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A highly specific radioimmunoassay (RIA) for the measurement of prolactin (PRL) in the plasma and pituitary of salmonid fishes was developed using a rabbit antiserum to chinook salmon (*Oncorhynchus tschawytscha*) PRL. The PRLs purified from chinook salmon and chum salmon (*O. keta*) pituitaries showed exactly the same competitive inhibition curves in the RIA, regardless of iodination of either hormone. The displacement curves for pituitary extracts and plasma from several salmonids, including chum, coho, and amago salmon, rainbow trout, and Japanese charr, were parallel to the salmon PRL standard, whereas those from the eel, goldfish, carp, and tilapia showed negligible cross-reactivity. Negligible cross-reactivity was also seen with plasma from hypophysectomized rainbow trout or coho salmon. None of the mammalian PRL or growth hormone (GH) preparations, bullfrog PRL, or presumptive chum salmon "gonadotropin" and eel "PRL" cross-reacted in the PRL RIA. Presumptive chum salmon GH showed less than 0.05% cross-reactivity. The RIA sensitivity was less than 0.1 ng of the salmon PRL standard per milliliter. The immunoreactive plasma PRL levels in mature chum salmon were below 1 ng/ml in seawater. The plasma PRL in females increased to about 8 ng/ml 1 day after transfer to fresh water, and high levels (2–4 ng/ml) were maintained during 3–7 days after the transfer. In contrast, when males were transferred to fresh water, an increase in plasma PRL was seen only 1 day after the transfer. A significant decrease in plasma osmolality was observed in both males and females after transfer to fresh water. No change was observed either in plasma PRL or osmolality, when fish were transferred from seawater to seawater.

It is well established that prolactin (PRL) is an important hormone for freshwater adaptation of certain euryhaline teleosts, and that hypophysectomy without PRL therapy impairs their survival in fresh water (see Hirano and Mayer-Gostan, 1978; de Vlaming, 1979; Clarke and Bern, 1980; Loretz and Bern, 1982). In salmonids, however, little information is available on the hypophyseal control of osmoregulation. Hypophysectomy has only been accomplished in rainbow trout (Donaldson and McBride, 1967; Komourdjian and Idler, 1977; Björnsson and Hansson, 1983) and in brown trout (Oduleye, 1975, 1976). Although these studies have shown that the pituitary or PRL is involved in regulation of hydromineral balance in trout, hypophysectomy does not impair freshwater survival when rainbow trout are allowed to recover in one-third seawater (Komourdjian and Idler, 1977; Björnsson and Hansson, 1983).

The osmoregulatory role of PRL in salmonids may be partially evaluated by measuring the blood concentration of the hormone and its metabolic clearance rate. Several attempts have been made to use radioimmunoassay (RIA) procedures for ovine PRL for measurement of circulating

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PRL in several salmonid species such as sockeye (McKeown and van Overbeeke, 1972), kokanee (Leatherland and McKeown, 1974; Leatherland *et al.*, 1974), and coho (McKeown and Brewer, 1978). However, without rigorous validation of the RIA, the substance(s) measured in the heterologous RIA remains unidentified (see Nicoll, 1975). A homologous RIA for teleost PRL has only been reported for tilapia, *Oreochromis* (*Sarotherodon*) *mossambicus*, by Nicoll *et al.* (1981).

Recently, highly purified preparations of PRLs have been obtained from the pituitaries of chum salmon (Kawauchi *et al.*, 1983) and chinook salmon (Prunet and Houdebine, 1984). This paper describes our results on the development and validation of a RIA suitable for the measurement of both plasma and pituitary immunoreactive PRL levels in salmonid fishes.

MATERIALS AND METHODS

Production of antisera. The procedures employed for the purification of chum and chinook salmon PRL (chum and chinook PRL, respectively) used in this study have been described previously (Kawauchi *et al.*, 1983; Prunet and Houdebine, 1984).

Antisera against chum PRL were raised in young rabbits as described previously (Kawauchi *et al.*, 1983). An antiserum against chinook PRL was also raised in a rabbit. Chinook PRL (150 μg) was dissolved in 300 μl 0.05 *M* phosphate buffer, pH 6.0, and emulsified with 300 μl of complete Freund's adjuvant. Two injections of the above were given subcutaneously into foot pads at 2-week intervals followed by eight injections of the same dose of PRL using incomplete Freund's adjuvant at 2-week intervals. They were completely bled by cardiac puncture 1 week after the last injection.

