e.g. lepidopteran midgut[12], the movement of ions, particularly K^+, is thought to involve a K^+ modulated Mg^{2+} ATPase. Although the existence and nature of the ion transport across the hindgut of *G.m.morsitans* remains to be established, it would nevertheless be of interest to determine whether other types of ATPases are present in these tissues.

Finally, the barely significant difference ($p < 0.05$) in rectal (Na^++K^+)-ATPase activity between fed and unfed flies probably indicates that in unfed flies, the remnants of the previous blood meal may have affected, the determination of enzyme activity adversely by interfering with the protein assay. Thus the (Na^++K^+)-ATPase activity of unfed flies was depressed.

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Studies of total and protein-bound plasma Mg^{++} in wild and hatchery-reared coho salmon smolts in freshwater and in seawater

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Summary. Total plasma Mg^{++} and Ca^{++}, Mg^{++} in erythrocytes as well as protein-bound plasma Mg^{++} were investigated in wild and hatchery-reared smolts. The proportion of plasma Mg^{++} which was bound to plasma protein did not change significantly during entry into seawater, even though the in vitro addition of exogenous Mg^{++} to the plasma showed that additional binding was possible.
Key words. Plasma Mg^{++}; RBC Mg^{++}; coho salmon; ion binding; smolting; seawater entry.

Magnesium ion is a necessary activator of about 300 enzymes which are widely involved in the metabolism of proteins, carbohydrates, lipids, and nucleic acids of higher vertebrates[1,7]. In fish, Mg^{++} is also essential, but is not well understood. In hatchery-reared coho salmon, migrating into seawater has been associated with moderate to heavy mortality rates, sometimes as high as 80%. Problems with Mg^{++} regulation have been suspected because dying fish are often lethargic and die in a state of flaccid paralysis (L. Smith, personal observation). Suggestions have been made that plasma Mg^{++} levels greater than 7–10 meq/l should be lethal to coho salmon (Gary Wedemeyer, USFWS, Seattle, WA 98115, in preparation), but the exact toxicity limits and Mg^{++} toxicity mechanisms are largely unknown. However, the lower vertebrates typically show some temporary increases in plasma Mg^{++} when exposed to a high Mg^{++} environment such as seawater[2].

This project originated from observations of hatchery-reared coho smolts in which fish in apparently good health after 20–30 h in seawater had plasma Mg^{++} levels which we would have expected to be toxic. We wondered whether part of their plasma Mg^{++} had somehow been inactivated. The objective of this project was to test whether the high level of plasma Mg^{++} found in hatchery coho smolts after transfer into seawater was not harmful because of binding the excess amount of Mg^{++} to plasma proteins in a way not found in wild coho smolts.

Materials and methods. Plasma from four stocks of coho salmon were tested for Mg^{++} binding: 1) Wild yearling coho smolts (10–12 g) were captured during their downstream migration in late May and early June 1984 at a weir just above the estuary of Big Beef Creek which empties into Hood Canal about 25 km west of Seattle, WA. The fish were transported in oxygenated fresh water to our University of Washington lab. This is the same stock of fish investigated by Miles and Smith[5] several years earlier.

2) Hatchery-reared yearling coho smolts (11–18 g) were obtained in freshwater in June 1984 from the NMFS facility adjacent to the UW campus and were transported briefly in freshwater to our UW lab. This stock was originally from the Issaquah hatchery about 50 km east of Seattle.

Both groups of fish were adapted to laboratory conditions in freshwater for one week before being put into natural seawater in a recirculating system of about 30,000 liters capacity which was kept at a temperature of about 15 °C. Blood data from these two groups of fish were statistically comparable.

3) The stock of coho salmon in which we observed the high levels of plasma Mg^{++} in 1982 was available to us only in early July 1984 after the yearling smolts had been in seawater for about one month. We therefore took blood samples at their estuarine rearing site near Coos Bay, Oregon, and transported them to Seattle on dry ice.

4) We obtained blood samples from regular production of zero-age smolts in freshwater in early July from Oregon Aqua Foods, Springfield, Oregon.

