CALCIUM AND CALCITONIN STUDIES IN PACIFIC SALMON, GENUS ONCORHYNCHUS, AND RAINBOW TROUT, SALMO GAIRDNERI

by

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ABSTRACT

In mammals, calcium homeostasis is under the control of parathyroid hormone and calcitonin. Fish lack parathyroid glands but large amounts of calcitonin are located in the ultimobranchial gland. The objective of this thesis was to examine calcium metabolism and the possible physiological role of calcitonin in rainbow trout, Salmo gairdneri, and Pacific salmon, genus Oncorhynchus.

Ultimobranchial gland calcitonin concentrations were measured in trout and salmon under a variety of conditions, using the rat bioassay. Assays indicated that calcitonin concentrations in the ultimobranchial glands varied widely and showed no consistent relationship to plasma calcium and phosphate levels, sex, sexual maturation, environmental calcium concentration or species. The ultimobranchial gland calcitonin concentrations of fingerling trout (age 7 - 8 months) were lower than adult trout, suggesting a possible relationship between calcitonin and growth.

The biological half-life of salmon calcitonin was measured in free-swimming cannulated trout and salmon. Results indicate that the half-life of salmon calcitonin in fish (trout 27.6 min., salmon 48.0 min.) is considerably longer than that found in mammals.

The effect of salmon calcitonin on plasma calcium and phosphate was examined in trout and salmon. Salmon calcitonin injection did not cause hypocalcaemia or hypophosphatemia in fingerling trout and was also ineffective in cannulated adult trout.

No significant change in plasma electrolytes or urinary electrolyte excretion was observed following infusion of salmon calcitonin into cannulated adult female salmon.

A migration study on the Chilko race of sockeye salmon was carried out to investigate plasma electrolyte and tissue changes as these fish migrate from sea to fresh water. Ionic and total serum calcium were determined and results indicate that the sockeye maintain a relatively constant serum ionic calcium level throughout their spawning migration, indicating effective homeostatic control.

Measurements using a sensitive and specific radioimmunoassay, revealed that calcitonin can be detected in the plasma of salmon and that this hormone was continuously secreted under basal conditions. The levels of calcitonin detected in salmon plasma were higher than those found in most mammals.

A sex difference in plasma calcitonin levels (females higher than males) was found in sockeye, as well as in the coho and chinook adult salmon. This sex difference appears to be unique to salmonids. Female plasma calcitonin levels were found to rise during the migration and to decrease following spawning. Plasma calcitonin changes followed a different pattern in the migrating male sockeye. The plasma calcitonin changes were clearly not related to plasma calcium and phosphate alterations. In the female sockeye, calcitonin appears to be involved in sexual maturation and spawning.

Removal of the gonads from mature female sockeye resulted

in a marked drop in circulating plasma calcitonin levels. Estrogen injection into these gonadectomized salmon dramatically elevated plasma calcium but did not restore the plasma calcitonin levels.

These investigations indicate that the physiological role of calcitonin in calcium metabolism in fish may be different from that in mammals.

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PREFACE

"There they were," he said, pointing at the huge fish; "nearly two hundred years old; perfectly healthy; no symptoms of senility; no apparent reason why they shouldn't go on for another three or four centuries . . . . . ."

He paused and stood for a moment in silence, drumming with his fingers on the glass of the aquarium. Poised between mud and air, the two obese and aged carp hung in their greenish twilight, serenely unaware of him. Dr. Obispo shook his head at them. "The worst experimental animals in the world," he said in a tone of resentment mingled with a certain gloomy pride. "Nobody had a right to talk about technical difficulties who hadn't tried to work with fish. Take the simplest operation; it was a nightmare. Had you ever tried to keep its gills properly wet while it was anaesthetized on the operating table? Or, alternatively, to do your surgery under water? Had you ever set out to determine a fish's basal metabolism, or take an electrocardiograph of its heart action, or measure its excreta? And, if so, did you know how hard it was even to collect them? . . . . . ."