Iodination of salmon PRL. The chum and chinook PRLs were iodinated using a modification of the method of Thorell and Johansson (1971). Two hundred microcuries $Na^{125}I$ in 2 μl of 1 m*M* NaOH, pH 8–11 (IMS-30, Amersham), 0.5 μg lactoperoxidase (90 U/mg, Sigma) in 5 μl of distilled water and 0.037 μg H_2O_2 in 5 μl of 0.2 *M* phosphate buffer, pH 6.0, were added to 5 μg of salmon PRL in 25 μl 0.05 *M* phosphate buffer, pH 6.0. After 90 sec, the reaction was stopped by adding 75 μl chilled 0.05 *M* phosphate buffer, pH 6.0, containing 0.14 *M* NaCl. The specific activity of the iodinated PRL ranged from 10 to 20 μCi/μg. Phosphate buffer at pH 6.0 was essential to solubilize and to yield high specific activity of the salmon PRLs, isoelectric points of which are pH 10.3 for chum PRL (Kawauchi *et al.*, 1983) and 9.4 for chinook PRL (Prunet and Houdebine, 1984). Unreacted iodide was removed by gel filtration on a Sephadex G-75 column (0.6 × 20 cm), previously saturated with 3 ml of 2% BSA. The column was then washed with a further 10 ml 0.05 *M* phosphate buffer, pH 6.0, containing 0.14 *M* NaCl. The iodination mixture was eluted with the buffer, and 400 to 500-μl fractions were collected in tubes containing 50 μl 2% BSA in the elution buffer. The ^{125}I-salmon PRL was stable for about 1 month when stored at −20°.

Radioimmunoassay procedure. RIA was performed using a double antibody method under disequilibrium conditions, largely following the procedures developed for chicken FSH (Sakai and Ishii, 1983). The assay buffer was 0.01 *M* phosphate buffer, pH 7.3, containing 0.14 *M* NaCl, 1% BSA, 0.1% Triton X-100, and 0.1% NaN_3. Standards, plasma, and homogenized pituitaries were serially diluted with assay buffer and added to plastic assay tubes in 100-μl volumes. Each preparation was assayed in duplicate. The salmon PRL antiserum was diluted 1:20,000–1:100,000 with 0.01 *M* phosphate buffer, pH 7.3, containing 0.14 *M* NaCl, 0.05 *M* EDTA, and 1% normal rabbit serum (NRS–EDTA–PBS) and added to assay tubes in 100-μl volumes. After 24-hr incubation at 5°, 100 μl of iodinated chum or chinook PRL (15,000–20,000 cpm) were added to each tube and incubation was continued for 24 hr at 5°. The antibody-bound hormone was precipitated by addition of 200 μl of a 1:15 dilution of goat anti-rabbit γ-globulin (dilution with 0.01 *M* phosphate buffer, pH 7.3, containing 0.14 *M* NaCl and 0.05 *M* EDTA) and incubated overnight at 5°. After addition of 200 μl of the assay buffer, the tubes were vortexed and then centrifuged for 60 min at 2000*g* at 5°. The supernatant was decanted and the radioactivity in the bound fraction was determined in a Packard Multi-Prias gamma counter.

All values obtained were corrected for nonspecific binding of the labeled hormone by substituting the NRS–EDTA–PBS for the antiserum and the assay buffer (buffer blank) or 50–100 μl of plasma (plasma blank) for the sample. Triton X-100 was necessary in the assay buffer to reduce the radioactivity of the buffer blank and plasma blank to less than 1.5% (250–300 cpm) of the total activity added to each tube; without Triton X-100, the buffer blank was always greater than the plasma blank, resulting in overestimation of the blood concentration. Nonionic detergents such as Triton X-100 have been shown to have little or no detectable effect on antigen–antibody reaction (see Dimitriadis, 1979). The total radioactivity bound by the antibody in the absence of unlabeled hormone (B_0/T) was about 30–40%. The results were

calculated using a computer program, based on log-logit transformation.

Immunocytochemistry. PRL-producing cells in the chum salmon pituitary were identified immunocytochemically with the unlabeled peroxidase–antiperoxidase (PAP) method as described by Naito *et al.* (1983). The pituitaries used for this study were removed from mature chum salmon females, weighing 3–4 kg, captured just before spawning in the Otsuchi River, Iwate, Japan.

