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## The Effects of Changes in External Sodium, Calcium, and Magnesium Concentrations on Prolactin Cells, Skin, and Plasma Electrolytes of *Gasterosteus aculeatus*

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Accepted September 16, 1977

Exposure of freshwater fish to freshwater containing calcium and magnesium ions in concentrations found in seawater reduces prolactin cell activity to the low values characteristic for seawater fish. When applied in the same concentrations, calcium is more effective than magnesium. High sodium concentrations have only small effects. Transfer of fish to freshwater or seawater with reduced ionic calcium and magnesium levels activates the prolactin cells. In these experiments plasma ionic calcium and sodium levels were negatively correlated with prolactin cell activity, while thickness of the epidermis and density of the epidermal mucocytes were positively correlated with prolactin cell activity. It is concluded that ionic calcium concentration, and not osmolarity or sodium content, is the main environmental factor in the control of prolactin secretion in sticklebacks.

One of the most prominent functions of teleost prolactin is its role in hydromineral regulation of freshwater fish. The hormone controls the permeability of skin and gills for ions and water and promotes sodium retention and water release by the kidneys. In most euryhaline species prolactin cells are more active in freshwater than in seawater. Considerable activation of these cells after transfer of seawater-adapted fish to freshwater has been frequently reported (Nagahama *et al.*, 1973; Schreibman *et al.*, 1973). It has been suggested that changes in sodium content and total osmolarity of the environment account for the enhanced prolactin cell activity. This effect is supposed to be mediated by plasma sodium concentration and plasma osmolarity (Ball and Ingleton, 1973; Nagahama *et al.*, 1974, 1975; Wigham and Ball, 1977). The importance of these blood factors for the control of prolactin secretion is mainly based on *in vitro* studies (Ingleton *et al.*, 1973; Zambrano *et al.*, 1974; Nagahama *et al.*, 1975).

For several teleost species it has been demonstrated that prolactin cells are under inhibitory hypothalamic control (Zambrano *et al.*, 1974). This does not exclude the possibility that these cells are influenced directly by various blood factors, but it may implicate that the *in vitro* results do not fully apply to the *in vivo* situation. The possibility that ions other than sodium affect prolactin secretion deserves attention, since there is evidence that prolactin is involved in the control of several ions, including calcium (Chan *et al.*, 1968; Ogawa, 1968; Pang *et al.*, 1973). Pang and co-workers have suggested that prolactin has a specific hypercalcemic effect, distinct from its influence on plasma sodium concentration. The effects of internal as well as external calcium levels on prolactin cell activity are hardly known, however.

In this paper an attempt is made to identify the environmental factors responsible for the differences in prolactin cell activity between freshwater and seawater fish.

Three-spined sticklebacks were exposed to media of varying osmotic and ionic composition, and the effects on prolactin cell structure were analyzed. In addition, skin, epidermal mucocytes, and plasma electrolytes were studied. The layer of mucus as well as the epidermis determine the osmoregulatory properties of the integument and are supposed to be under control of prolactin, at least under freshwater conditions (Mattheij and Stroband, 1971; Marshall, 1976). Special attention was paid to the effects of external sodium, calcium, and magnesium ions.

### MATERIALS AND METHODS

Adult female sticklebacks (60–70 mm in body length) were collected along the coast of the Wadden Sea, in late winter and early spring. They were acclimated for at least 6 weeks to either freshwater or Wimex artificial seawater at 15° and 8 hr of light. The concentrations of the main electrolytes of these media have been reported (Wendelaar Bonga *et al.*, 1976).

Freshwater fish were exposed for a period of 16 days to the following solutions.

(F1) *Calcium- and magnesium-free freshwater*: NaCl, 2.1 mmol/liter; KCl, 0.06 mmol/liter, dissolved in demineralized water.

(F2) *Freshwater*: tap water containing (in millimoles per liter) Na<sup>+</sup>, 2.1; K<sup>+</sup>, 0.06; Ca<sup>2+</sup>, 0.1; Mg<sup>2+</sup>, 0.2; Cl<sup>-</sup>, 2.8.

(F3) *Sodium-enriched freshwater*: Fish were adapted for 6 days to increasing concentrations of NaCl in freshwater. Adaptation started in one-sixth of the final concentration, 410 mmol/liter. The concentration was regularly increased. Subsequently the fish were exposed for 10 days to the final concentration.

(F4) *Magnesium-enriched freshwater*: as F3, with a final concentration of 10.2 mmol/liter of MgCl<sub>2</sub>·6H<sub>2</sub>O.

(F5) *Magnesium-enriched freshwater*: as F3, with a final concentration of 56.0 mmol/liter of MgCl<sub>2</sub>·6H<sub>2</sub>O.

(F6) *Calcium-enriched freshwater*: as F3, with a final concentration of 10.2 mmol/liter of CaCl<sub>2</sub>·2H<sub>2</sub>O.

The fish acclimated to seawater were exposed for 16 days to the following solutions.

(S1) *Hale's seawater*: a solution of the following salts in demineralized water (in millimoles/liter): NaCl, 410.0; KCl, 9.7; CaCl<sub>2</sub>·2H<sub>2</sub>O, 10.2;

MgCl<sub>2</sub>·6H<sub>2</sub>O, 56.0; Na<sub>2</sub>SO<sub>4</sub>, 28.2; and NaHCO<sub>3</sub>, 2.3. This solution was modified after Hale (Lockwood, 1963).

