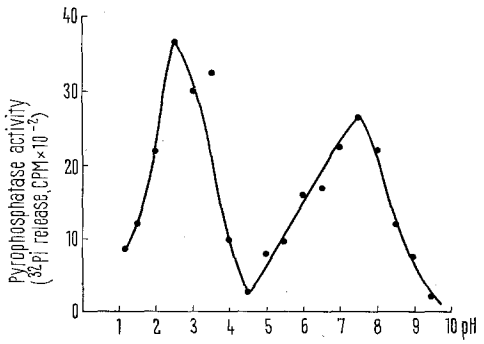

Fig. 1. pH profile. The reaction mixtures containing 48 μg protein of bone homogenate in buffer solutions of various pH were incubated at 37 °C for 30 min. The enzyme activity is expressed as Cpm of released ^{32}Pi.

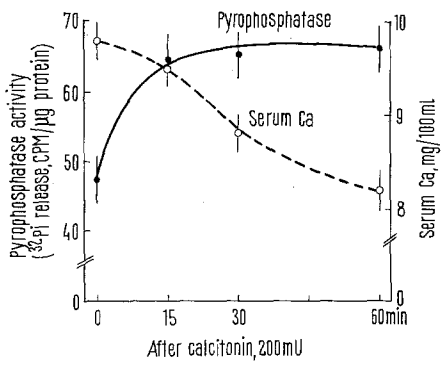

Fig. 2. A significant rapid increase in alkaline pyrophosphatase activity of rat bone after 200 mU of calcitonin. Note that change in pyrophosphatase activity occurs prior to the change in serum calcium. Each point represents the mean of 5 samples.

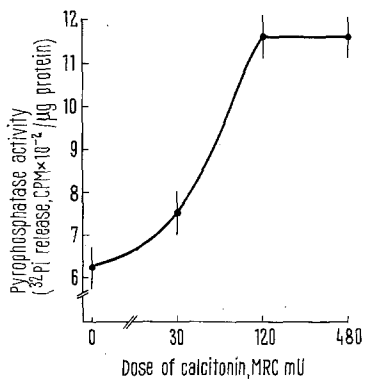

Fig. 3. Dose related increase in alkaline pyrophosphatase activity of the bone of thyroparathyroidectomized rat after calcitonin.

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

Table I. Mean melanophore responses of *Ictalurus melas* during transition from white to black background adaptation

Time interval	Mean δ MI of 15 intact fishes			Mean δ MI of 15 hypophysectomized fishes		
	Epidermal	Upper dermal	Lower dermal	Epidermal	Upper dermal	Lower dermal
0 min	1.10 (±0.14)	1.26 (±0.29)	1.50 (±0.28)	1.00 (±0.00)	1.25 (±0.31)	1.74 (±0.30)
15 min	2.74 (±0.50)	2.81 (±0.39)	3.15 (±0.29)			
30 min	3.31 (±0.43)	3.40 (±0.42)	3.61 (±0.31)	1.90 (±0.50)	2.38 (±0.51)	3.24 (±0.47)
1 h	3.70 (±0.40)	3.83 (±0.38)	4.10 (±0.26)	2.05 (±0.56)	2.57 (±0.46)	3.40 (±0.44)
$1^1/_2$ h	4.00 (±0.28)	4.16 (±0.38)	4.28 (±0.29)	2.15 (±0.57)	2.65 (±0.12)	3.52 (±0.41)
2 h	4.20 (±0.28)	4.30 (±0.36)	4.40 (±0.29)	2.28 (±0.64)	2.80 (±0.44)	3.55 (±0.38)
3 h	4.40 (±0.39)	4.50 (±0.33)	4.60 (±0.29)	2.31 (±0.61)	2.87 (±0.45)	3.58 (±0.36)
4 h	4.54 (±0.32)	4.62 (±0.29)	4.74 (±0.29)	2.40 (±0.56)	2.90 (±0.20)	3.63 (±0.33)
6 h	4.77 (±0.17)	4.88 (±0.13)	4.94 (±0.13)	2.40 (±0.52)	2.90 (±0.26)	3.63 (±0.31)
24 h	5.00	5.00	5.00	2.42 (±0.66)	2.90 (±0.40)	3.63 (±0.40)

Temperature 20±1 °C. Overhead illumination 40 W, 75 cm.