Source of plasma and pituitaries. Mature male and female chum salmon (*Oncorhynchus keta*), weighing about 3–5 kg were trapped in a salmon set-net in Otsuchi Bay in early December 1980. Since the spawning ground of the salmon is less than 1 km from the mouth of the Otsuchi River, all fish had matured testes or ovulated eggs while in the bay (see Hirano *et al.*, 1978; Morisawa *et al.*, 1979). Five males and five females were transferred to running freshwater aquaria at 8° at Otsuchi Marine Research Center of the Ocean Research Institute, University of Tokyo. Three males and three females were kept in seawater aquaria at 10°. Males and females were kept in separate aquaria to prevent spontaneous spawning. After anesthesia with 0.02% tricaine methane sulphonate, about 5 ml of blood were taken from the caudal vessels of each fish with a hypodermic syringe at the time of transfer, and then 1, 3, 5, and 7 days after the transfer. The blood plasma was immediately separated by centrifugation at 10,000 rpm for 60 min at 5°, and kept frozen at −30° until assay. Osmolality of the plasma was measured immediately (without freezing) with a Knauer osmometer.

Rainbow trout (*Salmo gairdneri*), carp (*Cyprinus carpio*), goldfish (*Carassius auratus*), Japanese eel (*Anguilla japonica*), and tilapia (*Oreochromis* (*Sarotherodon*) *mossambicus*) were obtained from commercial dealers in Tokyo. Japanese charr (*Salvelinus leucomaenis*) were obtained from Niigata Freshwater Fisheries Station, Koide, Niigata. They were all kept in freshwater aquaria at the Ocean Research Institute before blood sampling. Plasma samples from masu salmon (*O. masou*) and amago salmon (*O. rhodurus*) were supplied by Dr. K. Aida (Faculty of Agriculture, University of Tokyo) and by Dr. S. Fushiki (Samegai Trout Hatchery, Shiga), respectively. Pituitaries were removed from freshly killed chum salmon, rainbow trout, charr, eel, tilapia, goldfish, and carp. They were quickly weighed and homogenized with 0.9% NaCl in a glass homogenizer. The homogenates were centrifuged at 12,000 rpm for 5 min, and the supernatants were used for RIA. Coho salmon (*O. kisutch*) were obtained from the Iron Gate Hatchery (California Department of Fish and Game).

Hypophysectomy of rainbow trout and coho salmon was conducted by a modification of the transorbital approach of Nishioka (1980). Trout (weighing about 100 g) and coho salmon (weighing 20 to 35 g) were anesthetized with 0.01% each of tricaine methane sulphonate and $NaHCO_3$ at 2–5° (trout) and 12–14° (coho). Thereafter, the gills were continually irrigated with aerated cold water or anesthetic during the operation. The hypophysectomized and sham-operated fish were kept in one-third seawater for 7–14 days before blood sampling. Completeness of ablation was verified microscopically at time of autopsy.

Source of hormones. Ovine prolactin (NIH-P-S14) and growth hormone (NIH-GH-S12) were supplied by the National Hormone and Pituitary Program of the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases (Bethesda, Md.). Bullfrog (*Rana catesbeiana*) PRL (frog PRL) (see Yamamoto and Kikuyama, 1981) was generously provided by Professor S. Kikuyama (School of Education, Waseda University). The procedures for purification of fin whale (*Balaenoptera physalus*) PRL and GH have been described (Kawauchi and Tsubokawa, 1979; Tsubokawa *et al.*, 1980). Presumptive chum salmon GH (chum "GH") and gonadotropin (chum "GTH") were isolated and purified from the chum salmon pituitary (Kawauchi, H., unpublished). Presumptive eel prolactin (eel "PRL") was obtained by culturing the eel pituitary in a defined medium (Kishida, M., and Hirano, T., unpublished).

RESULTS

Among all the antisera raised against chum PRL and chinook PRL, an anti-chinook PRL rabbit serum showed the best titer and most sensitive standard RIA curve. Thus, this antiserum was used for all of the following assays at an initial dilution of 1:100,000. The chum salmon and rainbow trout pituitaries incubated with the antiserum revealed a strong reaction specifically in follicle cells located in the rostral pars distalis (Fig. 1); these cells correspond with the PRL cells identified by Naito *et al.* (1983) using the anti-chum PRL rabbit serum.

