

## THE EFFECT OF HYPOPHYSECTOMY AND PROLACTIN THERAPY ON WATER BALANCE OF THE BROWN TROUT *SALMO TRUTTA*

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### SUMMARY

A technique for the hypophysectomy of the brown trout is described which might be applicable to other salmonids. Hypophysectomy produced a decrease in water turnover which was corrected by prolactin therapy.

Hypophysectomy resulted in a decrease in the net flux of osmotic water. Both osmotic and diffusional water permeabilities were evenly decreased after hypophysectomy, such that the ratio of the osmotic and diffusional permeability coefficients ( $P_{os}/P_d$ ) retained a value of approximately unity.

The data suggest that calcium and prolactin have opposing actions on water balance and that their modes of action are likely to be separate.

### INTRODUCTION

In higher vertebrates such as the mammals, the complex processes of homeostasis are under hormonal control, particularly by the pituitary hormones. The possibility of pituitary involvement in teleostean osmoregulation was first proposed by Fontaine, Callamand & Olivereau (1949). Since then, a great deal of evidence has accumulated showing the importance of pituitary hormones in hydromineral regulation in teleosts (Olivereau & Ball, 1970).

Attempts have been made to demonstrate the pituitary hormones involved in hydromineral regulation of teleosts and to explain the mechanism of their action. Pickford & Phillips (1959) were the first to demonstrate that prolactin is essential for the freshwater survival of hypophysectomized *Fundulus heteroclitus*. Prolactin has also been shown to prevent the fall in plasma electrolytes following hypophysectomy (Ball & Ensor, 1965). Apparently, prolactin, more so than any other pituitary hormone, is important in the maintenance of homeostasis in fresh water.

No clear picture has emerged from attempts to locate the site of action of prolactin in teleosts. In *Fundulus heteroclitus* (Burden, 1956) and *Beta splendens* (Schreibman & Kallman, 1965), prolactin acts on epidermal mucous cells. In *Fundulus kansae*, the kidney is the target organ for prolactin action (Stanley & Fleming, 1963, 1965) but not in *Fundulus heteroclitus* (Maetz *et al.* 1967).

Maetz (1970) suggested that the impairment of water balance is less important

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than the alteration of the electrolyte balance in freshwater-adapted hypophysectomized teleosts. That is, the primary effect of prolactin is on the electrolyte fluxes. Lahlou & Giordan (1970) observed a reduction in diffusion and osmotic water fluxes in the goldfish after hypophysectomy. In *Fundulus kansae*, there is a 20% reduction in tritiated water turnover following hypophysectomy (Potts & Fleming, 1970). There is no doubt therefore that water balance, as well as electrolyte balance, is affected by hypophysectomy and that both factors may be equally important.

This study was carried out to assess the relative roles of environmental calcium and the pituitary on water balance in the brown trout. This paper deals with the role of the pituitary.

#### MATERIALS AND METHODS

The source, maintenance of fish and ionic composition of media have been described elsewhere (Oduleye, 1975). Fish stocks were maintained in the aquarium at  $15 \pm 1$  °C. This was the temperature at which all experiments were performed. The experimental techniques for the determination and the calculation of water content, and diffusional and osmotic permeabilities have also been described (Oduleye, 1975).

The technique of hypophysectomy was similar to that of Donaldson & McBride (1967), except that it was not found necessary to drill through the eye muscles. A No. 3 round ash burr (Claudius Ash Ltd.) was used to drill through the parasphenoid. The drilled hole was large enough to allow two fine probing needles to be used to part the underlying muscle and reveal the pituitary foramen. The tip of a 14 G hypodermic needle was then used to suck out the pituitary.

In preliminary experiments, drilling through the eye muscle caused severe trauma, unnecessary damage and impaired swimming ability after the operation. The undesirable effects of the operation were severe postural disability resulting from brain damage, brain haemorrhage, and excessive loss of blood. The first of these was prevented by probing for the pituitary fossa very cautiously, breaking the membrane gently and applying very gentle suction. Brain haemorrhage and excessive blood loss were prevented by performing the operation in two stages: first, drilling the parasphenoid, then removing the pituitary one or two days later.

It must be emphasized that at no stage of the operation was it possible to view the pituitary directly with a microscope because it was obscured by the mass of tissue lying between it and the opening made on the parasphenoid. Only animals whose whole pituitary were removed at the first attempt were used for experiments. Extirpated organs were viewed under a microscope immediately to ensure that they were intact and that hypophysectomy was complete. When an organ was not intact, the fish was abandoned and not used in the experiments.