"No, you had not," said Dr. Obispo contemptuously. "And until you had, you had no right to complain about anything."

<u>After Many A Summer Dies the Swan</u>

Aldous Huxley

GENERAL INTRODUCTION

According to Romer (1962), teleosts are unquestionably the most numerous and versatile of all the vertebrates. This fact was acknowledged by Bern (1967) who, when discussing problems in fish endocrinology remarked, "In their endocrine systems, as in all other aspects of their anatomy and physiology, the fishes reveal a broader range of variation and a longer history of adaptation than do the 'land-living' (tetrapod) vertebrates."[1]

It has now been determined that calcium metabolism in teleosts is under endocrine control (Hoar, 1957a; Simmons, 1971; Chan, 1972). The discovery of calcitonin in fish (Copp et al, 1967a) gave rise to the question, "Is calcitonin involved in this hormonal regulation of calcium metabolism?" The purpose of the present thesis was to investigate calcium metabolism in fish and the physiological role of calcitonin in this process.

With regard to the endocrine control of calcium homeostasis, fish are unique among the vertebrates in that they lack a parathyroid gland (Fleischmann, 1951; Pickford, 1953; Hoar, 1951, 1957a; Bern, 1967). Most teleosts possess an endocrine gland, the corpuscles of Stannius, which appears to be intimately involved in calcium and other electrolyte homeostasis (Chan, 1969, 1972; Pang, 1971a). The pituitary gland, adrenal cortex, gonads and

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[1]Bern, H.A. Hormones and Endocrine Glands of Fishes, Science, N.Y. 158: (1967) pg. 455.

thyroid gland are also definitely involved in calcium metabolism in fish (Hoar, 1957a; Henderson et al, 1970; Simmons, 1971; Chan, 1972).

The importance of the calcium ion to fish was first reported by Ringer (1883) who noted that while unfed fish live for weeks in tapwater, they soon die if placed in distilled water. Uptake of calcium from the environment occurs at the gills, fins and oral epithelia (Moss, 1965; see Simmons, 1971). This uptake appears to be more efficient in freshwater fish and is facilitated by the presence of phosphate (see Love, 1970).

The freshwater fish solves the problems of electrolyte loss and water influx by absorbing ions from the environment, maintaining an impermeable integument (scales, skin, mucous) and excreting a hypo-osmotic urine (Black, 1957; Bentley, 1971). The problems of calcium regulation in seawater teleosts are quite different from those in freshwater. Marine teleosts drink seawater. This means that electrolytes, including a significant quantity of calcium, are absorbed by the gastrointestinal tract (Chan et al, 1967; Henderson et al, 1970). Excess salts are then excreted directly from the blood through the gills, retaining water in the fish (Smith, 1930; see Parry, 1966; Potts, 1968). Again, a dilute urine is formed and divalent ion excretion occurs. Thus, the environment plays an important role in calcium regulation in fish. For this reason, both the freshwater trout, Salmo gairdneri, and the anadromous Pacific salmon, genus Oncorhynchus, were studied to ascertain the effect of environmental

water calcium concentration on calcium homeostasis.

The osmoregulatory problems of a freshwater or seawater fish are enormous but these same problems encountered by a migrating anadromous or catadromous fish are even more complex. A major portion of this thesis concerns research done on the migrating sockeye salmon, Oncorhynchus nerka, and its ability to control its internal ionic environment as the external environment changes from sea to freshwater.

Since many of the hormones which affect calcium metabolism in fish are also involved in migration and sexual maturation, there are numerous endocrine inter-relationships. Many female teleosts develop hypercalcaemia during the breeding season and this condition can be produced by estrogen injection in the laboratory. The pituitary hormones, thyroxine and gonadal steroids appear to be involved in migratory behaviour changes as well as in sexual maturation (Hoar, 1953, 1957b, 1963; Woodhead and Woodhead, 1965). Environmental factors such as temperature, light or rainfall may serve to initiate, potentiate, and integrate the hormonal activities in the above processes (Hoar, 1965a,b; Henderson et al, 1970).