(S2) *Low-calcium, low-magnesium seawater*: During a 6-day period, seawater fish were adapted to Hale's seawater with daily decreasing content of CaCl<sub>2</sub>·H<sub>2</sub>O and MgCl<sub>2</sub>·6H<sub>2</sub>O. Afterwards they were exposed for 10 days to the final concentration, 10% of the normal calcium and magnesium concentration in Hale's seawater.

Animals were killed by severing the spinal cord. From the caudal artery 20–40 μl of blood was collected per animal into 50-μl heparinized hematocrit tubes. After centrifugation, plasma was stored at -20°C. Sodium concentrations were determined by atomic absorption spectrophotometry after appropriate dilution. Calcium concentrations were determined by microtitration in a Marius Calcium Titrator. The plasma osmolarity of 50 μl of pooled samples from two or three fish was measured in a Vogel Micro Osmometer. The means given in Table 1 refer to five pooled samples for each. At the end of the experimental period, the sodium and calcium concentrations and the osmolarity of the media were determined by the same techniques.

For light microscopy the brains and parts of the skin of the lateral body wall in the anal region were removed and fixed for 24 hr in Bouin-Hollande fluid, dehydrated, and embedded in paraffin. Longitudinal sections of the brain and cross-sections of the skin (7 μm thick) were stained with Periodic acid-Schiff (PAS) and Mayer's hemalum. Cell and nuclear indexes, i.e., (maximal length + maximal width)/2, were determined by means of a Leitz ocular micrometer, and the respective volumes were calculated. Twenty-five cells and nuclei were measured per animal, in median sections of the pituitary. The density of the epidermal mucocytes was estimated by counting the number of cells in sections of the skin epithelium with a total length of 150 mm per animal. The thickness of the epidermis was determined in 25 sections per animal. Five fish per group were studied.

For evaluation of the data on cell and nuclear size Wilcoxon's test was used. The other data, as well as all correlation coefficients (Pearson's *r*) presented, were statistically analyzed by Student's *t* test. All tests were two tailed at the 5% level.

For electron microscopy the pituitaries were excised and prefixed in phosphate-buffered (0.1 M; pH 7.2) 3% glutaraldehyde for 10 min at room temperature. The rostral pars distalis was separated from the pituitary and fixed in a similarly buffered solution of 1% osmium tetroxide, 1.5% glutaraldehyde, and 2.5% potassium dichromate, for 1 hr at 0°C. It was post-fixed in 1% uranyl acetate in distilled water, dehydrated in mixtures of ethanol and 1% uranyl acetate,

TABLE 1  
SODIUM AND CALCIUM CONCENTRATIONS AND OSMOLARITY OF BLOOD PLASMA AND EXTERNAL MEDIA<sup>a</sup>

	Na <sup>+</sup> (meq/liter)	Ca <sup>2+</sup> (meq/liter)	Osmolarity (mosm/liter)
(F1) FW, Ca <sup>2+</sup> and Mg <sup>2+</sup> free			
Medium	2.2	< 0.01	5
Plasma <sup>b</sup>	148 ± 6	3.8 ± 0.3	310 ± 6
(F2) FW, controls			
Medium	2.1	0.2	5
Plasma	156 ± 4	4.3 ± 0.4	311 ± 5
(F3) FW + NaCl			
Medium	460	0.3	895
Plasma	163 ± 3	4.2 ± 0.2	343 ± 5
(F4) FW + MgCl <sub>2</sub> (10.2 mmol/liter)			
Medium	2.0	0.3	33
Plasma	153 ± 4	4.5 ± 0.3	314 ± 4
(F5) FW + MgCl <sub>2</sub> (56.0 mmol/liter)			
Medium	2.3	0.1	158
Plasma	168 ± 4	6.4 ± 0.2	327 ± 4
(F6) FW + CaCl <sub>2</sub>			
Medium	2.1	20.6	31
Plasma	159 ± 6	7.5 ± 0.4	316 ± 5
(S1) SW, controls			
Medium	468	21.2	980
Plasma	167 ± 3	6.5 ± 0.4	346 ± 6
(S2) SW, low Ca <sup>2+</sup> /Mg <sup>2+</sup>			
Medium	452	2.3	785
Plasma	153 ± 5	4.6 ± 0.4	338 ± 3

<sup>a</sup> For composition of media, see Materials and Methods. FW, Freshwater; SW, seawater.

<sup>b</sup> Values for blood plasma are mean ± SEM; *n* = 5.

and embedded in Spurr's resin. Ultrathin sections were examined under a Philips EM 200 electron microscope.

The relative extent of the Golgi areas, the length of the membranes of the granular endoplasmic reticulum, and the volume of the mitochondria were determined by morphometrical analysis of electron micrographs. Samples of 500 μm<sup>2</sup> of cytoplasm of each of three animals of groups F2 and S1 were analyzed at a final magnification of 15,000×, using Kontron MOP AMO1 equipment.