For taking blood samples, we first anesthetized fish with tricaine methane sulfonate (MS-222) at a concentration of 100 mg/l. Blood was drawn from the caudal vein behind the anal fin using a small syringe which had been rinsed in citrate. Blood was centrifuged for 5 min at 13,000 × g, the plasma decanted immediately and frozen at −20 °C. Erythrocytes which were to be analyzed for total Mg^{++} were washed and centrifuged three times with equal volumes of cold 0.9% NaCl solution, after which they were frozen at −20 °C for assay on the next day. A plasma ultrafiltrate was prepared according to the method described by D'Costa and Cheng[3], using the Amicon microparti-

tion system MPS-1 with the new YMT membrane which retains up to 99.9% of the plasma proteins.

Since none of the blood samples showed potentially toxic levels of Mg^{++}, we tested for the possibility of additional Mg^{++} binding capability by adding a solution of 12.5 meq/l Mg^{++} to the plasma at the rate of 5 µl per 100 µl of plasma. We then measured total plasma Mg^{++} and Ca^{++}, total Mg^{++} in erythrocytes, and Mg^{++} in the ultrafiltrate by atomic absorption spectrophotometry. The protein-bound Mg^{++} was then calculated as the difference between the total plasma Mg^{++} value and the ultrafiltrate value. We calculated mean and standard errors as well as performing T-tests to determine statistical significance using standard computer programs[4].

Results. Total and protein-bound plasma Mg^{++}. The basic data from stocks 1 and 2 are presented graphically in figure 1 and the percentages of protein-bound Mg^{++} appear in figure 2. Total plasma Mg^{++} in hatchery coho increased 58% after 24 h in seawater and the portion of the Mg^{++} in the ultrafiltrate increased by 81%. In wild coho after 24 h in seawater, total plasma Mg^{++} increased by 196% and the Mg^{++} in the ultrafiltrate by 202% (fig. 1). The protein-bound Mg^{++}, including that in samples with added Mg^{++}, expressed as a percentage of total plasma Mg^{++}, changed in only minor amounts, none of which were statistically significant (fig. 2).

While not directly comparable with the data above, plasma Mg^{++} measurements from stocks 3 and 4 allow further extension of the significance of the data. In the yearling coho smolts which had shown the potentially toxic Mg^{++} level in 1982 (unpublished), in 1984 the total mean Mg^{++} was 5.88+1.70 meq/l and the protein-bound Mg^{++} was 0.56+0.50 meq/l after one month in seawater. We also measured Mg^{++} in zero-age (underyearling) coho smolts in freshwater (mean total Mg^{++} = 1.68+0.43 meq/l, mean protein bound Mg^{++} = 0.56+0.50 meq/l).

Other data. Total plasma Ca^{++} levels were statistically similar in all groups, including the zero-age smolts and the smolts which had been in seawater for a month. Not only did Mg^{++} levels in erythrocytes increase during exposure to seawater, but exceeded those in plasma both in freshwater and seawater.

Discussion. The proportion of the plasma Mg^{++} which was bound to plasma protein did not change significantly in either wild or hatchery stocks. Even the addition of Mg^{++} in vitro in excess of naturally occuring levels did not elicit any special protein-binding mechanism which might suggest how the earlier coho smolts survived the supposedly-lethal Mg^{++} levels.

Nevertheless, hatchery smolts differed significantly from wild smolts. Since our two data points for wild smolts were the same as those seen earlier[5], we can assume that they also quickly adjusted their Mg^{++} levels down to essentially their prior freshwater levels. Hatchery smolts showed significantly less plasma Mg^{++} after 24 h in seawater than wild smolts, but did not get their plasma Mg^{++} down again to freshwater levels. Similarly, in the yearling coho smolts in which we observed the high total plasma Mg^{++} levels of 13–14 meq/l, these fish after three weeks in seawater still had total plasma Mg^{++} levels of 4–5 meq/l. This contrasts dramatically with plasma Mg^{++} in wild coho smolts which peaked at 3–4 meq/l and returned to around 1 meq/l after only 30–36 h in seawater[5]. From this, one might suggest that hatchery fish could be more likely to have problems with Mg^{++} regulation in seawater than wild fish.