The degree of involvement of bone in calcium homeostasis in fish has been a subject of controversy for some time (Fleming, 1967; Moss, 1965; Simmons, 1971). Teleost bone exhibits a wide range of histo-morphology, from acellular to cellular bone (Moss, 1961). The degree of calcification of the teleost skeleton appears to be independent of bone type (cellular versus acellular) and histology (Moss and Freilich, 1963). Moss (1961, 1962, 1963, 1965)

and others (see Fleming, 1967) have proposed that calcium in the environmental water is important in calcium homeostasis and that mineral stores in the fish skeleton play only a minor role in this process. In contrast, Urist (1962, 1966) believes that the teleost skeleton is involved in calcium homeostasis and that the bone and tissues of the fish form a "bone-body fluid continuum" which acts as a closed-cycle system. Resorption of teleostean bone has seldom been reported but it may be that cellular-boned species, such as the eel and salmon, are able to draw on the bone mineral under certain conditions (Simmons, 1971). Resorption of fish scales, which contain substantial amounts of calcium, has been observed in starved fish (Simmons, 1971) and during salmon migration (van Someren, 1937).

The importance of the soft tissues, such as muscle and skin, in teleost calcium regulation has been emphasized by several researchers (Norris et al, 1963; Rosenthal, 1963; Podoliak and Holden, 1965). These tissues serve as important storage depots for exchangeable calcium, the mobilization of which, at least in the eel, appears to be under the endocrine control of the pituitary, adrenal cortex, corpuscles of Stannius and the ultimobranchial glands (Chan, 1969, 1972; Simmons, 1971).

The dietary supply of calcium, even in marine fish, is generally not as important as direct absorption from water (Moss, 1962; Berg, 1968, 1970; Simmons, 1971) although food can supply calcium in freshwater fish under certain experimental conditions (Ophel and Judd, 1967). The main excretory routes for calcium are the gills and kidneys, fecal loss of calcium probably being

smaller than in mammals (Simmons, 1971).

Although the parathyroid gland is absent in fish, they do possess ultimobranchial glands which contain a rich supply of calcitonin (Copp and Parkes, 1968b; Copp et al, 1968b; Copp, 1969a). In fact, calcitonin began phylogenetically in fish (Copp, 1969a; Copp et al, 1972a; Copp, 1972). The ultimobranchial origin of calcitonin was discovered in 1967 and salmon calcitonin (SCT), the first non-mammalian calcitonin to be characterized, became available in purified form in 1969 (O'Dor et al, 1969a, b). The amino acid sequence of salmon calcitonin was reported shortly thereafter (Niall et al, 1969). Salmon calcitonin has been shown to exert a longer-lasting, more powerful hypocalcaemic effect than mammalian calcitonins when tested in young mammals. This effect is due primarily to the inhibition of bone resorption (Copp, 1970a).

When work on the present study commenced, porcine calcitonin had been injected into a few species of teleosts and with variable results (Pang and Pickford, 1967; Louw et al, 1967; Chan et al, 1968). Salmon calcitonin had not been tested in any fish prior to 1969. Therefore, experiments were designed to collect basic information on calcitonin in fish in an attempt to answer the following questions:

1. What is the effect of salmon calcitonin injection on plasma and renal electrolytes in salmonids?
2. How much calcitonin is stored in fish ultimobranchial glands?

3. Does the UB gland calcitonin concentration vary with age, sex or salinity?
4. What is the circulating level of plasma calcitonin in fish and what factors govern this level, i.e. migration, sexual maturation, gonadectomy?
5. Is calcitonin involved in calcium and phosphate homeostasis in fish?
6. How do the actions of calcitonin in fish compare to those in mammals?

Thus, the investigation of calcium metabolism in fish and the physiological role of calcitonin was begun on this broad base.