## RESULTS

Prolactin cells and skin were studied by light and electron microscopy in freshwater- and seawater-adapted sticklebacks and in specimens adapted to Hale's seawater. This solution of six salts is similar to seawater as far as osmolarity and concentration of the quantitatively most important ions are

concerned. In addition the effects of calcium- and magnesium-free artificial freshwater and seawater were studied, as well as the effects of solutions, in freshwater, of chloride salts of sodium, calcium, and magnesium.

### *Prolactin Cells*

The prolactin-producing acidophilic  $\eta$  cells are located in the rostral pars distalis of the pituitary gland. The secretory activity of these cells was assessed by calculation of cell and nuclear volumes. The results are presented in Fig. 1a and b. In freshwater-adapted fish (F2), cells and nuclei were large. Arrays of granular endoplasmic reticulum were found at the periphery of the cells and around the nu-

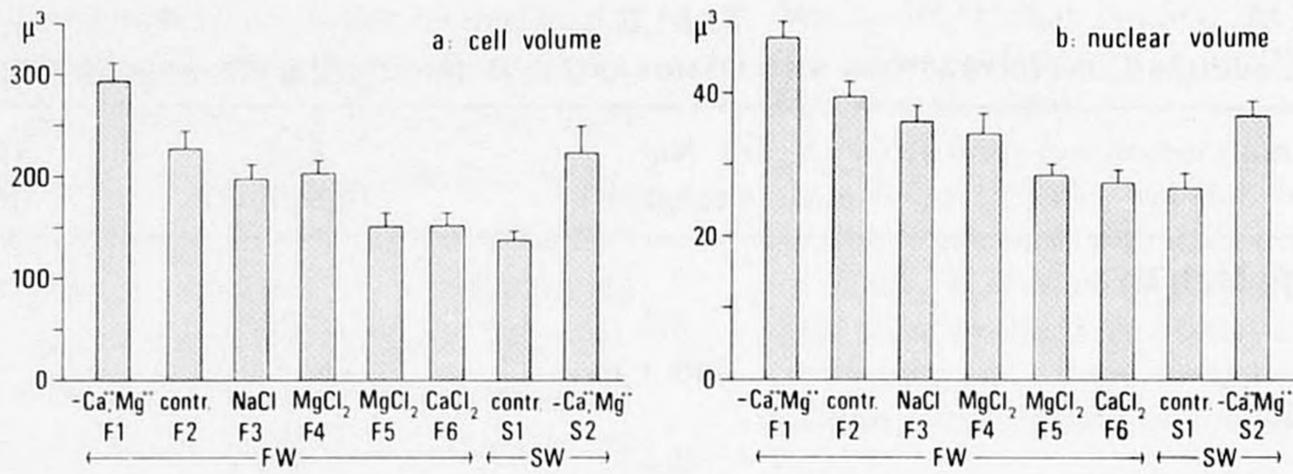


FIG. 1. Cell (a) and nuclear (b) volumes of prolactin cells of fish exposed to several media (mean + SEM;  $n = 5$ ). For details on the media, see Materials and Methods. FW, freshwater; SW, seawater; contr., controls.

cleus. Most Golgi elements were concentrated in a central area (Fig. 2). The elongated Golgi saccules were surrounded by many small clear vesicles, some multivesicular bodies, and a few presecretory granules. The mitochondria were large and showed well-developed cristae. Some mitochondria contained cytoplasmic inclusions when viewed in cross-sections (Fig. 4). These inclusions may represent indentations of the cytoplasm which penetrate deep into the mitochondria. Similar structures have been described as multilamellar organelles by Leatherland (1970). Occasionally, indentations of the outer cell membranes were observed that contained electron-dense material at the outside of the cells. They are suggestive of hormone secretion by exocytosis (Leatherland, 1970).

In seawater fish (S1) cell and nuclear volumes were considerably smaller than in freshwater fish, namely, by 39 and 32% ( $P < 0.01$ ). Stereological analysis of small samples indicated that, per cell, the amount of granular endoplasmic reticulum and the extent of the Golgi areas were less than 40% of the freshwater values. Values for granular endoplasmic reticulum, the extent of the Golgi zones, and total volume of the mitochondria amounted to less than 40% of the values found in freshwater fish. The individual mitochondria were also smaller than those in freshwater fish and the cristae were less extensive. Mitochondria containing cytoplasmic inclusions were not found (Fig.

3). Multivesicular bodies were scarce, and phenomena indicative of exocytosis were hardly observed.

In calcium- and magnesium-free freshwater (F1) the values for cell and nuclear volume of the prolactin cells surpassed the high values found for freshwater fish. In fish exposed to freshwater enriched with sodium (F3) at a concentration present in seawater (410 mmol/liter), cell and nuclear volumes were not significantly different from those for freshwater controls, while at the ultrastructural level the cells showed the normal freshwater appearance. In freshwater containing a relatively low magnesium concentration (10 mmol/liter; F4) the mean values for cell and nuclear volume were slightly below the values of the freshwater controls. However, in freshwater enriched with magnesium (F5) or calcium (F6) at concentrations present in seawater (56 and 10 mmol/liter, respectively), cell and nuclear volumes were about 30% smaller ( $P < 0.01$ ) than those of the freshwater controls and reached values similar to those of the seawater controls. The ultrastructure of the cells showed the features typical for seawater fish (Fig. 6), such as small amounts of granular endoplasmic reticulum, small Golgi areas with low numbers of Golgi vesicles, and absence of the phenomena of exocytosis. In addition, it was observed that presecretory granules were exceptionally scarce, while autophagous vacuoles were occasionally seen (Fig.