Table II. Mean melanophore responses during transition from black to white background adaptation

Time interval	Mean δ MI of 15 intact fishes			Mean δ MI of 15 hypophysectomized fishes		
	Epidermal	Upper dermal	Lower dermal	Epidermal	Upper dermal	Lower dermal
15 min	3.14 (±0.45)	3.50 (±0.30)	3.70 (±0.27)			
30 min	2.82 (±0.40)	3.10 (±0.40)	3.20 (±0.38)	1.32 (±0.27)	2.15 (±0.46)	2.62 (±0.38)
1 h	2.36 (±0.43)	2.33 (±0.34)	2.62 (±0.33)	1.25 (±0.24)	1.95 (±0.49)	2.48 (±0.42)
$1^1/_2$ h	1.96 (±0.35)	2.14 (±0.40)	2.40 (±0.40)	1.15 (±0.25)	1.73 (±0.29)	2.31 (±0.38)
2 h	1.60 (±0.35)	1.80 (±0.35)	2.12 (±0.24)	1.10 (±0.25)	1.65 (±0.40)	2.20 (±0.36)
3 h	1.30 (±0.27)	1.57 (±0.30)	1.83 (±0.30)	1.00 (±0.20)	1.56 (±0.37)	2.10 (±0.34)
4 h	1.24 (±0.30)	1.46 (±0.29)	1.70 (±0.21)	1.00 (±0.20)	1.50 (±0.35)	2.02 (±0.37)
6 h	1.10 (±0.10)	1.32 (±0.20)	1.57 (±0.21)	1.00 (±0.20)	1.47 (±0.40)	1.95 (±0.26)
24 h	1.10 (±0.19)	1.20 (±0.22)	1.40 (±0.28)	1.00	1.40 (±0.31)	1.84 (±0.35)

and in darkness. 3. Hypophysectomized specimens transferred to the same conditions as the unoperated animals in (1). 4. Injection of pituitary extracts.

Results. 1. *Responses of normal intact fish.* Black and white adaptation and background reversal: In a black-adapted fish the melanophores are in a fully dispersed condition. The epidermal melanophores show a marked difference in their pattern of dispersion from the rest of the melanophores in that they send out thin and slender branches in all 3 dimensions, the branches of the neighbouring melanophores overlapping to give the appearance of a continuous lace-work. The upper and the lower dermal melanophores, on the other hand, give out quite thick branches at one level only. Complete adaptation in both directions to black/white illuminated backgrounds (melanophore index (MI)[1] 5.00 and 1.00) in all layers is accomplished in 6–8 h. The pigment movements are very rapid for about the first 30 min. Thereafter they become much slower. Moreover, the melanophores of different layers react differentially to background changes, the rate of pigment dispersion being slower and the rate of aggregation faster in the epidermal melanophores than those of the dermis.

Adaptation to greys: The differential responses are also observed on equilibration to different shades of grey (derived Ostwald Index[2] 2, 4 and 6 having per cent reflection 28, 11 and 4.5 respectively), the epidermal melanophores having the most aggregated pigment and the lower dermal ones the least aggregated.

Chromatic behaviour in darkness: The lower dermal melanophores of normal fish in darkness equilibrate at MI 3.10–3.20 after 6–8 h. The reactions of these melanophores in darkness were found to be more uniform than those of the epidermis. Moreover it was more convenient to read the MI of this layer.

2. *Blinded animals.* All the melanophores of eyeless animals have fully dispersed pigment when the animal is illuminated. In darkness the lower dermal melanophores equilibrate as they do in the intact fish, at MI 3.10–3.20.

3. *Responses of hypophysectomized animals.* Black and white background adaptation and reversal: Hypophysectomy considerably limits black background adaptation whereas adaptation to a white background does not appear to be affected[3] (Table I). The extent and the rate of dispersion of the lower dermal melanophores of white-adapted hypophysectomized catfish on transfer to a black background after 30 min differs only slightly from the values observed in intact fish and controls (Table I). However, in hypophysectomized animals these melanophores do not attain dispersion beyond a mean MI 3.65 on a black background. Another interesting result of the removal of the pituitary is the increased differential responses of the different layers of melanophores. The responses to background changes are on the whole accomplished in 4–6 h but a slight further aggregation of dermal melanophores on a white background continues for over 48 h after transfer from a black background. The aggregation of the pigment during first 1–$1^1/_2$ h after a change from black to white background adaptation appears to

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be accelerated in hypophysectomized animals in comparison to intact ones (Table II). Controls in which the hypothalamic region of the brain is exposed with the pituitary remaining intact respond like unoperated animals to background changes.