GENERAL MATERIALS AND METHODS

In this chapter, those materials and methods which were common to all studies are described. Materials and methods specific to individual studies will be outlined in the appropriate chapter.

1. Choice of Experimental Animal

The fish was chosen as the experimental animal in this study for several reasons. Phylogenetically, the ultimobranchial (UB) glands first originate in fish. Almost nothing was known about the function of calcitonin in non-mammals until Copp (Copp et al, 1967a, b; Copp and Parkes, 1968a, b) demonstrated that the ultimobranchial gland was a rich source of calcitonin. Salmon and trout were readily available in British Columbia and the surgical techniques for working on these fish were well documented. Finally, a very sensitive and specific radioimmunoassay for salmon calcitonin became available and provided an important tool to investigate the physiological changes of calcitonin in these fish.

2. Experimental Animals

Several types of fish, obtained from a variety of sources, were studied. The types of fish included rainbow trout (Salmo gairdneri), coho salmon (Oncorhynchus kisutch), chinook salmon (Oncorhynchus tshawytscha) and sockeye salmon (Oncorhynchus nerka).

a) Rainbow Trout

Rainbow trout used in this study were purchased from the Sun Valley Trout Farm, Mission, British Columbia. The trout weighed between 60 and 260 g. The fish were transported to the University of British Columbia in oxygenated 100 gallon tanks and kept in a 250 gallon self-cleaning, fibreglass, holding tank (Everlast Plastics Co., Vancouver) supplied with fresh running water. Tapwater was filtered and dechlorinated using a filter containing activated charcoal, limestone, oyster shells and sand. Water temperatures varied seasonally over a range of 4 - 16^{o}C, but remained fairly stable over any one experimental time course. The trout were fed regularly with commercial trout pellets (Purina Trout Chow, Ralston Purina Co.).

The light regimen in the fish laboratory was controlled using an Inter-Matic Time Switch (Model T 101). This switch was adjusted regularly to the seasonal light conditions and was kept constant during any one acclimation and experimental period. All fish were acclimated to laboratory conditions for at least one week before being used in any experiments.

b) Coho Salmon

Mature adult coho salmon were obtained from the Washington State Department of Fisheries at the Samish River holding ponds in Washington, U.S.A. Immature seawater and freshwater coho, were obtained from the Fisheries Research Board of Canada, Vancouver, B. C.

c) Chinook Salmon

Mature adult chinook salmon were obtained from the Washington State Department of Fisheries at the Deschutes River holding ponds in Olympia, Washington, U.S.A.

d) Sockeye Salmon

The sockeye salmon were obtained from two sources, the Chilko Lake race of sockeye and the Great Central Lake race of sockeye.

The Chilko sockeye were caught at various stages in their migration during the summers of 1971 (July 23 to September 22) and 1972 (July 21 to September 24).

Sexually immature adult sockeye salmon were caught by trap when entering Great Central Lake on Vancouver Island, B.C. in June and July of 1970 and 1971. These fish normally spawn from late September until the end of November in the lake and in creeks feeding the lake. The method of capture and transport to the Vancouver Laboratory, Fisheries Research Board of Canada, have been described (McBride et al, 1963). The fish were held at the Vancouver Laboratory in large fiberglass holding tanks at seasonal water temperatures and on a natural photoperiod. Fungal infections were treated with salt baths of 3% aqueous sodium chloride and 2-Phenoxyethanol as described by Idler (1961a). A volume of 0.5 ml of a 50 mg per ml solution of Terramycin (Phizer, Canada) was injected intramuscularly to control bacterial infection.

Prior to an experiment, the sockeye were transported to the Physiology Department fish laboratory as required, using a

1:15,000 solution of MS-222 anesthetic (tricaine methanesulfonate, Fraser Medical Supplies Ltd.) and portable air pumps. Unless indicated otherwise, the salmon were not fed in the laboratory.

3. Operating Procedures

a) Operating Table and Operating Techniques

This study involved the serial sampling of blood from a free-swimming quiet fish, over extended periods of time. For this reason, the dorsal aorta cannulation technique (Smith and Bell, 1964) was employed.