Hypophysectomized fish were routinely maintained in 33% sea water to recover before being used for an experiment. Survival in this medium was indefinite (Oduleye, 1973).

Ovine prolactin was administered as a solution in 0.6% saline and was injected into the peritoneal cavity with a fine gauge micrometer-driven syringe. 0.2 i.u./g fish in 5  $\mu$ l saline was injected. Prolactin was administered every other day for 2 weeks, beginning the day after operation. The highly purified ovine prolactin preparation was obtained from Ferring AB Malmö, Sweden. In experiments where

prolactin was administered, it was necessary to start the therapy the day after hypophysectomy. In preliminary experiments when therapy was delayed for 1 week to allow the fish to recover from the shock of the operation, there was no response to the therapy. In the eel *Anguilla anguilla* similar maintenance therapy is necessary to prevent the fall in plasma sodium and calcium after hypophysectomy (Olivereau & Chartier-Baraduc, 1966).

Water content of prolactin-treated hypophysectomized fish was determined the day after the 7th injection. In order to assess the effect of calcium on the water content of hypophysectomized trout in fresh water, the following procedure was followed. Fish were hypophysectomized and kept in 33% sea water containing varying concentrations of calcium for 2 weeks. One group was maintained in 33% sea water containing 0.3 mM-Ca<sup>2+</sup> (the same as in normal tap water), another group was maintained in 33% sea water containing 5 mM-Ca<sup>2+</sup> and a third in 33% sea water containing 10 mM-Ca<sup>2+</sup>. A sham-operated group was kept in 33% seawater containing 0.3 mM-Ca<sup>2+</sup> as control. After 2 weeks, each group was transferred separately into a freshwater tank containing the appropriate concentration of calcium. Two days after this, the fish were killed and their water content established. The fish were allowed only 2 days in fresh water because survival was limited to only a few days.

The maintenance of hypophysectomized fish prior to the determination of tritiated water flux rate, urine flow rate and drinking rate was similar to the above procedure. For urine flow rate, due to the difficulties of cannulation, fairly large fish were required. As a result, the effect of prolactin therapy on urine flow rate was not examined, as fairly large doses of prolactin would be required and the supply of the hormone was limited.

## RESULTS

### (1) *Diffusion permeability*

#### (a) *Water space*

The effect of hypophysectomy and prolactin therapy on total body water in the brown trout is shown in Table 1. Hypophysectomy produced an increase in body water from 76 to 78%. The slight increase was not statistically significant. When prolactin was administered, there was no change in water content. Maintenance of hypophysectomized fish in media of varying calcium content also had little effect on total body water (Table 2).

#### (b) *Tritiated water flux*

The effect of hypophysectomy and prolactin therapy on tritiated water flux is shown in Table 3. Intact fish in fresh water had an exchange rate of 81% h<sup>-1</sup> while sham-hypophysectomized ones exchanged at a rate of 78% h<sup>-1</sup>. There was no statistically significant difference between the two groups. On the other hand, both hypophysectomized and saline-injected hypophysectomized groups exchanged at the rates of 59 and 62% h<sup>-1</sup> respectively. This is a decrease of about 30%. The exchange rate for the prolactin-injected group was 77% h<sup>-1</sup>. This was lower than the intact and sham-operated groups, although there was no significant difference between the three groups.

Table 1. *Water content in intact and hypophysectomized brown trout*

Group	Body water (%)
Unoperated $n = 5$	*75.8 ± 0.2
Operated and prolactin-treated, $n = 6$	*76.0 ± 0.3
Operated and saline-injected, $n = 9$	*78.2 ± 0.6

The means ± s.e. are given.  $n$  = number of measurements.

\* No significant difference ( $P > 0.05$ ).

Table 2. *Effect of calcium on water space of hypophysectomized brown trout*

Group	Body water (%)
Intact (FW; 0.3 mM-Ca <sup>2+</sup> ), $n = 5$	*75.8 ± 0.2
Sham-operated (FW; 0.3 mM-Ca <sup>2+</sup> ), $n = 8$	*75.2 ± 0.07
Operated (FW; 0.3 mM-Ca <sup>2+</sup> ), $n = 9$	*77.1 ± 0.09
Operated (FW; 5.0 mM-Ca <sup>2+</sup> ), $n = 8$	*76.4 ± 0.06
Operated (FW; 10 mM-Ca <sup>2+</sup> ), $n = 7$	*75.5 ± 0.04

The mean ± s.e. are given.  $n$  = number of determinations.