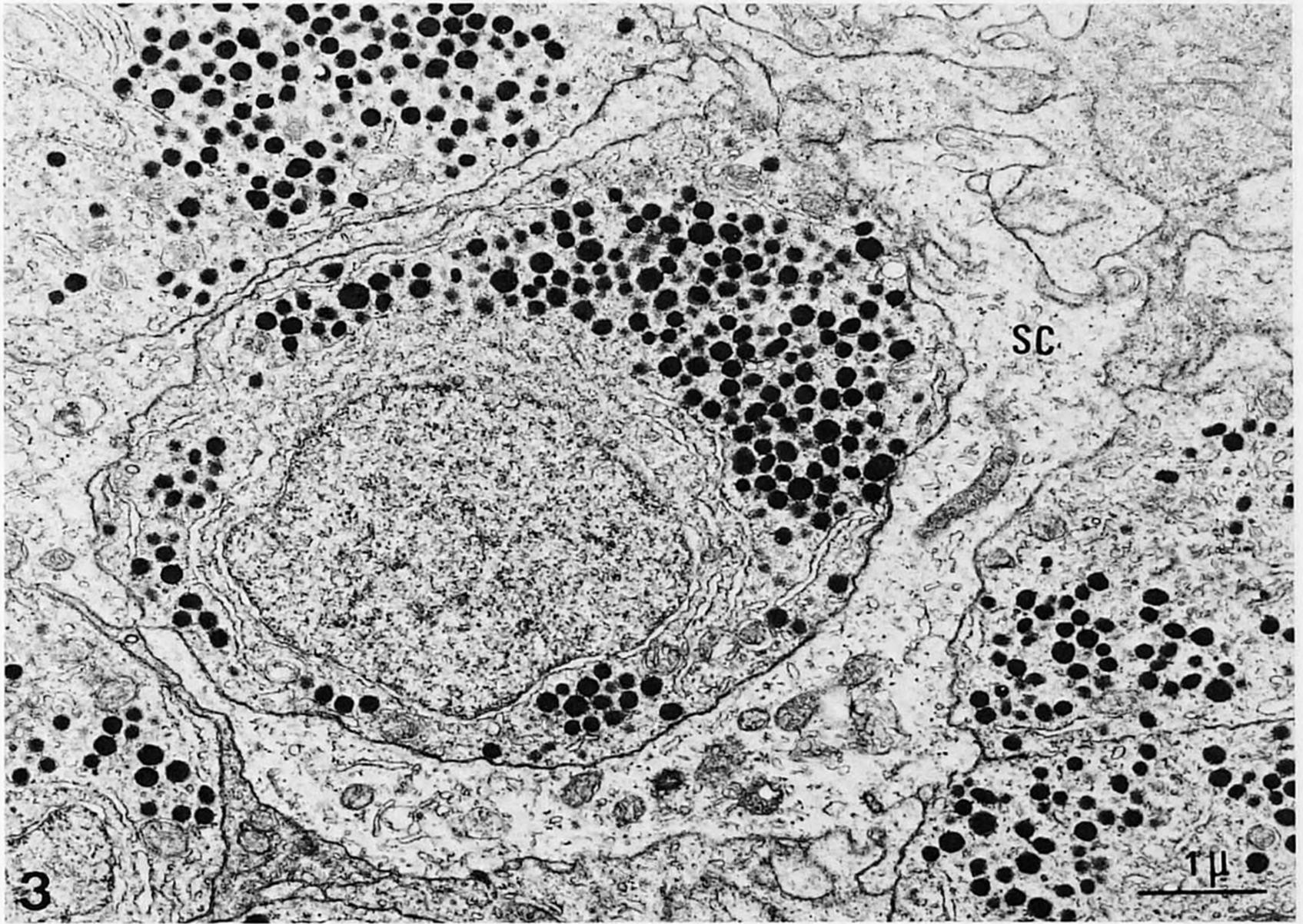
