and had no effect on the brain, heart, liver and stomach, may indicate a tissue specificity of the hormone.
In conclusion, the results of the present investigation suggest that, unlike in brain, heart, liver and stomach, the changes in pancreatic, renal and intestinal functions following calcitonin treatment may in part be due to the change in the capacity of these tissues to synthesize protein.

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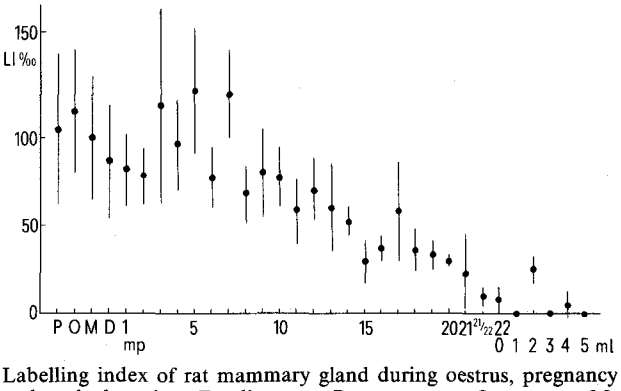
Variations of the labelling index in vitro of rat mammary gland in pregnancy and early lactation

J. van Marle, A. Lind and J. van Weeren-Kramer[1]

Pharmacological Laboratory, University of Amsterdam, Polderweg 104, NL-Amsterdam KP 1093 (The Netherlands), 26 January 1979

Summary. The labelling index of rat mammary gland during oestrus, pregnancy and early lactation was studied in vitro. The implications concerning the existence of a critical cell division are discussed.

The size of the mammary gland increases during pregnancy, on the one hand by an increase in the number of cells, on the other hand by differentiation and increase in cell size as part of this differentiation. Both systems, proliferation and differentiation, are determined by hormones[2,3]. It has been assumed that a critical division must take place before differentiation can start[4]. The cell(s) formed after this division would not divide further, but they would differentiate.
The aim of this investigation was to study the changes taking place in the population of cells still capable of division during pregnancy and at the beginning of lactation.
Material and methods. Rat mammary gland explants taken at various stages of the oestrus, from day 0 to day 22 of pregnancy and from the first 5 days of lactation were cultured in a tissue culture system for 24 h. For each experiment (i.e. per day) 2 Wistar rats (180 g at the moment of prooestrus) were used. From each rat 6 explants were cultured.
A medium consisting of 50% serum of non-pregnant rats, 50% synthetic medium (t16) and insulin (50 µg/ml) was used[5]. 4 h before the experiments were finished 5 µCi/ml ^{3}H-thymidine was added to the medium. For histological examination the explants were fixed in Bouin and stained with haematoxylin-phloxin. Autoradiographs were prepared according to Rogers[6]. The labelling index (LI) was determined by counting at least 2000 nuclei in each explant.
Results and discussion. During the various stages of the oestrus cycle the LI does not change significantly (figure). This LI, approximately 100‰, is maintained during the first 8 days of pregnancy. Thereafter the LI slowly decreases until it has reached 7‰ on day 22. In the first 5 days of lactation the LI fluctuates considerably. It is known that the culture medium ensures a good maintenance of the explants during the 24-h culture period, whereas it does not stimulate growth[7]. Therefore, it may be assumed that the course of the measured LI reflects the change in the population of dividing cells in vivo.
The constant LI during the 1st part of pregnancy may indicate that the chance of cell division remains equal for each cell in the population during this period. In other words there is no proliferating subpopulation. Bresciani[8] described a cell cycle time for the mammary gland in oestrogen stimulated mice of only 13 h. If this figure holds for rats too, then an existing subpopulation of dividing cells should result in an increased LI, at least during the first 7 days of pregnancy, in which this subpopulation divides about 12 times. If after each division 1 of the daughter cells does not divide anymore, a decreased LI on account of the increasing number of non-dividing cells would be the result. Furthermore, no morphological indications exist for such a population of stem cells[3]. In contrast to LI the mitotic index increases during the 1 half of pregnancy[9] and shows significant changes during the oestrus cycle (a peak in dioestrus)[10]. These 2 findings taken together show that the generation period of the cells of the mammary gland has been shortened, possibly due to the effect of oestrogens which are known to shorten the generation period. Not only the G_1 phase is shortened but also the S phase (from 27 to 9 h)[8].
The decrease in LI starting at day 8 indicates that the population of dividing cells diminishes. This decrease of



Labelling index of rat mammary gland during oestrus, pregnancy and early lactation. D: dioestrus, P: prooestrus, O: oestrus, M: metoestrus. mp 1-22: mammary glands from day 1 to day 22 of pregnancy. ml 1-5: mammary glands from day 1 to 5 of lactation. Each point shows the mean ± SD. 12 determinations per group.

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*

F. Van Herp and H.J.M. Van Buggenum

Zoological Laboratory, Faculty of Sciences, Catholic University, Toernooiveld, NL-6525 ED Nijmegen (The Netherlands), 23 January 1979

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