Further, we looked at only three hatchery stocks and only one of those in detail. However, since total plasma Mg^{++} levels in the zero-age smolts in freshwater and in yearling smolts after a month in seawater resembled the third hatchery stock, it seemed likely that all three hatchery stocks were also comparable in protein binding of Mg^{++}. We also believe that the higher levels of plasma Mg^{++} than in wild smolts after full adaptation to seawater may be a general phenomenon.

The ultrafilterable fraction typically contains phosphates, citrates, and other anions which could complex with Mg^{++} and thus reduce the activity of ionized magnesium similarly to plasma protein binding. Thus some complexing or binding of Mg^{++} is still not completely excluded as a mechanism for dealing with high levels of Mg^{++}.

We measured Ca^{++} for two reasons. If Ca^{++} levels differed significantly between stocks, then changes in membrane permeability might explain part of the differences in Mg^{++} levels, since Ca^{++} levels are suggested as being involved in controlling membrane permeability. Also, Ca^{++} is chemically similar to Mg^{++} and measurement methods are reliable, thus serving as a check on our Mg^{++} measurements. However, all fish had essentially the same Ca^{++} levels and therefore Ca^{++} probably had no effect on differences in Mg^{++} levels. Plasma Ca^{++} levels were comparable to those measured earlier[5].

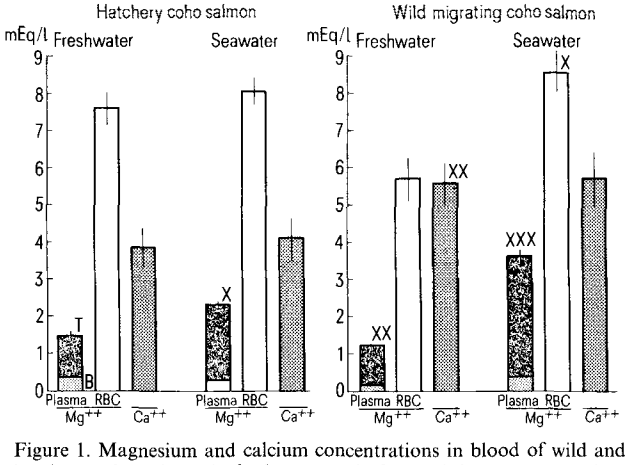

Figure 1. Magnesium and calcium concentrations in blood of wild and hatchery coho salmon in freshwater and after 24 h in seawater. Values represent means and standard errors. *X* signifies values which are significantly different ($p < 0.05$) from values in freshwater. *XX* signifies values which are significantly different ($p < 0.05$) from those in hatchery fish. In all cases, n = 10 with all tests being run on one blood sample from each fish.

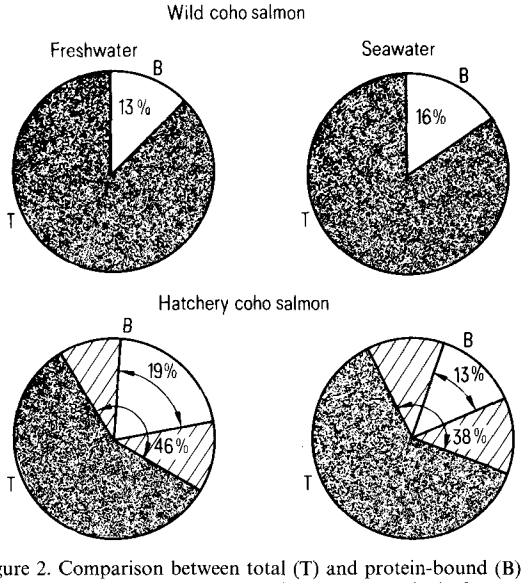

Figure 2. Comparison between total (T) and protein-bound (B) plasma magnesium in wild and hatchery coho salmon smolts in freshwater and after 24 h in seawater. In hatchery smolts, additional Mg^{++} was added to the plasma (see Methods) before ultrafiltration. For hatchery fish, the circle represents total plasma Mg^{++} after adding exogenous Mg^{++}, black represents total endogenous Mg^{++}, white shows the binding of endogenous Mg^{++}, and cross-hatched indicates the added binding of exogenous Mg^{++}.