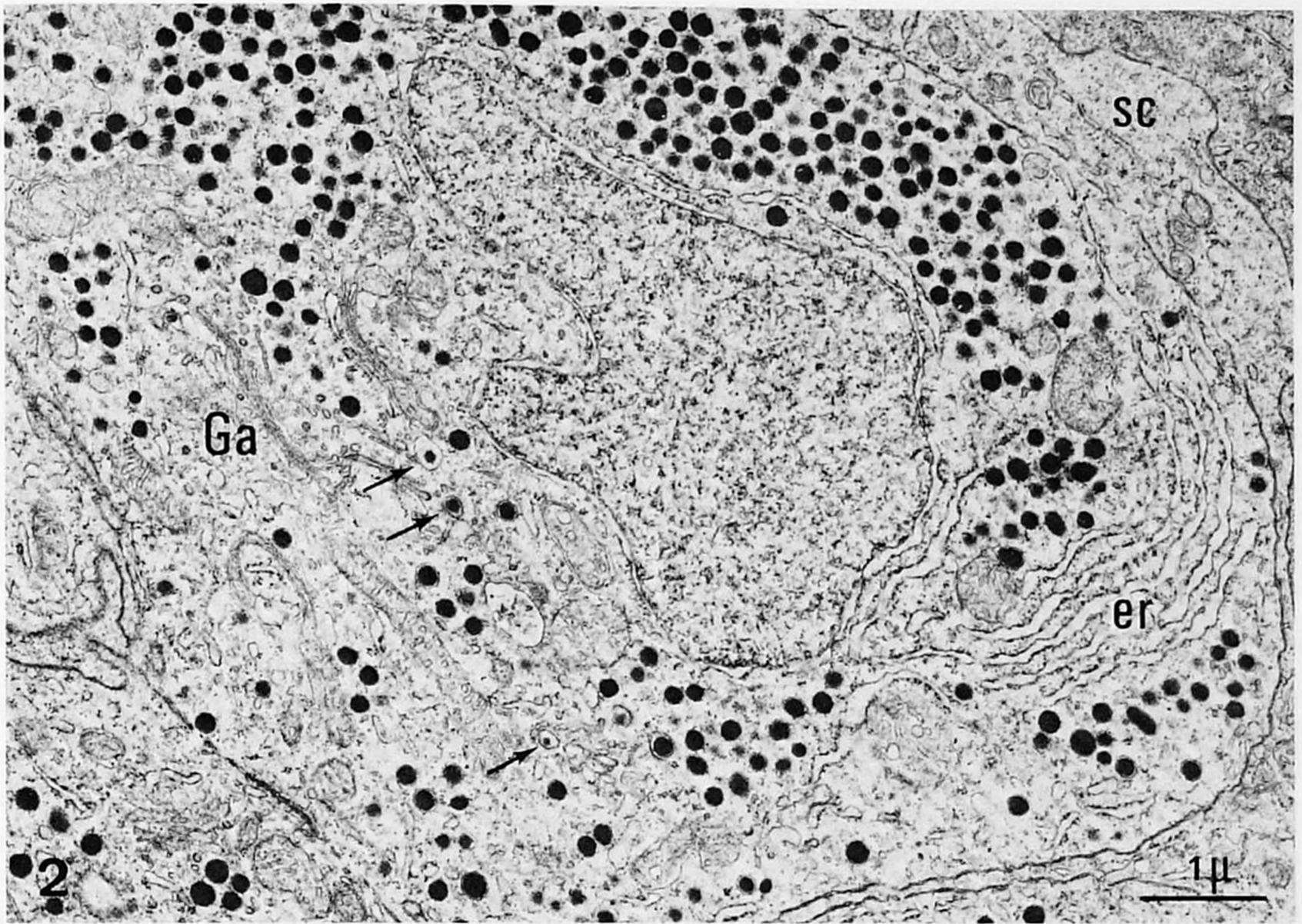