Since plasma from hypophysectomized chum salmon or chinook salmon were not available, specificity of the antisera to salmon PRL was examined using plasma samples from hypophysectomized rainbow trout and coho salmon. As shown in Table 1, no significant cross-reactivity was detected in hypophysectomized trout kept in one-third seawater; a significant amount

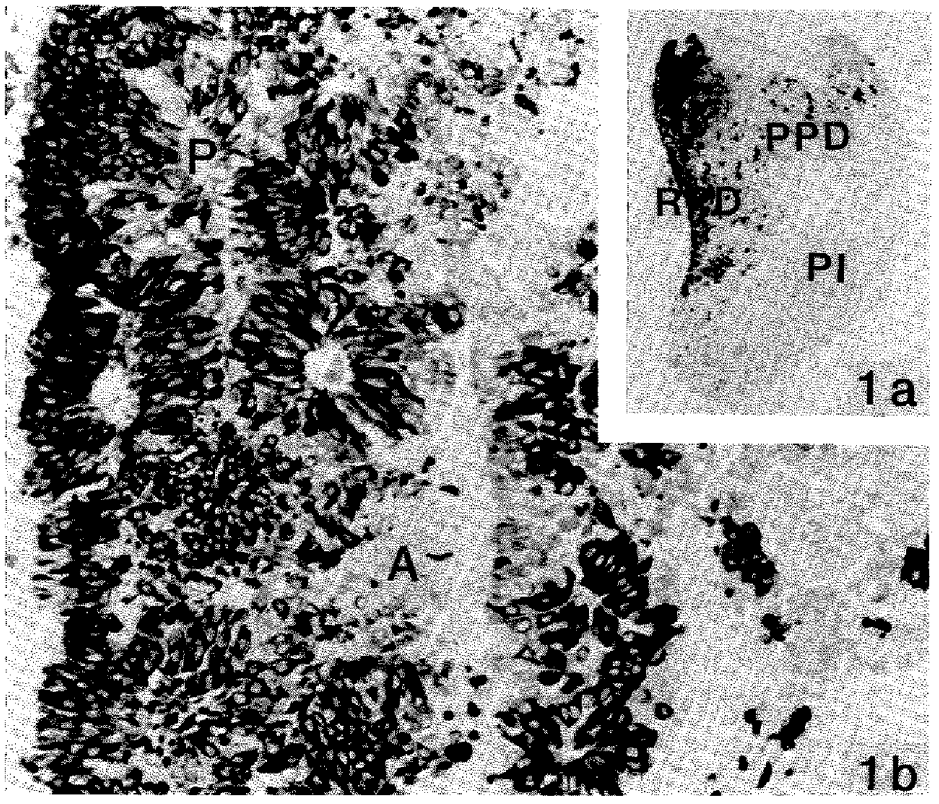

FIG. 1. Sagittal section of the rostral pars distalis of chum salmon pituitary stained with anti-chinook salmon PRL rabbit serum at a concentration of 1/5000, counterstained with Mayer's hematoxylin. RPD; rostral pars distalis; PPD, proximal pars distalis; PI, pars intermedia; A, ACTH cells; P, PRL cells. (a) ×15, (b) ×250.

(0.7 ng/ml) was detected in only one plasma sample out of six. Plasma of sham-operated trout in one-third seawater also showed low PRL immunoreactivity, with significant amounts being detected in five out of seven samples. Significant amounts were also detected in plasma of hypophysectomized coho salmon, although the levels were lower than in intact fish kept in one-third seawater or those in fresh water.

As is clearly shown in Fig. 2, the anti-chinook PRL rabbit serum did not distinguish between chum PRL and chinook PRL; serial dilutions of chum PRL and chinook PRL caused displacements of the chum PRL label or the chinook PRL label, which were not different from each other, and the displacement curves for plasma samples from mature chum salmon were exactly the same in both assays.

Figure 3 illustrates the displacement curves for pituitary extracts from several teleost species. Serial dilutions of pituitary extracts from the chum salmon, rainbow trout, and Japanese charr gave inhibition slopes which were not significantly different from the chum PRL standard. Pituitary extracts from the eel, goldfish, carp, and tilapia caused only limited displacement of antibody-bound labeled chum PRL.