We believe that the data presented on total Mg^{++} levels in erythrocytes are the first from any teleost fish during migration from freshwater into seawater. The functional significance of the changes remains to be determined.

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Somatic growth in hypophysectomized pituitary-homografted rats is promoted by prolactin

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Summary. Hypophysectomized male rats bearing a homograft of two adenopituitaries under the kidney capsule showed a significant increase in b.wt as compared to hypophysectomized non-homografted animals. Radioimmunoassay of growth hormone (GH), ACTH, α-MSH, β-endorphin and prolactin (PRL) revealed that only the latter was highly increased in the plasma of hypophysectomized homografted rats. These animals showed also increased levels of plasma corticosterone. However, daily injection of corticosterone failed to promote somatic growth in hypophysectomized non-homografted rats. These results suggest that PRL, and not other hormonal factors, promotes somatic growth in hypophysectomized homografted rats, and stress the concept that only PRL is secreted in significant amounts by pituitary homografts.
Key words. Prolactin; somatic growth; hypophysectomy; corticosterone; pituitary hormones.

It has been reported that prolactin (PRL) stimulates somatic growth in various species, including amphibians[1,2], reptiles[3] and birds[4]. In these species, PRL increases food consumption and promotes feeding behavior[5]. A stimulation of somatic growth has also been described in hypophysectomized pituitary-homografted rats[6–8]. These pituitary homografts are believed to secrete high amounts of PRL and little, if any, of the other hypophyseal hormones[9,10]. Although the rat implanted with extra anterior pituitary glands under the kidney capsule has been widely used as a model of chronic hyperprolactinemia, its hormonal status has not been fully characterized[11]. Various authors have described a progressive decrease in growth hormone (GH) plasma levels in hypophysectomized animals made hyperprolactinemic by adenopituitary homografts[12,13]. As hyperprolactinemic rats have been reported to exhibit also high levels of corticosterone[14,15], the present experiments were undertaken to investigate whether somatic growth in hypophysectomized homografted rats is indeed promoted by PRL or by other hormonal factors.

Male rats of the Wistar strain, weighing about 180 (± 20) g, were hypophysectomized by the trans-auricular route under ether anesthesia. During the same surgical session, the animals received a homograft of two adenopituitaries from animals of an identical strain under the kidney capsule. Control rats were sham-hypophysectomized and/or sham-homografted. All animals were kept at room temperature (21 °C) under a light-dark cycle (lights on between 08.00 and 20.00 h) with food and water available ad libitum. Water containing NaCl 0.9% was given to hypophysectomized rats. The completeness of hypophysectomy was checked after sacrifice.

A group of hypophysectomized and non-hypophysectomized non-homografted rats were injected s.c. daily with corticosterone (1 mg/kg, in corn oil) or with the vehicle. Somatic growth was checked by weighing the rats every week for 6 weeks after operation. At the end of this procedure, all animals were killed by decapitation and the blood was collected for radioimmunoassay (RIA).

PRL and GH were measured by RIA using reagents and proto-

Table 1. B. wt of male rats subjected to hypophysectomy and/or adenopituitary homografts under the kidney capsule (values are expressed in g)

Experimental groups	(n)	Weeks after surgery 0	1	2	3	4	5	6
1 Sham-hypophysectomy + sham-homografts	(8)	125 ± 1.8	160 ± 1.9	181 ± 2.4	210 ± 3.4	232 ± 3.6	241 ± 4.6	262 ± 4.8
2 Sham-hypophysectomy + homografts	(8)	126 ± 1.7	162 ± 2.1	179 ± 2.6	212 ± 3.6	234 ± 3.8	242 ± 4.2	263 ± 4.6
3 Hypophysectomy + sham-homografts	(8)	126 ± 1.5	108 ± 1.2	106 ± 1.3	108 ± 1.4	108 ± 1.6	107 ± 1.7	108 ± 1.6
4 Hypophysectomy + homografts	(8)	126 ± 1.8	107 ± 1.5	118 ± 1.3*	130 ± 2.0*	136 ± 2.3*	140 ± 2.7*	140 ± 2.6*

Values are mean ± SEM. * Significantly different as compared to group 3 ($p < 0.05$, Student's t-test, two-tailed).