FIG. 2. Prolactin cell of freshwater control: er, endoplasmic reticulum; Ga, Golgi area; arrows, presecretory granules; sc, stellate cells.

FIG. 3. Prolactin cells of seawater control: sc, stellate cells.

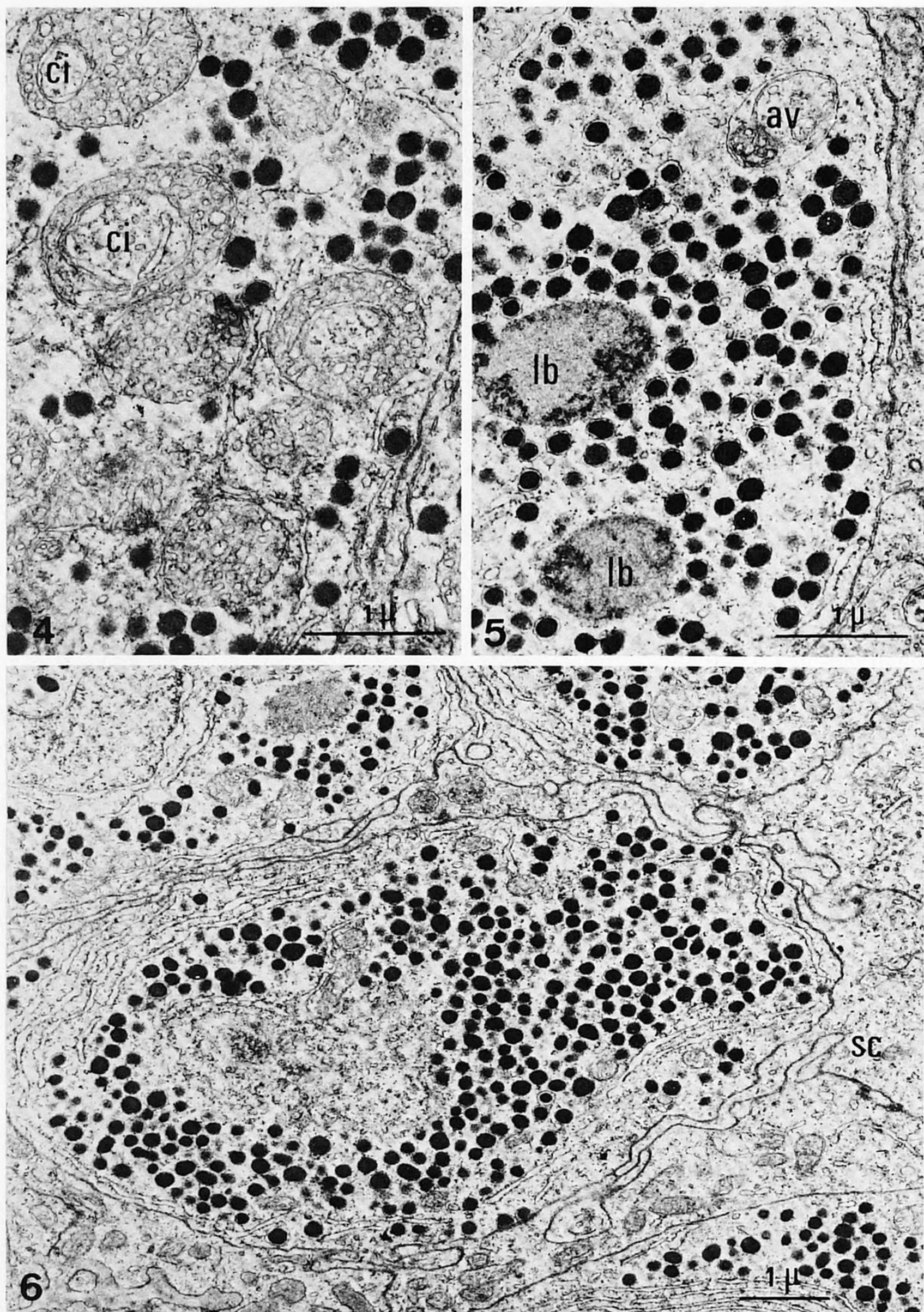


FIG. 4. Mitochondria with cytoplasmic inclusions (ci) in a prolactin cell of a freshwater fish.

FIG. 5. Lysosome-like bodies (lb) and autophagous vacuole (av) in a prolactin cell of a fish adapted to calcium-enriched freshwater.

FIG. 6. Prolactin cells of specimen adapted to calcium enriched freshwater. Sc, Stellate cells.

5). Such vacuoles were not found in seawater or freshwater controls. Lysosome-like dense bodies were also more numerous than in seawater or freshwater fish. These observations point to intracellular digestion of superfluous cell organelles in the prolactin cells during exposure of fish to high levels of calcium and magnesium. In fish from seawater with low calcium and magnesium levels (S2) cell and nuclear volumes differed significantly from those of the seawater controls ( $P < 0.01$ ) and reached values as high as those of freshwater fish.

#### Mucocytes and Skin

The multilayered epithelium of the skin contained many cells producing mucus with a strong affinity for PAS. They were distributed in the upper layers of the epithelium, often in contact with the exterior. The density of the mucocytes varied considerably in different parts of the epidermis, but proved to be fairly constant in the lateral body wall around the anal opening. It was in this area that the density was determined. In sticklebacks adapted to freshwater (F2) the density of these cells (Fig. 7a) was higher than in seawater fish (S1;  $P < 0.01$ ). In the skin of fish exposed to calcium- and magnesium-free freshwater (F1) these cells were more numerous than in freshwater controls ( $P < 0.001$ ). In sodium-enriched freshwater (F3) the density of these cells was unchanged when compared to the con-

trol value, but in magnesium-enriched and, more especially, calcium-enriched freshwater (F5, 6) the numbers of these cells had decreased ( $P < 0.01$ ). The cells occurred at the low densities characteristic of seawater fish. The thickness of the skin epithelium (Fig. 7b) showed differences between the various experimental groups which paralleled the differences in the density of the mucocytes (correlation coefficient  $r$ : 0.88;  $P < 0.01$ ). The differences ( $P < 0.01$ ) between the low epithelium heights in fish in the calcium- and magnesium-rich solutions (F5, F6, S1), as compared to those of fish in the media with low levels of these cations (F1, F2, S2), were due to flattening of the cells in the upper epithelial layers. The density of the mucocytes as well as the thickness of the epithelium were both highly correlated with prolactin cell size ( $r$ : 0.96 and 0.93;  $P < 0.001$ ).

#### Plasma Sodium, Calcium, and Osmolarity

Despite the large variations in sodium content and osmolarity of the media, the corresponding plasma values showed only relatively small differences (Table 1). The plasma osmolarities of fish exposed to the high sodium solution (F3) and to both seawater solutions (S1, S2) differed significantly from the value of the freshwater control group ( $P < 0.01$ ). Relatively large variations occurred in plasma calcium levels. In fish from media with high calcium

