Hypocalcemia and Tetanic Seizures in Hypophysectomized Killifish, *Fundulus heteroclitus*[1] (35199)

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All present evidence indicates that fish lack parathyroid glands (1), although the ultimobranchial body (UB) has been suggested to be the homologue in teleosts (2). However, recent evidence has shown that the teleostean ultimobranchial body is a potent source of calcitonin which lowers serum calcium in mammals. This is true of preparations from salmon, cod, and *Fundulus heteroclitus*, when tested on white rats (3–5). Calcitonin administration elicits hypocalcemia in some species of teleosts (6, 7) but repeated studies have failed to reveal any such effect on the killifish, *F. heteroclitus*, either with porcine calcitonin (8) or with teleostean preparations (4, 5). Therefore, in this species, the two main calcium regulating hormones are either absent or ineffective and other controlling mechanisms must be investigated. In the present studies, repeatable tetanic convulsions were induced in hypophysectomized (hypect.) killifish and they were related to a marked hypocalcemia.

Methods. Large female *F. heteroclitus* from the New York Aquarium and males from the vicinity of New Haven, Conn. were adapted to artificial sea water with, or without, calcium for at least 1 month before they were used in experiments. They were kept in standing water with 8 hr artificial day light at 20° and were fed on a modified Aronson mixture in which calcium-rich ingredients were omitted (no dry shrimp and oatmeal substituted for Pablum). In the first experiment, females were used. Two groups of fish were maintained in seawater (SW) and two in calcium-deficient seawater (SW-Ca). After the adaptation period, one group in each environment was hypophysectomized and they were returned to their respective media. No food was offered to the fish for 3 days to allow wound healing in the hypophysectomized groups. All groups were fed on the fourth day and all fish ate except the hypects. in SW-Ca. All fish were anesthetized with tricaine methanesulfonate and autopsied on the seventh day. The autopsy procedures and methods for serum analysis were similar to those described in Pickford *et al.* (9).

In the second experiment, male fish were hypophysectomized after 1 month's adaptation to SW. They were allowed to recover from the operation for 1 week. The fish were then divided into three groups, two of which were transferred to SW-Ca and the third remained in SW. These fish were kept in aerated 2-gallon jars and the water was changed daily. Two weeks later, one SW-Ca group was transferred back to SW.

In the third experiment, all fish were adapted to SW-Ca for 1 month. One group was mock-operated and two groups were hypophysectomized. They were all returned to SW-Ca. Four days after the operation, one group of the hypects. was transferred to SW.

Results. The SW hypects. in the first experiment were normal in their behavior. However, 3 days after the operation the hypects. in SW-Ca showed hyperexcitability. On the fourth day, a few of them exhibited tetanic convulsions when disturbed. Initial symptoms included erratic flipping on the water surface, inability to close the mouth and opercula and loss of balance. These were followed by complete loss of control and the fish lay on their backs at the bottom of the

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TABLE I. The Effects of Hypophysectomy and Environmental Calcium on *Fundulus heteroclitus*.

Serum electrolytes (mmoles/liter).

Expt.	Group	Na^+	K^+	Cl^-	Total Ca^{2+}	Inorg. PO_4^{3-}
1	SW, control, intact	181.2[a] ±3.8 (5)	7.8 ±0.2 (5)	142.1 ±3.2 (5)	3.68 ±0.13 (5)	2.93 ±0.22 (5)
	SW, hypect.	175.4 ±1.2 (5)	5.9 ±0.3 (5)	124.2 ±5.2 (5)	3.22 ±0.24 (5)	2.93 ±0.32 (5)
	SW-Ca, control, intact	175.2 ±5.3 (5)	7.2 ±0.5 (5)	138.0 ±2.7 (5)	2.46 ±0.21 (5)	3.20 ±0.28 (5)
	SW-Ca, hypect.	180.3 ±3.0 (5)	6.2 ±0.6 (5)	131.2 ±3.3 (5)	1.50[b] ±0.14 (5)	2.37[b] ±0.15 (5)
2	SW, hypect.	197.5 ±7.9 (5)	10.0 ±0.9 (5)	144.9 ±6.4 (5)	3.01 ±0.16 (7)	2.76 ±0.24 (7)
	SW-Ca, hypect.	183.3 ±2.8 (6)	7.0 ±1.1 (6)	134.0 ±5.9 (6)	1.55[b] ±0.15 (6)	2.79 ±0.63 (6)
	SW-Ca, hypect., returned to SW	180.5 ±2.5 (5)	5.56[b] ±0.28 (5)	137.3 ±3.6 (5)	2.68 ±0.11 (5)	2.36 ±0.15 (5)
3	SW-Ca, mock-operated control	175.1 ±1.5 (5)	3.0 ±0.8 (5)	142.0 ±1.7 (5)	2.29 ±0.10 (5)	3.66 ±0.35 (5)
	SW-Ca, hypect.	167.9[b] ±1.4 (5)	4.9 ±1.2 (5)	129.7[b] ±4.7 (5)	1.34[b] ±0.08 (5)	2.49[b] ±0.20 (5)
	SW-Ca, hypect., returned to SW	174.7 ±6.1 (4)	5.8 ±0.3 (4)	147.0 ±4.4 (4)	3.04 ±0.29 (4)	2.84 ±0.14 (4)