An operating table, similar to the one described by Smith and Bell (1967), was constructed and used for all surgical operations (Plate 1, page 11). The fish was first anesthetized in a bucket of a 1:5,000 solution of MS-222 and then placed ventral side up on the operating table. The gills were perfused through the mouth or opercular openings with a 1:15,000 solution of MS-222 (Bell, 1967). The fish was kept moist at all times with wet fish netting. Throughout the procedure, the operating table anesthetic was aerated and the water temperature kept constant by placing plastic bags of ice into the reservoir.

Following the operation, the fish was allowed to recover in fresh, aerated running water. The procedures were completed in less than 30 minutes and recovery time was approximately 5 minutes. Complete recovery was indicated by twitching, fin movements, resumption of normal respiratory movements and swimming efforts. The fish was placed in the appropriate experimental apparatus (aquarium,

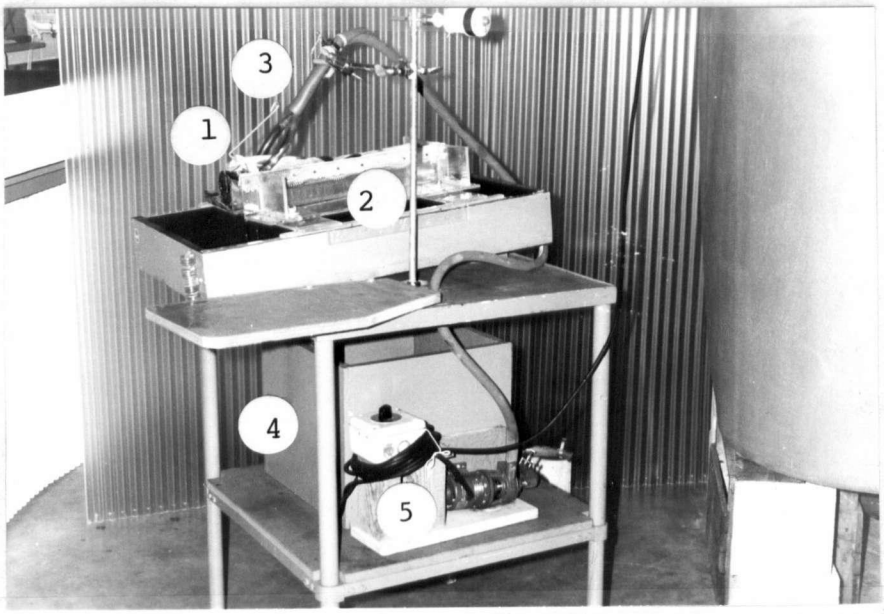

PLATE 1. Fish Operating Table

1. Trout (upside down) in position for cannulation
2. Adjustable holding device for fish
3. Rubber hose attachment to direct anesthetic over gills
4. Anesthetic reservoir
5. Variable-flow pump

urine box) immediately following the operation and at least 12 to 24 hours recovery time allowed before use. Since surgical operations can lead to considerable physiological trauma (Houston et al, 1969), a recovery period of this length is considered necessary. The experimental apparatus was partially covered with black plastic to prevent the fish from being disturbed by the movements of the investigator.

b) Dorsal Aortic Cannulation Procedure

The dorsal aorta was cannulated at its point of intersection with the second efferent branchial arteries as described by Smith and Bell (1964). The cannula consisted of a 60 cm length of Clay-Adams PE 60 (ID. 0.762 mm) plastic tubing into which a 2-3 cm 21-G (Huber Point with Closed Bevel-B-D Yale Luer Lok) needle had been inserted. A hole was made mid-dorsally in the tip of the snout of the fish with a 12-G needle. Caution was taken to avoid the olfactory lobes and no injury was evident from this procedure. A 3 cm length of Clay-Adams PE 200 plastic tubing ("sleeve"), heat flared on one end, was passed through this hole in the snout from inside the mouth. This PE 200 sleeve was used to secure the PE 60 cannula in place. The cannula was filled with heparinized (10 USP units Heparin (Ammonium Salt) per ml of saline) Cortland Saline (Wolf, 1963) and plugged with a tapered stainless steel pin when not in use. The cannula needle was inserted at the midline junction of the first gill arch in the roof of the mouth at an angle of approximately 15-20^{o}. Successful cannulation was indicated by bright red blood rushing into the cannula tubing, dis-