Figure 4 presents a typical RIA dose–response curve for chum PRL and serial dilutions of plasma samples from seveal te-

TABLE 1
IMMUNOREACTIVE PROLACTIN IN PLASMA OF HYPOPHYSECTOMIZED RAINBOW TROUT AND COHO SALMON

Treatment	*N*	Plasma PRL (ng/ml)
Rainbow trout		
Hypophysectomized (1/3 seawater)	6	0.18 ± 0.101*
Sham operated (1/3 seawater)	7	0.20 ± 0.037
Intact (fresh water)	5	2.85 ± 0.805
Coho salmon		
Hypophysectomized (1/3 seawater)	10	0.78 ± 0.117
Intact (1/3 seawater)	3	4.04 ± 0.621
Intact (fresh water)	5	2.84 ± 0.844

Note. The results are presented as the mean ± SEM.
* Not significantly different from 0 ($P < 0.05$).

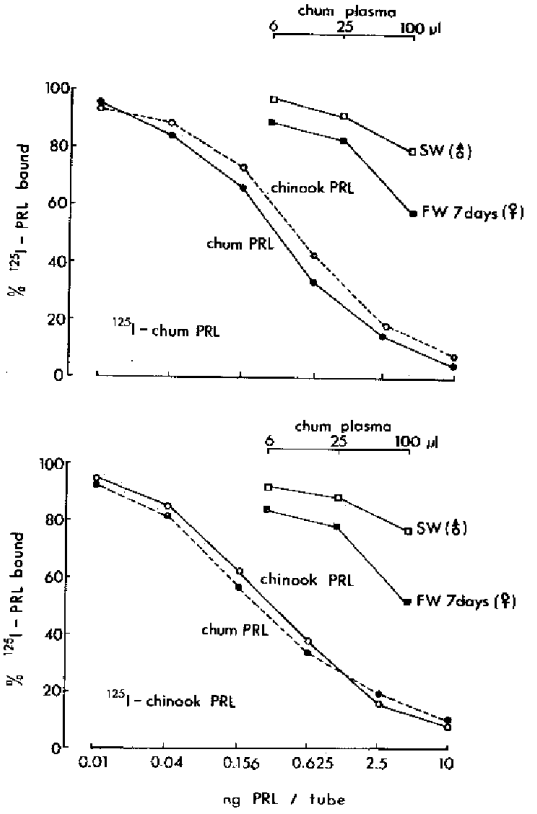

FIG. 2. Competitive binding curves for preparations of purified chum and chinook PRLs and also for plasma samples from mature chum salmon kept in seawater (SW) or fresh water (FW), in two radioimmunoassays with an anti-chinook salmon rabbit serum, one employing chum PRL label (above) and the other chinook PRL label (below). Each point represents the average of duplicate determinations.

leost fishes. The displacement curves for plasma from chum salmon (female), coho salmon, amago salmon, masu salmon, rainbow trout, and Japanese charr in fresh water were parallel to the chum PRL standard, whereas plasma from hypophysectomized rainbow trout caused a displacement which was not significantly different from the zero-dose response. The plasma samples from the hypophysectomized coho salmon and sham-operated trout kept in one-third seawater showed less displacement of the chum PRL label than those of the intact fishes in fresh water. No displacement of the chum PRL level was seen in plasma from the eel, goldfish, carp, and tilapia.

The specificity of the salmon PRL RIA was determined also using several independent procedures. As shown in Fig. 5, none of the mammalian PRL or GH preparations or frog PRL cross-reacted in the PRL RIA. Chum "GTH" and eel "PRL" did not significantly interfere in the assay. Although the slope of the inhibition curve for serial dilution of chum "GH" was not significantly different from that of the salmon PRL standard, chum "GH" had only limited immunological potency in the salmon PRL RIA, consisting of less than 0.05% cross-reactivity. Recovery experiments were conducted by measuring chinook PRL added in increasing concentrations to 50 µl of trout plasma (Fig. 6). The mean recovery was 122%, and the slope of the regression line was equal to 1 at $P < 0.01$.

The midrange of the RIA calculated as the amount of the chum PRL standard that will bind 50% of the zero-hormone standard

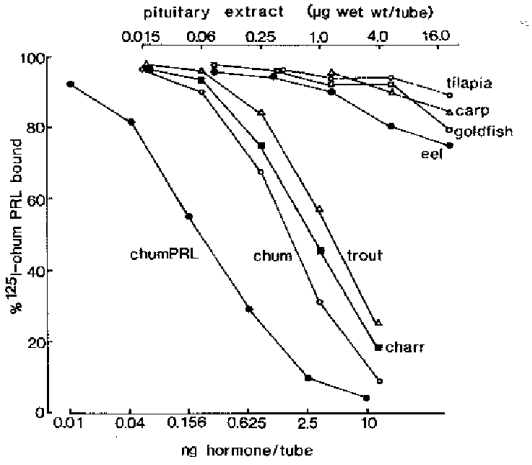

FIG. 3. Competitive binding curves for chum PRL standard and serial dilutions of crude pituitary extracts from chum salmon, Japanese charr, rainbow trout, eel, goldfish, and tilapia. Each point represents the average of duplicate determinations.