[a] Results are expressed as the mean ± SE (no. of samples).

[b] Significantly lower than the control group, $p < 0.001$.

aquarium without observable movements of the fins or the extended opercula. After a few minutes, a slow opercular movement started again and the fish gradually regained equilibrium. Complete recovery was achieved in about 10 min. The newly recovered fish would not be induced to go into tetany again until much later. On the seventh day, all except one of the SW-Ca hypects. went into tetany while the other groups appeared normal.

In the second experiment, the hypects. were normal in SW. However, 2 weeks after the transfer to SW-Ca, several fish went into tetany. The rest showed hyperexcitability, failure to close the mouth and opercula and loss of muscular control but did not go into typical tetanic seizures. The group that was transferred back to SW recovered from the abnormal behavior within 24 hr.

The mock-operated controls in the third experiment did not suffer any ill effects from the operation. However, repeated tetany was seen in all the hypects. The tetanic group that was transferred to SW on the fourth day recovered within 24 hr.

The data from the serum analysis are summarized in Table I. Tetanic fish had low levels of serum calcium in all three experiments (1.34–1.55 mmoles/liter). Serum inorganic phosphate was lowered in two experiments when compared with intact or mock-operated controls but there was no change when the comparison was made with hypophysectomized fish in SW. Moreover, return of tetanic fish to SW restored serum calcium but serum phosphate remained low. Serum sodium, potassium, and chloride was either unchanged or decreased. Clearly tetanic convulsions are specifically related to low levels of serum calcium. The one SW-Ca hypect. which failed to show tetany was found to have an incomplete operation and a pituitary remnant was seen on autopsy. This fish had normal calcium and phosphate levels.

Discussion. Repeated tetany has been induced for the first time in fish. Breder de-

scribed "convulsions" in marine fishes, kept in Ca-enriched tap water (10). This response might be due to osmotic stress. Low serum calcium level was not demonstrated and was unlikely as the environment was rich in calcium. Neuhold and Sigler reported "tetanic death" in some goldfish suffering from fluoride poisoning (11). Again serum calcium level was not studied and there was no indication that the fish recovered. In the present studies, the tetanic convulsions were not lethal and were repeatable in individuals. They were compared to those reported in mammals and reptiles after parathyroidectomy (12, 13). Furthermore, these symptoms were correlated with a marked fall in serum calcium and could be corrected by putting the fish in a high calcium environment in which the phosphate level was the same.

The involvement of the pituitary in maintaining the serum calcium level in the hypocalcemic hyperosmotic environment is obvious. The failure of the hypects. to show tetany in SW is understandable, as the main problem in such a hypercalcemic environment is to get rid of the excess calcium. A fall in serum calcium in hypophysectomized eels adapted to freshwater has been reported by Fontaine (14) and Chan and Chester Jones who believed that the decrease was a result of osmoregulatory problems (15). The marked fall in serum calcium observed in the present studies in an independent function of the pituitary as no consistent changes in serum chloride, sodium, and potassium were evident. Hypercalcemia was reported by Fleming in hypophysectomized *Fundulus kansae* in tap water (16). However, the freshwater he used was high in calcium and the increase in serum calcium might be due to an influx of external calcium into the organism as shown by the simultaneous fall in serum sodium.

To conclude, the present investigations established a pituitary hypercalcemic mechanism essential in maintaining normal serum calcium level in a hypocalcemic environment. This factor is independent of the processes regulating sodium, potassium, and chloride. The fact that *F. heteroclitus* showed tetany and marked hypocalcemia indicates that it is very unlikely that this fish has a functional parathyroid gland which would otherwise correct the defects in calcium metabolism.

Summary. Tetanic convulsions were observed in hypophysectomized *Fundulus heteroclitus* kept in calcium deficient but hyperosmotic conditions. Analysis of sera revealed a large decrease in total calcium and inorganic phosphate although sodium, potassium, and chloride were unaffected. These defects in calcium metabolism were corrected by returning the tetanic fish to a calcium-rich environment.

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