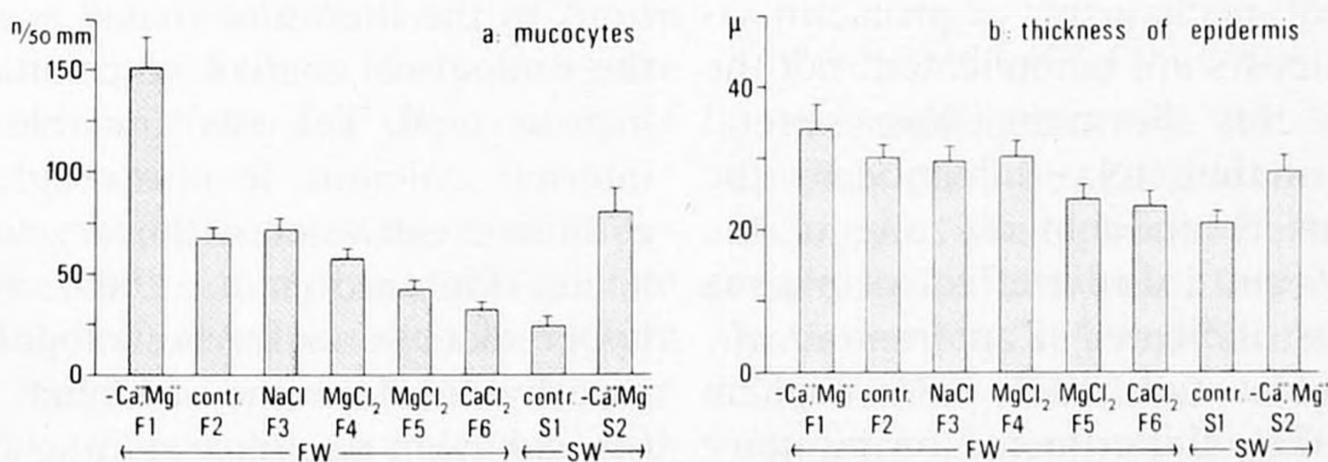


FIG. 7. Density (a) of mucocytes (number of cells found in cross-sections of the epidermis with a total length of 50 mm/animal) and thickness (b) of the epidermis of fish exposed to various media (mean + SEM;  $n = 5$ ). For details on the media see Materials and Methods. FW, freshwater; SW, seawater; contr., controls.

and/or magnesium concentrations (F5, F6, S1) plasma calcium levels were substantially higher ( $P < 0.01$ ) than in low calcium and magnesium solutions (F1, F2, F3, S2). Internal ionic calcium as well as sodium concentrations, but not plasma osmolarity, were negatively correlated with prolactin cell volume ( $r: -0.85$  and  $-0.86; P < 0.01$ ).

## DISCUSSION

### *Control of Prolactin Secretion*

Prolactin cells in sticklebacks appeared to be better developed in freshwater than in seawater specimens. Our structural data agree with those reported for the same species (Leatherland, 1970; Benjamin, 1974) and many other teleosts (Schreibman *et al.*, 1973). They point to a higher rate of prolactin secretion in freshwater fish. The low activity of prolactin cells in teleosts from the sea or from hypersaline lagoons has been related to the high salinity of the environment (Schreibman *et al.*, 1973; Nagahama *et al.*, 1975). Our data on sticklebacks show that low prolactin secretion in seawater is related to the presence of high external concentrations of calcium and magnesium. In freshwater solution, both ions reduce prolactin cell activity, although in solutions of the same concentration calcium is more effective. Thus, in a natural freshwater environment ionic calcium is likely to be the decisive environmental factor in the control of prolactin secretion, as far as the role of this hormone in osmoregulation is concerned.

The control mechanisms of prolactin secretion in teleosts are complicated, not the least since this hormone has several functions. An inhibitory influence by the hypothalamus is undoubtedly one of the mechanisms, but a direct effect of plasma factors is also indicated (Zambrano *et al.*, 1974; Nagahama *et al.*, 1974, 1975; Wigham and Ball, 1977). Experiments on pituitary glands *in vitro* and on pituitary transplants have led to the hypothesis that the effect of environmental salinity on prolactin secre-

tion is mediated by changes in plasma osmolarity or sodium concentration (Nagahama *et al.*, 1974, 1975; Wigham and Ball, 1977). Incubation of teleost pituitary glands in hyposmotic fluids activates the prolactin cells, as was concluded from the observation of proliferation of cellular organelles, increase in exocytosis, reduction of prolactin content of the pituitary glands, and a rise in bioassayable prolactin in the incubation fluid (Zambrano *et al.*, 1974; Nagahama *et al.*, 1975). A reduction of prolactin cell activity has been reported after an increase in the sodium content of the culture medium, although not for the prolactin cells of all species examined (Nagahama *et al.*, 1974; Baker and Ingleton, 1975). Our results show a negative correlation of prolactin cell activity to both plasma sodium and plasma ionic calcium. No indications were found that plasma osmolarity is involved in the control of prolactin cell activity. Thus, in this respect the above mentioned *in vitro* results do not apply to the *in vivo* situation of sticklebacks. Whether sodium or calcium is the primary plasma factor in the control of prolactin secretion cannot be concluded from the present experiments. This problem is under investigation.