(ED_{50}) was 0.275 ± 0.013 ng chum PRL/ml (mean ± SEM, $N = 6$). The sensitivity of the assay defined as twice the standard deviation at zero dose was 3–7 pg/assay tube or 0.03–0.07 ng/ml plasma (when 100 μl plasma was used). Thus, all the plasma values calculated as less than 0.1 ng/ml by a computer were assumed to be 0.1 ng/ml. Repeated determinations of 0.15 and 0.6 ng of chum PRL gave an intraassay coefficient of variation of 5.9 and 6.8%, respectively. Using the ED_{50} of the chum PRL standard in six assays, the interassay coefficient of variation was 13.5%.

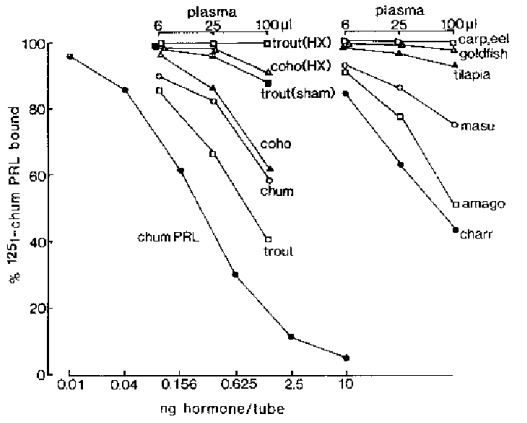

FIG. 4. Dose–response inhibition curves for chum PRL and serial dilution of plasma obtained from chum salmon (mature female), rainbow trout, coho salmon, amago salmon, masu salmon, Japanese charr, eel, goldfish, carp, and tilapia. All the plasma samples were taken from fish adapted to fresh water, except for those from hypophysectomized (HX) or sham-operated (sham) rainbow trout or coho salmon, which were acclimated to one-third seawater. Each point represents the average of duplicate determinations.

Results of the PRL RIA on plasma samples of mature chum salmon transferred from seawater to fresh water are shown in Fig. 7. The immunoreactive PRL levels in seawater fish, including those transferred to seawater aquaria were below 1 ng/ml. The plasma PRL in the female increased greatly to about 8 ng/ml 1 day after transfer to fresh water, decreased to about 4–5 ng/ml 3–5 days after the transfer, and decreased further to 2 ng/ml after the seventh day. In contrast, when the males were transferred to fresh water an increase was apparent only 1 day after the transfer. The difference of PRL levels among all the groups transferred to fresh water was tested first by Friedman's two-way analysis of variance (Seagel, 1956), and then the differences between the control seawater value and each of the freshwater values was tested by the one-side randomization test for matched pairs (Seagel, 1956). Calculations were performed with the aid of a microcomputer (NEC-PC8801) using programs made by one of the authors (Ishii, 1983). In both males and females, the overall difference among all the groups was significant ($P = 0.016$ in females and $P = 0.0325$ in males). The difference from the seawater level was significant ($P = 0.031$) in all of the freshwater values in females. In males, a significant increase ($P = 0.031$) was seen only after 1 day in fresh water.

On the other hand, plasma osmolality decreased from 360–370 mOsm/kg in seawater to about 320 mOsm/kg 1 day after transfer to fresh water in both males and females, and the level was maintained until the seventh day. Statistical significance in