\* No significant difference ( $P > 0.05$ ).

Table 3. *Effect of hypophysectomy and prolactin therapy on diffusion water flux*

Group	K	Diffusion flux (ml h <sup>-1</sup> 100 g <sup>-1</sup> )	$P_d$ (μm.sec <sup>-1</sup> )
Unoperated, $n = 5$	81 ± 1.0	60.5	0.5
Sham-operated, $n = 5$	78 ± 0.5	58.8	0.48
Operated and prolactin-treated, $n = 9$	77 ± 1.4	57.8	0.47
Operated and saline injected, $n = 9$	*62 ± 2.0	46.5	0.38
Operated, $n = 7$	*59 ± 1.9	44.3	0.36

K = efflux constant (% h<sup>-1</sup> ± s.e.m.).  $n$  = number of determinations.

\* Significantly different from corresponding values in other groups ( $P < 0.05$ ) but not significantly different from each other ( $P > 0.05$ ).

$P_d$  = Diffusional permeability coefficient.

Table 4. *Effect of calcium on diffusion water flux of hypophysectomized brown trout*

Group	K	Diffusion flux (ml h <sup>-1</sup> 100 g <sup>-1</sup> )	$P_d$ (μm.sec <sup>-1</sup> )
Sham-operated (FW; 0.3 mM-Ca <sup>2+</sup> ), $n = 11$	80 ± 0.3	60.0	0.49
Operated (FW; 0.3 mM-Ca <sup>2+</sup> ), $n = 10$	*56 ± 0.2	42.0	0.34
Operated (FW; 5.0 mM-Ca <sup>2+</sup> ), $n = 9$	*†42 ± 0.7	31.5	0.26
Operated (FW; 10 mM-Ca <sup>2+</sup> ), $n = 10$	*†41 ± 1.0	30.8	0.25

K = efflux constant (% h<sup>-1</sup> ± s.e.m.).  $n$  = number of determinations.

\* Significantly lower than the sham-operated group ( $P < 0.05$ ).

† Not significantly different from one another ( $P > 0.05$ ) but significantly lower than corresponding values in the other two groups ( $P < 0.05$ ).

Table 5. *Effect of calcium on osmotic net flux in hypophysectomized brown trout*

Group	Drinking rate	Urine flow rate	Osmotic net flux
Sham-operated (FW; 0.3 mM-Ca <sup>2+</sup> )	39.2 ± 6.1 (5)	310 ± 18 (6)	270.8
Operated (FW; 0.3 mM-Ca <sup>2+</sup> )	17.9 ± 9.3 (6)	222 ± 5 (7)	204.1
Operated (FW; 5.0 mM-Ca <sup>2+</sup> )	20.3 ± 5.6 (6)	186 ± 10 (9)	165.7
Operated (FW; 10 mM-Ca <sup>2+</sup> )	16.5 ± 7.5 (5)	153 ± 6 (9)	136.5

The means ± s.e. are given. Number of measurements are in parentheses. All values given in  $\mu\text{l h}^{-1} (100\text{ g})^{-1}$ .

While sham-operated control fish had an efflux rate of 80% h<sup>-1</sup> in fresh water, the hypophysectomized group had a rate of 56% h<sup>-1</sup>. An increase in the calcium content in fresh water from 0.3–5 mM resulted in a significant decrease of water flux to 42% h<sup>-1</sup> (Table 4). Fish maintained at 10 mM-Ca<sup>2+</sup> had a slightly lower efflux rate (41% h<sup>-1</sup>) compared with those maintained in 5 mM-Ca<sup>2+</sup> fresh water. A comparison of Tables 3 and 4 shows that while calcium reduced the water permeability of hypophysectomized trout in fresh water, prolactin increased it.

(2) *Osmotic permeability*

(a) *Drinking rate*

Sham-operated fish had a drinking rate of 39.2  $\mu\text{l h}^{-1} (100\text{ g})^{-1}$  compared with 17.9 for hypophysectomized fish in the same medium (Table 5). In 5 mM-Ca<sup>2+</sup> fresh water, the rate of drinking was 20.3  $\mu\text{l h}^{-1} (100\text{ g})^{-1}$  while at 10 mM-Ca<sup>2+</sup> it was 16.5  $\mu\text{l h}^{-1} (100\text{ g})^{-1}$ . Drinking rate in all the groups was highly variable as shown by the magnitude of the s.e.m.

