = 1; n = 10). Interspersed among these neurosecretory cells, one can distinguish numerous smaller cells.

Ungrouped neurosecretory cells are more or less scattered throughout the other optic ganglia. The sinus gland is always well-defined because of its strong affinity for the aldehyde fuchsin stain. The whole gland has a more or less granular texture and contains a few nuclei of glial cells. It is not possible to distinguish any region in the gland using the aldehyde fuchsin staining procedure.

In our present investigation, we were able to localize the hyperglycemic hormone in the neurosecretory system of the eyestalk of *Astacus leptodactylus.* By performing the tests of specificity, as mentioned under 'material and methods', it was clear that only the sinus gland and a few cells in the x-organ of the medulla terminalis show an affinity for the immune reagents. As demonstrated in figure 1, an amorphous greyish-blue precipitate is found only in those sites where HGH-material is located. By choosing 4-Cl-1-naphthol instead of 3,3′-diaminobenzidine in the substrate solution, it was possible to distinguish this greyish-blue colour in the neurosecretory sites from the brownish-black pigment in the region of the ommatidia. Although the intensity of the background staining in the blood lacunes around the optic ganglion axis could be reduced, a diffuse minimal staining was always present in the tissue sections. Further investigations are set up to solve this problem.

In the x-organ of the MT, the HGH material can be visualized in the cytoplasm of a few cells in the extreme distal portion of the x-organ (figure 2). The cell bodies are large (mean diameter 47 µm; SE = 7; n = 20). The secretory product consists of a great number of aggregations which stain strongly greyish-blue and are embedded in a fine granular matrix. The nucleus measures about 22 µm





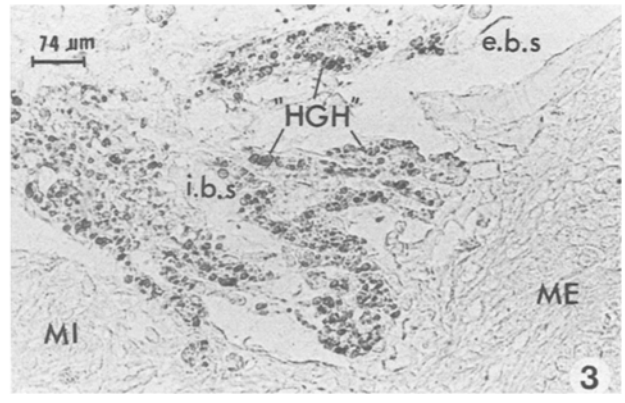


Fig. 1. Low power view of a longitudinal section through the right eyestalk.

Fig. 2. High power view of the x-organ region in the medulla terminalis.

Fig. 3. High power view of the sinus gland region.

Abbreviations used in the figures: e.b.s., external blood sinus; HGH cell, neurosecretory cell containing hyperglycemic hormone (HGH); i.b.s., internal blood sinus; LG, lamina ganglionaris; ME, medulla externa; MI, medulla interna; MT, medulla terminalis; mus, muscle in the eyestalk; om, ommatidia; SG, sinus gland; x-org, x-organ in the medulla terminalis; 1 and 2, neurosecretory cell type 1 and 2.

(SE = 2; n = 20) in diameter. It seems that these cells form a distinct subgroup of the neurosecretory cells 1, characterized by the aldehyde fuchsin method. Five to six of these cells show a large amount of the HGH-material, another six demonstrate a lower affinity for the immune reagent. This difference, however, can be expected in regard to different stages of their secretory process.

The sinus gland is homogeneously filled with granular clumps of greyish-blue HGH-material (figure 3). It is striking that a large portion of the gland is occupied by this secretory product. However, this is not uncommon, since Strolenberg et al.[5] indicate in an ultrastructural study of the sinus gland of *Astacus leptodactylus* that about 70% of the axon terminals in this neurohemal organ are filled with granulum type IV (between 100–170 nm; 49%) or type V (between 125–220 nm; 20%). In this respect we tentatively assign HGH to one of these two granulum types. To strengthen this assumption concerning the identification of HGH with one granulum type, we started an ultrastructural immunocytochemical study of the sinus gland of *Astacus leptodactylus*.

