tions. In the normal and sham-stimulated fish, the perikarya of the neurons are deeply stained by AF because of the accumulated neurosecretory material (Figure 1).

Stimulation of the olfactory tract for 30 sec has caused incomplete degranulation in the perikarya of the neurons of the nucleus preopticus. 60 sec after stimulation a large number of vacuoles are formed in the perikarya due to acute degranulation. Some of the neurosecretory cells have become completely degranulated and only very little amount of neurosecretory material is present along the nuclear margin (Figure 2). Most of the neuronal cell bodies are either AF-negative or weakly-stained because of depletion of the neurosecretory material. Electrical stimulation appears to have caused a higher degree of depletion of the neurosecretory material from the neuronal cells of the pars magnocellularis than from those of the pars parvocellularis. The axons in the vicinity of the nucleus preopticus contain a large number of neurosecretory droplets that appear to have been discharged from the neuronal cells (Figure 2).

After stimulation for 2 min, almost the entire neurosecretory material seems to have been discharged from the neuronal cells. Most of the perikarya have attained AF-negative nature due to which it becomes difficult to locate the boundary of the neurons in stained sections. However, traces of neurosecretory material may exist along the nuclear margin of a few neurosecretory cells (Figure 3). A 2-min stimulation led to depletion of the neurosecretory material even from most of the neurons of the pars parvocellularis. The axons also lose their identity, since the neurosecretory material which was present in them after 60 sec stimulation appears to have been transported further away from the vicinity of the nucleus preopticus (Figure 3).

The response of the nucleus preopticus to electrical stimulation of olfactory tract in *H. fossilis* has resulted in depletion of the neurosecretory material as reported for the goldfish by JASINSKY et al.[10]. 60 sec stimulation evoked complete depletion in the goldfish[10] while in *H. fossilis* it required a 2-min treatment, although the characteristics of the stimuli are the same. This may be because of higher capacity of tolerance of the catfish to electrical stimulation. The difference in responsiveness of the neurons of the pars magnocellularis and pars parvocellularis to stimulation of the olfactory tract is suggestive of their functional difference[11].

Résumé. Chez le *Heteropneustes fossilis* normal le périkaryon des neurons du noyau préoptique montrent une grande accumulation de substance neurosécrétaire AF-positive. Stimulation électrique du nerf olfacteur pendant une minute menait à une dégranulation incomplète, tandis qu'un traitement de dux minutes produisait un épuisement presque complet de la substance neurosécrétoire. Les neurons de la portion magnocellulaire du noyau préoptique paraissent répondre plus rapidement que ceux de la portion parvocellulaire.

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Calcitonin Stimulation of Pyrophosphatase Activity in Rat Bone

Calcitonin exerts its hypocalcemic effect through an action on bone[1]. However, the exact mechanism of such an action is not fully understood.

In view of the fact that inorganic pyrophosphate plays a key role in the regulation of bone formation and resorption, as has been proposed by FLEISCH et al.[2,3], one possibility is that calcitonin exerts its effect through the alteration of inorganic pyrophosphatase, an enzyme which degrades pyrophosphate.

TENENHOUSE and RASMUSSEN[4] have recently shown that calcitonin stimulates inorganic pyrophosphatase activity of the Ehrlich ascites tumor cells incubated in vitro.

We have therefore determined the inorganic pyrophosphatase activity of the rat bone after the administration of porcine calcitonin. Female Wistar rats of 50 g body wt. fasted overnight were given s.c. injection of 200 mU of porcine calcitonin (Armour pharmaceutical. Co. 8MRC mU/mg) dissolved in 16% gelatin. Left tibiae were dissected out, freed from adherring soft tissues, and blood samples were obtained from the cervical vein before, 15, 30 and 60 min after injection. In another experiment in which graded doses of porcine calcitonin was given s.c. to thyroparathyroidectomized rats, tibiae and blood samples were obtained 3 h after the injection. Thyroparathyroidectomy was performed 1 day before the experiment. Serum calcium was determined by the colorimetric autoanalyzer method of GITELMAN[5], and inorganic pyrophosphatase activity of the tibia was estimated by a highly sensitive assay method using ^{32}P-labeled inorganic pyrophosphate[6]. After removing the bone marrow, 1% homogenate of the tibia was prepared by homogenizing frozen tibia in ice-cold 0.25 M sucrose with bone crusher and Waring blender. The homogenate was then mixed with 0.1% Triton X-100, centrifuged at 3000g 3 min and the supernatant was used for the assay of pyrophosphatase activity and for the determination of protein content by LOWRY's method[7].