(b) *Urine flow rate*

The effect of hypophysectomy and environmental calcium on urine flow pattern is shown in Fig. 1. The average flow rates for each group are summarized in Table 5. Urine flow in the first few hours following cannulation was very high in the sham-operated group, reaching a value of about 10 ml kg<sup>-1</sup> h<sup>-1</sup> during the second hour. This gradually fell and became relatively stabilized after about 16 h. This marked diuresis is similar to that of intact fish (Oduleye, 1975).

Hypophysectomized fish in fresh water (0.3 mM-Ca<sup>2+</sup>) did not exhibit marked diuresis. In 5 mM-Ca<sup>2+</sup> fresh water, although the sharp peak in the first few hours after cannulation characteristic of diuresis was absent, urine flow rate in the first 24 h period was much higher than that of the following period.

Table 6 gives the permeability coefficient values of hypophysectomized fish in different calcium concentrations of fresh water. To a first approximation  $P_{\text{os}}/P_{\text{d}} = 1$  in all groups. Hypophysectomy and environmental calcium caused a reduction in both osmotic and diffusional water flows. The reduction in both water flows was

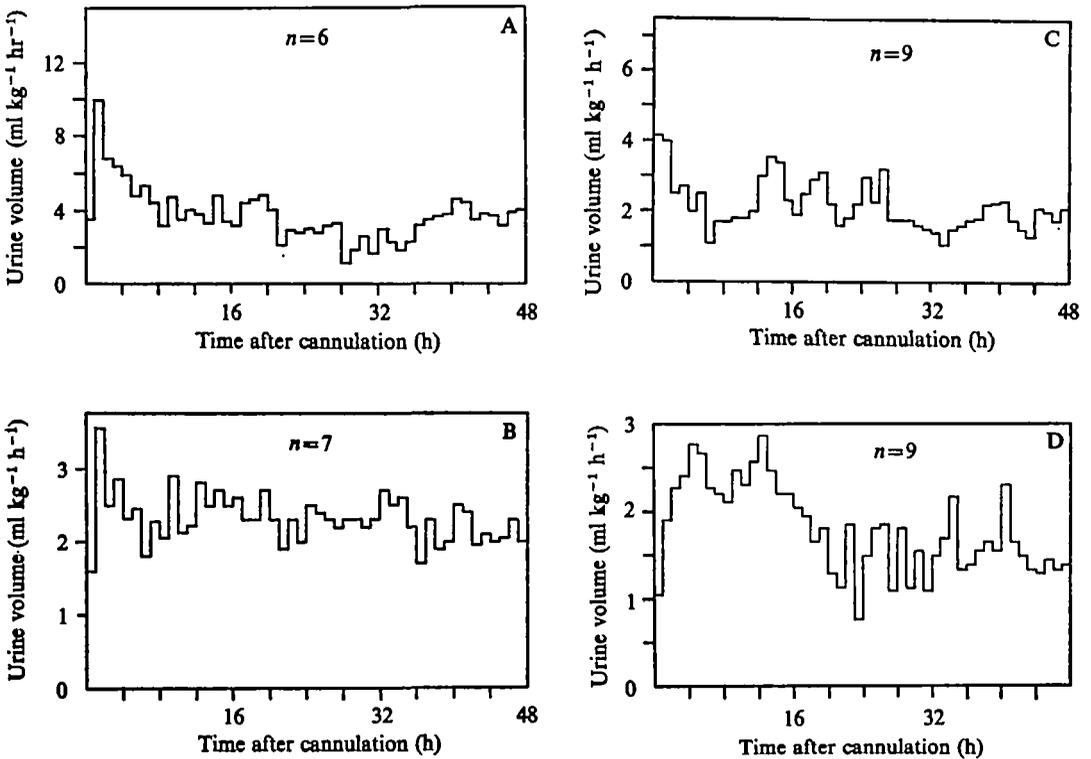


Fig. 1. The effect of calcium on urine flow pattern of hypophysectomized brown trout in fresh water. (A) Sham-operated (FW; 0.3 mM-Ca<sup>2+</sup>). (B) Operated (FW; 0.3 mM-Ca<sup>2+</sup>). (C) Operated (FW; 5 mM-Ca<sup>2+</sup>). (D) Operated (FW; 10 mM-Ca<sup>2+</sup>). *n* = number of individual fish.