placing the heparinized saline. The cannula was sutured securely in the roof of the mouth using black silk surgical suture (size 000 Davis & Geck Products) and on the PE 200 sleeve using white surgical cotton thread (Figure 1a, pg.14, and Plate 2, pg. 15). The cannula was flushed with fresh heparinized saline once a day and care was taken to ensure that the needle and cannula were completely filled with heparinized saline. The cannula was allowed to trail freely behind the fish and it did not appear to affect behaviour or swimming ability in any way.

c) Blood Sampling Procedure

The cannula was gently retrieved from the aquarium with long forceps, dried off with tissue paper, and the plug removed. A heparinized 1 ml syringe was used to withdraw 0.5 ml of the saline-blood mixture which filled the cannula. The dead space of the cannula amounted to 0.3 ml (heparin-saline) and so removal of 0.5 ml ensured that the succeeding sample would not be contaminated with heparin -saline. A second heparinized 1 ml syringe was used to withdraw the blood sample. Then the first syringe with the mixture of heparin-saline and blood was immediately returned to the fish and followed by clean heparin-saline so that no blood remained in the cannula. The steel plug was then replaced in the cannula and the cannula was returned to the aquarium so the fish would be free to move.

It should be noted that all syringes used in the sampling procedure were plastic (Roehr Monoject) and the dead space of the syringe was filled with concentrated heparin (1000 USP Units = 1 ml