Pyrophosphatase activity was estimated from ^{32}Pi released from ^{32}PPi in 0.033 M pH 7.5 veronal buffer or 0.05 M pH 2.5 glycine HCl buffer with 10^{-5} M $Na_4P_2O_7$ and 10^{-5} M $MgCl_2$ during 30 min incubation period at 37 °C. Reaction was stopped by the addition of 10% trichloroacetic acid and the aliquot was taken and mixed with 1 M acetate buffer (pH 5.0) and 0.1 M PPi. 0.1 M $MnCl_2$ was then added, shaken and centrifuged, thus precipitating the remaining ^{32}PPi. After the centrifugation, the released ^{32}Pi was counted by the liquid scintilla-

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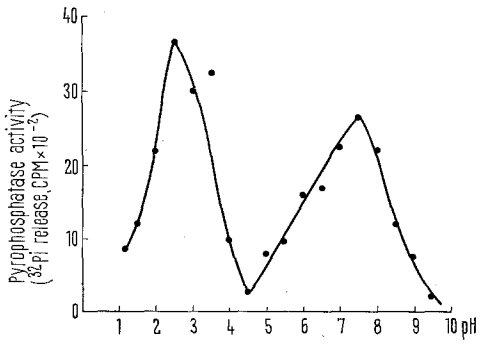

Fig. 1. pH profile. The reaction mixtures containing 48 μg protein of bone homogenate in buffer solutions of various pH were incubated at 37 °C for 30 min. The enzyme activity is expressed as Cpm of released ^{32}Pi.

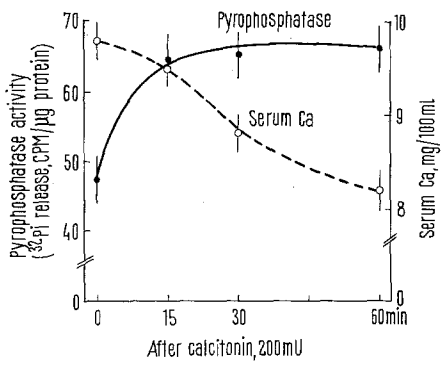

Fig. 2. A significant rapid increase in alkaline pyrophosphatase activity of rat bone after 200 mU of calcitonin. Note that change in pyrophosphatase activity occurs prior to the change in serum calcium. Each point represents the mean of 5 samples.

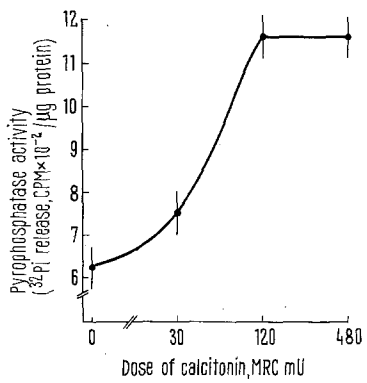

Fig. 3. Dose related increase in alkaline pyrophosphatase activity of the bone of thyroparathyroidectomized rat after calcitonin.

tion counter and inorganic pyrophosphatase activity was expressed as radioactivity of ^{32}Pi per μg of bone protein.

As shown in Figure 1, rat tibia contains two inorganic pyrophosphatases, namely acid and alkaline, which possess the optimum pH at 2.5 and 7.5 respectively.

Following the administration of porcine calcitonin, a significant and rapid increase in the alkaline pyrophosphatase activity of the tibia was noted (Figure 2). Since this increase in the enzyme activity is evident 15 min after calcitonin injection, preceding the development of significant hypocalcemia, it may be that this alteration of alkaline pyrophosphatase activity is directly involved in the mechanism of action of calcitonin. Calcitonin also stimulated alkaline pyrophosphatase activity of the tibia in thyroparathyroidectomized rats, as shown in Figure 3. The alkaline pyrophosphatase activity in the tibia of thyroparathyroidectomized rat was significantly lower than that of the intact rat. Acid pyrophosphatase activity, however, was not significantly changed after calcitonin both in intact and thyroparathyroidectomized rat.

Particularly interesting is the recent suggestion by FLEISCH et al.[2,3] that inorganic pyrophosphate is a physiological inhibitor of calcification and a physiological regulator of calcium homeostasis through its effect on bone formation and resorption.

If inorganic pyrophosphate is involved in such a system, its removal by pyrophosphatase would facilitate bone mineralization. At least, part of this removal may be brought about by the alkaline phosphatase of the bone, since alkaline phosphatase of bone is reported to possess pyrophosphatase activity[8,9].

Although it has been established that calcitonin inhibits bone resorption, there is still some evidence which suggests that calcitonin also stimulates bone formation[10,11]. Since calcitonin stimulates alkaline pyrophosphatase activity of the bone, it is possible that calcitonin stimulates bone formation through the removal of inorganic pyrophosphate.

Zusammenfassung. Calcitonin als neues kalzium- und phosphor-senkendes Hormon wurde auf seine Wirkung auf die inorganische Pyrophosphataseaktivität von Knochen untersucht. Eine Steigerung der alkalinen Pyrophosphataseaktivität wurde vor der starken Senkung des Serum-Kalziums beobachtet.

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The Reactions of Integumentary Melanophores to Background Changes in Intact and Hypophysectomized *Ictalurus melas* (Rafinesque)

In the fresh-water catfish *Ictalurus melas*, the chromatophores are all melanophores, evenly distributed in the integument and not forming any pattern. They occur in 3 layers, namely, in the epidermis and in the upper and lower regions of the dermis. The epidermal melanophores are the smallest and the lower dermal ones are the largest.

Material and method. Specimens of *I. melas* were subjected to the following experimental conditions: 1. On illuminated black and white backgrounds until full adaptation was reached followed by black/white background reversal; on illuminated grey backgrounds of known reflections; in darkness. 2. Blinded specimens illuminated