Table 6. Comparison of the effect of calcium on osmotic ( $P_{os}$ ) and diffusional ( $P_d$ ) permeability coefficients of the gill of hypophysectomized brown trout in fresh water

Group	$P_{os}$ ( $\mu\text{m} \cdot \text{sec}^{-1}$ )	$P_d$ ( $\mu\text{m} \cdot \text{sec}^{-1}$ )	$P_{os}/P_d$
Sham-operated (FW; 0.3 mM-Ca <sup>2+</sup> )	0.40	0.49	0.82
Operated (FW; 0.3 mM-Ca <sup>2+</sup> )	0.30	0.36	0.83
Operated (FW; 5.0 mM-Ca <sup>2+</sup> )	0.24	0.26	0.92
Operated (FW; 10 mM-Ca <sup>2+</sup> )	0.20	0.25	0.80

however nearly identical, resulting in very little shift in the  $P_{os}/P_d$  ratio. This is similar to the effect of calcium in intact fish (Oduleye, 1975).

## DISCUSSION

### Water content

Following hypophysectomy there was a slight increase in the body water of hypophysectomized brown trout. This increase in water content is in agreement with the results of Burden (1956), who observed an average weight increase of about 7% in hypophysectomized *Fundulus heteroclitus*. In the goldfish, there is a

weight increase of about 7% following hypophysectomy (Lahlou & Giordan, 1970). The parietal muscle of the eel becomes significantly hyperhydrated after hypophysectomy. This hyperhydration is corrected after prolactin injection although cortisol and ACTH are ineffective (Chan, Chester Jones & Mosley, 1968). Since the bulk of the water space in the brown trout is located in the muscles, it can be inferred that the increase in body weight is a result of the accumulation of water in the muscles. The weight increase following hypophysectomy could be due to a fall in plasma osmotic pressure leading to increased hydration of tissues.

#### *Diffusional water flux*

After hypophysectomy there was a reduction of about 25% in the tritiated water flux of the brown trout, which returned to normal after prolactin therapy. In the goldfish, hypophysectomy results in a reduction of water permeability and this is corrected by injections of ACTH, prolactin or cortisol. In contrast to the action of ACTH, prolactin and cortisol, arginine vasotocin (AVT) decreases tritiated water flux both in intact and hypophysectomized fish (Lahlou & Giordan, 1970). Potts & Fleming (1970) observed a 20% decrease in the tritiated water flux of freshwater-adapted *Fundulus kansae* following hypophysectomy. This hypophysectomy-induced reduction in water turnover was abolished by ovine prolactin therapy.

It is difficult to make a generalized statement about the effect of hypophysectomy and prolactin therapy on diffusional water flow in teleosts, as only a very few species have been investigated. It is very likely, however, that a decrease in branchial water permeability follows hypophysectomy and that this can be corrected by prolactin therapy. The increased accumulation of water in the tissues and the plasma will lead to haemodilution, but it is unlikely that this will completely explain the large drop in plasma electrolyte concentration which always follows hypophysectomy. Slight haemodilution and a state of negative electrolyte balance could, however, explain the phenomenon.

Maintenance of hypophysectomized brown trout in fresh water containing high levels of  $\text{Ca}^{2+}$  produced an even more drastic reduction in diffusional water flux (about 50% reduction compared with sham-operated control). In intact fish, external calcium produced a similar effect. In intact *Fundulus kansae*, external calcium reduces water permeability (Potts & Fleming, 1970). The effect of external calcium concentration on the rate of diffusional water flux in hypophysectomized teleosts has not been studied before. From the present results, increased environmental calcium apparently complicated the water-balance problem of the hypophysectomized brown trout in fresh water. This deduction may not be entirely true as the same treatment reduced the hypophysectomy-induced hydration and enabled hypophysectomized fish to maintain normal water content. The effect of calcium in reducing tritiated water permeability was in direct opposition to that of prolactin. It is probable that calcium and prolactin are to some extent antagonistic (Potts & Fleming, 1970). In the isolated urinary bladder of the toad *Bufo marinus*, pituitrin increases the rate of water movement (Bentley, 1958) while external calcium decreases it (Bentley, 1959). Anti-diuretic hormone (ADH) and calcium have similarly opposing effects on the equivalent pore radius of kidney slices of *Necturus maculosus* (Whittembury, Sugino & Solomon, 1960). It is difficult to substantiate a direct competition between