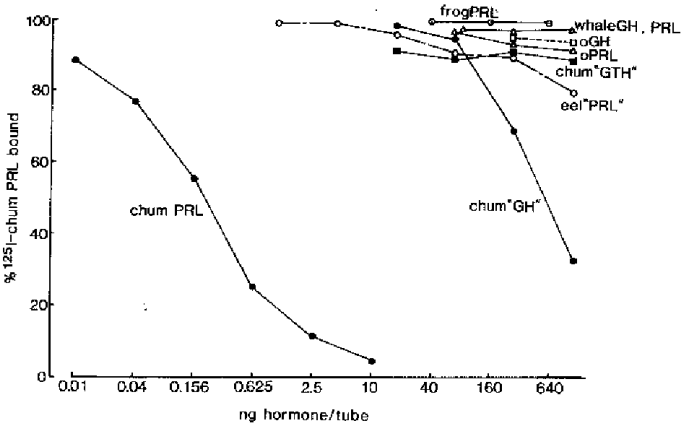

FIG. 5. Dose–response inhibition curves for chum PRL and presumptive chum growth hormone ("GH") and gonadotropin ("GTH"). The radioimmunoassay data for GHs and PRLs from sheep (○), whale, bullfrog as well as for presumptive eel PRL are as indicated. Each point represents the average of duplicate determinations.

the plasma osmolality was assessed using two-way layout analysis of variance (Campbell, 1967), and there was a significant difference ($P < 0.002$). When the fish were kept in seawater aquaria, there was a tendency toward an increase in plasma osmolality. One female died after 3 days, and only one female was left after 7 days; the mature chum salmon apparently adapted better to fresh water than to seawater.

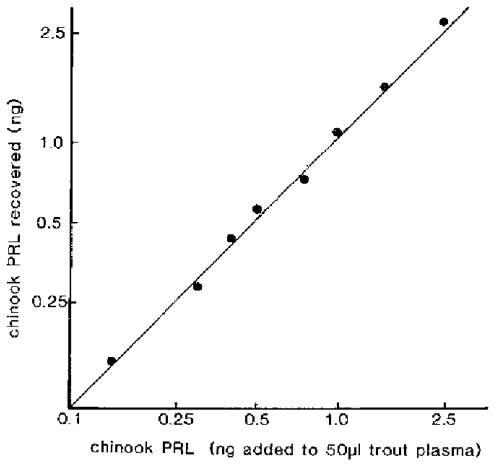

FIG. 6. Recovery of chinook PRL added to plasma from a rainbow trout (50 µl) in comparison with the theoretical amount expected. Each point is the average of duplicate determinations.

DISCUSSION

The salmon PRL RIA developed in the present study using an antiserum to chinook PRL was found to be highly specific and suitable for the measurement of immunoreactive PRL in plasma and pituitary of chum salmon as well as in those of other salmonid fishes. The specificity of the assay is established by several lines of evidence. The purified chum PRL and chinook PRL showed the same competitive inhibition curves in the RIA, regardless of iodination of either hormone. Close similarities between the chum and the chinook PRLs in physicochemical properties such as amino acid composition, molecular weight, and isoelectric point have been described (Kawauchi *et al.*, 1983; Prunet and Houdebine, 1984).

Pituitary extracts from several salmonid fishes such as the chum salmon, rainbow trout and Japanese charr showed strong cross-reactivity in the RIA, whereas those from the eel, goldfish, carp, and tilapia showed negligible cross-reactivity. The displacement curves for plasma from all the salmonids tested were also parallel to the salmon PRL standard, whereas plasma from the hypophysectomized rainbow trout

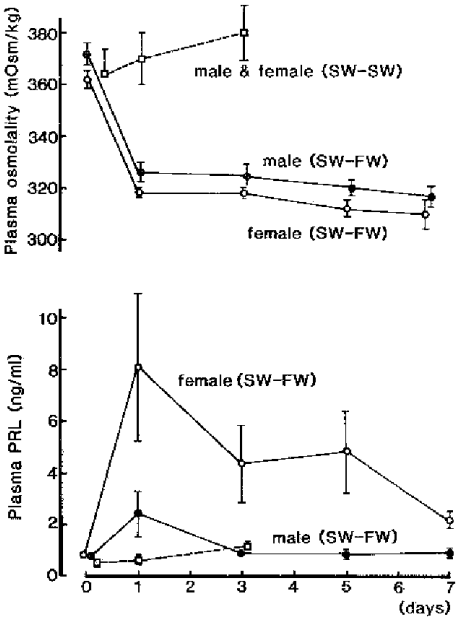

FIG. 7. Changes in plasma concentrations of immunoreactive PRL and plasma osmolality of mature chum salmon after transfer from seawater (SW) to fresh water (FW). Some fish were transferred to seawater aquaria as controls. Vertical bars represent standard errors of the means (N = 5, except for SW to SW transfer experiment, in which three males and three females were used).