At the light microscopic level, it was possible to distinguish transverse or oblique sections through the x-organ – sinus gland – tractus by studying serial tissue sections. Some parts of that 'neurosecretory pathway' were intensively stained greyish-blue by the immunocytochemical procedure.

Note added in proof. P.P. Jaros reports in Histochemistry *63* (1979) about the immunocytochemical demonstration of the neurosecretory x-organ complex in the eyestalk of the crab *Carcinus maenas*. Using an antiserum against sinus gland extract, he was able to demonstrate by the PAP staining method the hyperglycemic hormone (HGH) and/or the black pigment dispersing hormone (BPDH) in the eyestalk of *Carcinus* on the light and electron microscopical level.

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Photoperiodic effects in the Djungarian hamster: one minute of light during darktime mimics influence of long photoperiods on testicular recrudescence, body weight and pelage colour[1]

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Summary. In male Djungarian hamsters (Phodopus sungorus) short photoperiods (L/D 8/16) with additional 1- or 5-min light-pulses 8 h after light-off were as effective as long photoperiods (L/D 16/8) in stimulating testicular recrudescence, increase in body weight and moult into summer pelage. The results are discussed with regard to the hypothesis that the pattern of melatonin release from the pineal gland is important in mediating photoperiodic effects in mammals.

In many mammals the annual cycle of reproductive and other functions is regulated by photoperiod and its change. The participation of the pineal gland in the mediation of photoperiodic effects has been documented[2–4].There is no consensus as to which pineal compounds are responsible for the transduction of the light effects, but melatonin seems a likely candidate since its synthesis is dependent upon external conditions of illumination[5]. Serotonin N-acetyltransferase (NAT) is assumed to be the rate-limiting enzyme in the conversion of serotonin to melatonin[5]. In all mammals studied so far, pineal NAT activity and melatonin content as well as plasma melatonin levels were found to have a marked daily cycle with high values at night and low values during the day[5,6]. As shown in studies in the laboratory rat[5,7] and in the ewe[8,9] continuous illumination suppresses the nocturnal peak of pineal NAT activity and melatonin production, and light during the normal darktime drastically reduces NAT activity within minutes, while in continuous darkness the daily cycle is maintained.

More recently, Illnerova and coworkers[10,11] have shown that in rats maintained in L/D 12/12, 1 min of light applied at about the middle of the darktime induces a rapid decline of pineal NAT activity and melatonin content as well as of plasma melatonin levels, and also prevents a subsequent rise to normal nighttime levels during the remaining darktime. Thus, such a brief exposure to light greatly alters not only the amount of melatonin produced, but also the pattern of its production and release. Since experiments in which hamsters were injected with melatonin suggest that not the amount but the temporal pattern of melatonin release might be important in the photoperiodic mechanism[3,12–15], we studied the effect of brief light exposures interrupting the darktime in the Djungarian hamster Phodopus sungorus maintained in short photoperiods. In this species strong photoperiodic effects on gonadal size and activity, body weight and pelage colour were found, and the pineal gland was shown to be involved[3,16,17].

Materials and methods. On December 6, 75 adult male Djungarian hamsters (age 202–243 days) were taken from the animal house where they had been kept under natural illumination. All animals were in winter pelage and had regressed testes as ascertained by palpation. 5 groups of 15 animals each were formed, matched for age and body weight (table). 1 group (IC) was sacrificed at the beginning of the experiment. 4 groups were placed in dark rooms with constant temperature (20 ± 1 °C). In 3 of these rooms the illumination schedule was L/D 8/16; however, in 1 room the darktime was interrupted daily by 1 min of light in the middle of the dark period (L/D 8/16 + 1 min L, light 08.00–16.00 h and 23.59–24.00 h) and in another chamber the nightly lightpulse lasted 5 min, (L/D 8/16 + 5 min L, light 08.00–16.00 h and 23.55–24.00 h). A 4th group was placed into long photoperiods (L/D 16/8, light 04.00–20.00 h). Light intensities ranged 200–800 lx, depending on