calcium and prolactin or indeed any other hormones. It is highly probable, however, that calcium decreases membrane permeability to water by affecting the passive barrier to water flow (e.g. mucus). It has also been suggested that hypophysectomy decreases the activity of membrane mucous cells (Burden, 1956; Schreiberman & Kallman, 1965). If this and the hypothesis about mucus being a diffusion barrier are correct, then hypophysectomy should produce an increase in water permeability. On the contrary, permeability was decreased. The suggestion has been made that prolactin may increase water permeability by increasing membrane pore size (Potts & Fleming, 1970). This seems unlikely in view of the fact that prolactin reduces electrolyte loss in fresh water. The finding that both ACTH and cortisol have prolactin-like effect with respect to water permeability in hypophysectomized goldfish (Lahlou & Giordan, 1970) suggests interrenal involvement in branchial water permeability. Whether the changes in water turnover observed are due directly to prolactin or to the effect of prolactin on the interrenals or some other target organs remains to be established.

#### *Osmotic water flux*

The osmotic water flow deduced from the drinking and urine flow rates in fresh water was reduced in the brown trout after hypophysectomy. It is also curious that diuresis, which was marked in sham-operated and intact fish, was drastically reduced after hypophysectomy. This result is in agreement with those of Stanley & Fleming (1966) on *Fundulus kansae* and Lahlou & Giordan (1970) on *Carassius auratus*.

Hypophysectomy also results in a reduction in drinking rate. Drinking rate in hypophysectomized goldfish does not differ from that of intact fish (Lahlou & Giordan, 1970). In the European eel, however, hypophysectomy results in a reduction in both urine flow and drinking rates (Gaitskell & Chester Jones, 1971).

It is important to note that both osmotic and diffusion water permeabilities were reduced in the hypophysectomized trout in this study and also in the goldfish (Lahlou & Giordan, 1970). The effect of prolactin on urine flow rate was not tested in the present study. In *Carassius*, however, the decrease in urine flow rate after hypophysectomy is inhibited by prolactin therapy (Lahlou & Giordan, 1970). It is probable that this is also true in most teleosts. Neurohypophysial hormones, especially AVT, reduce branchial water permeability (Lahlou & Giordan, 1970) and at the same time induce diuresis in both normal and hypophysectomized fish (Holmes & McBean, 1963). On the basis of the results on the trout and those obtained by Lahlou & Giordan (1970) for the goldfish, water balance appears to be maintained in normal animals by the antagonistic effects of prolactin, ACTH and cortisol on the one hand and the neurohypophysial hormone (AVT) on the other. The suggestion by Lahlou & Giordan (1970) that renal water regulation is distinct and does not reflect branchial water permeability changes is difficult to prove. Since increased external calcium decreased both branchial and renal water flow in both intact and hypophysectomized brown trout, one is tempted to assume that the reduction in urine output was a water conservation response to the decrease in branchial water permeability. It will certainly be disadvantageous even for a fresh-water teleost to continue to run down its water reserve as urine when branchial water influx has been reduced by as much as a half.

The results of this work do not permit any proposal on the direct effect of prolactin on urine production, although the reduction in renal water flow following hypophysectomy of the goldfish was restored to normal after prolactin therapy (Lahlou & Giordan, 1970). Similarly, it cannot be said that environmental calcium has a direct effect on renal function. The decrease in urine output following hypophysectomy could be a passive response to the reduction in branchial water permeability. It may also be attributed to the suppression of the pituitary-interrenal axis following hypophysectomy, since the interrenal glucocorticoid, cortisol, affects renal function (Holmes & Stainer, 1966). Other factors such as changes in blood volume and extracellular space, which have not so far been shown to be controlled by prolactin, will affect renal function. Undoubtedly, renal function in teleosts is a complex process involving three and probably more hormones.

Prolactin is involved directly or indirectly in both branchial and renal water regulation in teleosts. In the brown trout, hypophysectomy decreased branchial and renal water loss while prolactin therapy increased the former and probably the latter as well. Lahlou & Giordan (1970) reached a similar conclusion for the goldfish. Prolactin action directly opposes that of calcium in these respects. It is tempting to suggest that prolactin reverses the effect of calcium on membrane mucus. Unfortunately, the present evidence on the probable effect of prolactin on membrane mucus is contrary to such a suggestion. It is also unlikely that prolactin acts by increasing membrane pore size and consequently branchial water loss. This is due to the fact that although it increased water loss, it also reduced electrolyte loss in fresh water (Oduleye, 1973).

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