as well as those from the eel, goldfish, carp, and tilapia showed virtually no displacement activity. The reason why small but significant amounts of PRL were detected in plasma of the hypophysectomized coho salmon is not clear. None of the mammalian PRL or GH preparations, bullfrog PRL or eel "PRL" cross-reacted in the PRL RIA, thus indicating major immunological differences between the salmon PRL and PRLs from the other species. The finding that the chum "GH" does not cross-react to an appreciable extent in this RIA is of considerable significance in view of the biological, chemical, and immunological data indicating similarities between PRLs and GHs prepared from many species (see Farmer and Papkoff, 1979). In accordance with the present observation, a homologous RIA for tilapia PRL exhibited only 0.05% cross-reactivity with tilapia GH (Nicoll *et al.*, 1981). More recently, Cook *et al.* (1983) reported a specific RIA for carp and goldfish GHs, with insignificant cross-reactivity with the goldfish PRL. It is also to be noted that the antiserum used in the present study specifically stained the putative PRL cells in the rostral pars distalis of the chum salmon and the rainbow trout pituitaries. There seems to be greater antigenic differences between teleost PRL and GH than was previously assumed. Furthermore, a clear distinction has been shown in biological activity between PRL and GH in teleosts (Doneen, 1976; Clarke *et al.*, 1977; Idler *et al.*, 1978; Komourdjian and Idler, 1979).

The salmon PRL RIA developed in the present study was sensitive enough to measure immunoreactive PRL in as little as 50 to 100 μl of salmon plasma. The precision of the assay compared favorably with PRL or GH RIAs developed for other teleost species (Nicoll *et al.*, 1981; Cook *et al.*, 1983), with an overall coefficient of variation (average of within and between assay CV) in the useful range of the RIA. In the present study, plasma PRL levels in mature chum salmon caught in the bay and in those transferred to seawater aquaria were below 1 ng/ml. The plasma PRL in the female increased to 4–8 ng/ml after transfer to fresh water, whereas no such increase was seen in the male except for a transient increase after 1 day in fresh water. The chum PRL used for the standard and the label in the RIA was extracted exclusively from female salmon (Kawauchi *et al.*, 1983), whereas the chinook PRL used to raise the antiserum was purified from a mixed stock (50% each) of pituitaries from male and female salmon (Prunet and Houdebine, 1984). Thus, it might be possible that male PRL is immunologically distinguishable from female PRL. However, this possibility seems to be ruled out, as there was no significant difference in the pituitary PRL content between the male (0.97 ± 0.338 μg/mg, N =

5) and the female (0.63 ± 0.613 μg/mg, N = 5) chum salmon adapted to fresh water for 7 days, and also as the antiserum against the chinook PRL did not distinguish between chinook and chum PRLs as shown above.

The present finding that the immunoreactive plasma PRL in the mature female chum salmon increased significantly after transfer to fresh water is consistent with a large body of evidence indicating important osmoregulatory roles of PRL in freshwater adaptation (Hirano and Mayer-Gostan, 1978; Clarke and Bern, 1980; Loretz and Bern, 1982). In males, however, an increase was seen only 1 day after transfer to fresh water. Using an RIA for ovine PRL, a decrease in plasma "PRL" has been observed after entry or transfer of some salmonids from seawater to fresh water (Leatherland and McKeown, 1974; McKeown and van Overbeeke, 1974; McKeown and Brewer, 1978). They ascribed the lower plasma levels to a rate of degradation of the hormone than the rate of secretion, although the validity of the data obtained with the heterologous RIA remains problematic (see Nicoll, 1975; Nicoll *et al.*, 1981). On the other hand, Komourdjian and Idler (1977) and Björnsson and Hansson (1983) reported that hypophysectomy does not impair freshwater survival if rainbow trout are allowed to recover in one-third seawater. Similar observations have been made in the coho salmon and the chinook salmon (R. S. Nishioka, N. H. Richman, and H. A. Bern, unpublished). Recently, Edery *et al.* (1984) reported high PRL receptor levels in the gonads of tilapia. Together with the low plasma PRL levels in male chum salmon, these observations may indicate that PRL is involved in female reproduction rather than osmoregulation in hypotonic environment. However, as indicated above, an increase in secretion rate may be accompanied by an increase in clearance rate, thus resulting in low circulating levels. Further studies are needed on changes in plasma level as well as turnover rate of salmon PRL under various physiological conditions such as osmoregulation, smoltification, migration, maturation. Examination of possible circadian and circannual fluctuations is also indicated.

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