Darkness: In darkness the lower dermal melanophores of white- and black-adapted hypophysectomized catfish equilibrate at a mean MI 2.76 in about 6 h, suggesting that the contribution of the pituitary to equilibrium in darkness is not appreciable.

4. *Injection of pituitary extract.* Intraperitoneal injection of 0.3 ml of crude pituitary extract (2 glands from long white-adapted fish in 4 ml Ringer) into white-adapted hypophysectomized fish of the same size induces dispersion to MI 4.00 (mean of 3 animals) in 2 h whereas the same injection causes only slight dispersion (MI 2.20) in unoperated white-adapted controls.

Discussion. Comparison of the time relations of the chromatic changes of other teleosts shows that *I. melas* fills the gap between the species in which these changes are entirely or primarily neurally coordinated (e.g. *Macropodus*[4], *Fundulus*[5], *Gasterosteus*[6], *Lebistes*[7], and *Phoxinus*[8]) and those in which their control is mainly hormonal (e.g. *Anguilla*[9]).

The pituitary appears to be essential for full dispersion of melanophores in black background adaptation[10]. Of all the layers the epidermal melanophores appear to be most influenced by pituitary hormones. Furthermore, adaptation of hypophysectomized fish to a white background shows no statistically significant difference from that of the normal fish, and therefore indicates that no pigment aggregating hormone is playing a part in the normal colour changes of *I. melas*.

Zusammenfassung. Beim Zwergwels *Ictalurus melas* liegen die Melanophoren im Ektoderm sowie im oberen und tieferen Entoderm und zeigen ein schichttypisches Verhalten. Nach Hypophysektomie bleibt das unterschiedliche Verhalten bestehen. Postoperativ wird der Fisch auf schwarzem Untergrund weniger dunkel als das normale Tier, während die Anpassung auf weissem Untergrund unverändert bleibt.

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An Unusual Trabecular Thyroid Cancer Producing Calcitonin

We have established that thyroid medullary carcinoma with amyloid stroma[1,2] is a differentiated tumour secreting calcitonin (CT)[3,4], the hypocalcemic hypophosphatemic hormone[5]. In the course of a systematic study of the histochemistry and biochemistry of thyroid carcinomas, we had the opportunity to observe a case of trabecular thyroid cancer lacking amyloid stroma and producing large amounts of CT.

The patient was a 60-year-old female. The left lobe of the thyroid increased in size within 9 months, was hard to palpation and the scan demonstrated a lack in ^{131}I uptake. The patient had no signs of diarrhoea, flush or cutaneous neurofibromatosis. Serum calcium levels fluctuated between 9.5 and 7.0 mg/100 ml; serum phosphate levels were 4.0 mg/100 ml. Total ablation of the thyroid was performed (April 1970): the left lobe (35 g) was the seat of a hard, white mass with a hemorrhagic center. Lymphnodes had a normal appearance and were left in situ. Different samples of the tumour were either extracted for CT or fixed for microscopic examination.

Methods. CT-bioassay: Tumour fragments were defatted with ether and extracted with butanol-acetic acid water[6]; the hormone content of the extracts and of fresh serum were assayed for hypocalcemic and hypophosphatemic[3] activity using a 4 point bioassay in the rat with a M.R.C. reference as a standard[18]. The results are expressed in mU/mg of tissue dry weight.

Histochemical stains: Periodic acid-Schiff (PAS), alcian blue, thioflavin T, Congo red, crystal violet, toluidine blue, lead hematoxyline before and after acid hydrolysis[7,8], and Daventport's silver impregnation.

Results. 1. CT: The hypocalcemic activity of the tumour is 137.2 mU/mg (85–214, $P < 0.05$), the hypophosphatemic activity is 126 mU/mg (94–158, $P < 0.05$). Hypocalcemic activity of the serum: 0.2 mU/ml.

2. Pathology: The tumour crosses the capsule of the thyroid, invades blood vessels and in particular a thyroidal vein, forms a paraisthmic metastatic nodule 3 mm in diameter. The right lobe of the thyroid is uninvolved and shows evidence of thyroiditis. The tumour is composed of closely packed neoplastic lobules, separated by thin zones of regressive parenchyma. The lobules are formed of trabeculi containing 5–50 cells, which are surrounded by a thin fibrous sheet and separated by voluminous capillaries. Microscopic appearance: the cells are often large, polyhedric or fusiform, the cytoplasm is clear and distinctly eosinophilic. Numerous atypical forms exist. The nuclei are polymorphic. Some intracellular eosinophilic deposits are found. All staining

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