### *Prolactin and Plasma Calcium Ions*

Although the majority of the investigations on teleost prolactin have been focused on the effects of this hormone on water and sodium balance, there are several indications in the literature that it is involved in the endocrine control of calcium metabolism as well. Teleosts are able to control internal calcium levels very efficiently, even in freshwater with very low calcium levels (Pickford *et al.*, 1969). However, a hypercalcemic factor homologous with the parathyroid hormone of higher vertebrates has not been demonstrated so far. In teleosts the pituitary gland is likely to be responsible for the maintenance of high plasma calcium levels in a low-calcium en-

vironment. Hypophysectomy leads to reduction of the ionic calcium concentration, in addition to low sodium and chloride levels. This effect has been demonstrated in several freshwater species (Chan *et al.*, 1968; Ogawa, 1968; Pang *et al.*, 1973). Injections of prolactin and, to a lesser extent, cortisol enhance the levels of all these ions (Pang *et al.*, 1973). Pang and co-workers concluded that prolactin has a distinct hypercalcemic action. This effect may be antagonistic to that of the hormone produced in the Stannius corpuscles, hypocalcin. Our finding in sticklebacks of an inverse relationship between prolactin cell activity and external as well as internal calcium levels is in line with the presumption of a hypercalcemic function for prolactin.

#### *Prolactin and Environmental Calcium*

The calcium concentration of the environment represents an important factor for the ecological distribution of teleosts (Evans, 1975). Maintenance of ionic balance in freshwater fish requires more energy when calcium levels are low (Eddy, 1975). Freshwater adaptation of euryhaline species is greatly facilitated by the presence of calcium ions and several stenohaline marine fish can even tolerate freshwater if the calcium concentration is sufficiently high (Hulet *et al.*, 1967; Carrier and Evans, 1976). The degree of euryhalinity of a fish is mainly determined by the efficiency of its mechanisms to regulate sodium permeability of the integument in the absence of high external calcium levels (Evans, 1975). In this condition prolactin is likely to be the predominant hormone for the control of these mechanisms.

There are some interesting similarities between the effects of high external calcium levels and the effects of prolactin on sodium balance. Pickford *et al.* (1966) demonstrated that, in hypophysectomized killifish (*Fundulus kansae*) exposed to deionized water, both the addition of calcium to the medium and injection of prolactin were ef-

fective in raising internal sodium levels. There is evidence from several freshwater species that external calcium and prolactin are able to maintain plasma osmolarity and sodium concentration, although to a varying extent in different species. The main mechanism involved is the reduction of ion fluxes over the integument (Potts and Fleming, 1971; Ensor and Ball, 1972; Evans, 1975; Carrier and Evans, 1976; Oduleye, 1976). Magnesium has a similar action, although it is less effective than calcium (Cuthbert and Maetz, 1972; Eddy, 1975). Both cations can influence structure and function of membranes by modifying their macromolecular organization, including enzymatic activities (Azzi *et al.*, 1975). Whether the actions of these ions and of prolactin on ion fluxes are similar at the molecular level remains to be established.

An increase in external calcium and magnesium reduces the permeability for water of the teleost integument (Potts and Fleming, 1970). In his studies of the isolated gills of the Japanese eel Ogawa (1975, 1977) showed that bovine prolactin has the same effect as external calcium ions on water balance. Both reduce osmotic water influx and osmotic permeability to water. However, in the killifish *Fundulus kansae* (Potts and Fleming, 1970) and brown trout (*Salmo trutta*; Oduleye, 1975), ovine prolactin has been found to increase water permeability and it has been concluded that prolactin and calcium ions have opposing actions on water balance.

#### *Prolactin and the Epidermis*

In sticklebacks the thickness of the epithelium of the skin and the density of the mucocytes are higher in freshwater than in seawater. A similar observation has been made by Mattheij and Stroband (1971) on *Cichlasoma biocellatum*. Mucocytes, like the nonsecretory epidermal cells, differentiate from cells originating in the basal layer of the epithelium of the skin. In trout (*Salmo trutta*) it takes about a week for the

cells to reach the superficial layers and start the release of mucus on the surface of the skin. They degenerate after some days (Pickering, 1976). Thus, it may be assumed that the life span of the mucocytes in sticklebacks is less than the duration of our experiments.

In trout and char (*Salvelinus alpinus*) a positive correlation has been found between the density of the epidermal mucocytes and the amount of mucus present on the skin (Pickering, 1974). This mucous layer has been thought to act as a protective barrier in an environment of low salinity (Johnson, 1973). Our data show, however, that, in sticklebacks, thickness of the epidermis and density of the mucocytes are not influenced by external osmolarity or sodium levels. Both parameters are inversely related to the external concentrations of calcium and, to a lesser extent, magnesium. Moreover, they are positively correlated with prolactin cell activity. These observations are in line with the presumption, based on the results of prolactin injections in intact and hypophysectomized fish (Mattheij and Stroband, 1971; Olivereau and Lemoine, 1971; Marshall, 1976), that thickness of the skin, density of the mucocytes, and thickness of the slime layer covering the skin are controlled by prolactin in many species. Control by prolactin was, however, not indicated in all species studied (Bern, 1967; Marshall, 1976).

The observed relationship among density of mucocytes, external calcium concentration, and prolactin cell activity in the stickleback is of interest since teleost mucus has specific calcium binding properties (Chartier, 1973). The presence of a mucous coating may therefore enhance the calcium concentration at the surface of the integument. It may maintain therefore a low rate of permeability for water and ions even in a low-calcium environment. As mucus secretion is likely to be controlled by prolactin, it is possible that this hormone is also responsible for the calcium binding activity

of the slime layer. Such a mechanism would explain the similarity between the effects of external calcium ions and prolactin on sodium permeability of the integument.

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