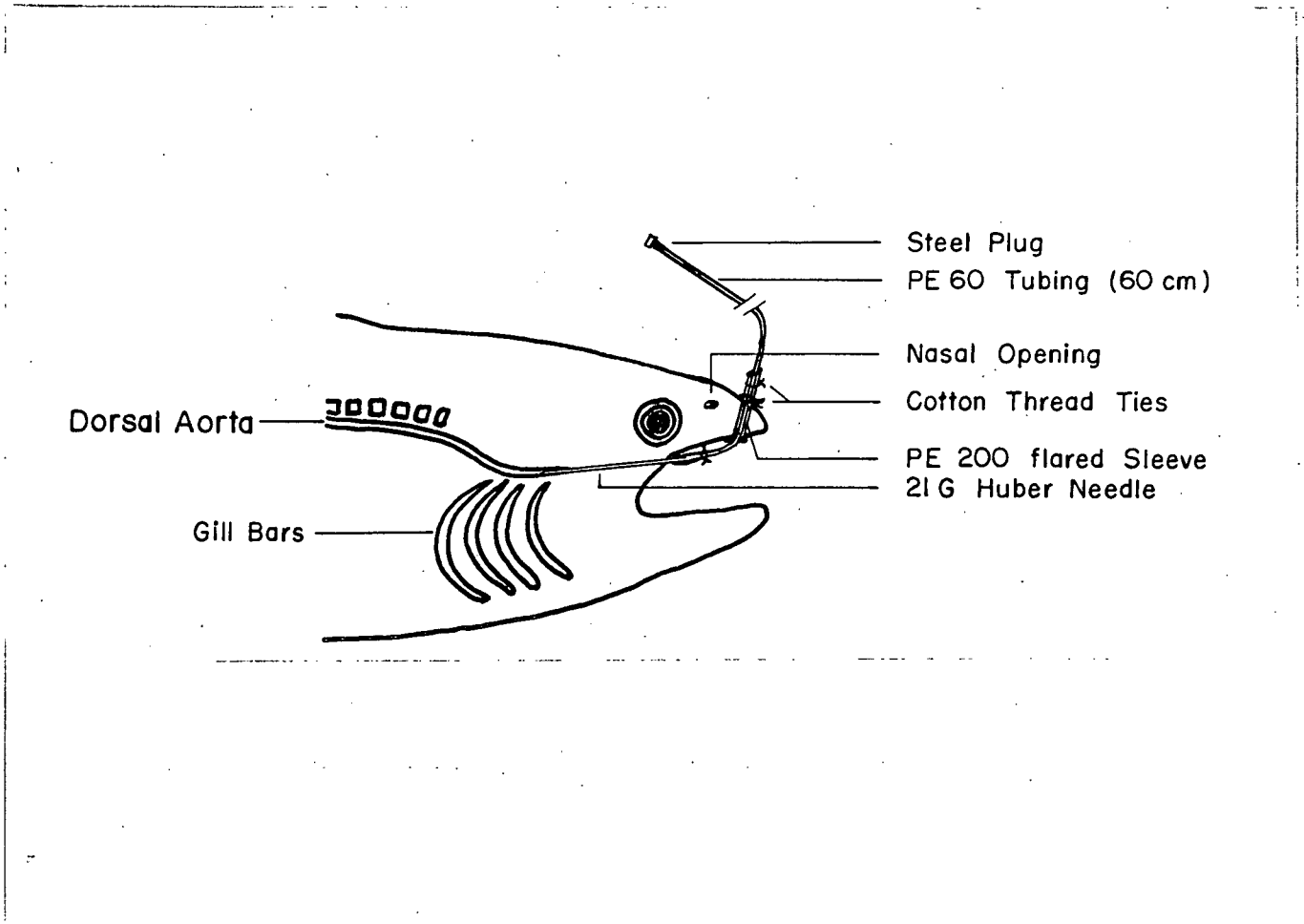

Figure 1a. Rainbow trout dorsal aortic cannulation

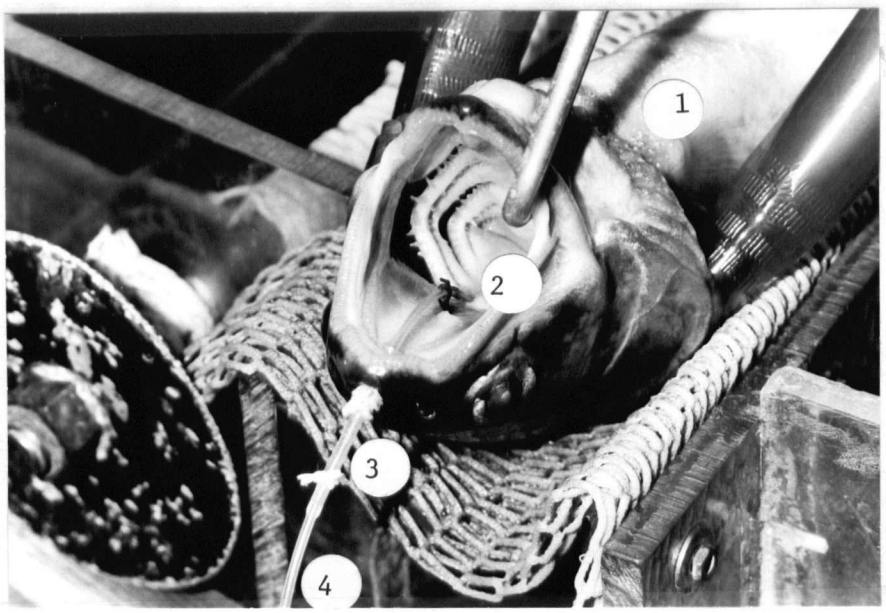

PLATE 2. Trout Dorsal Aortic Cannulation

1. Trout upside down on operating table
2. Huber needle (21-G) at first gill arch point of entry (tied in with silk thread)
3. PE 200 sleeve tied in place with cotton thread
4. PE 60 cannula

liquid Heparin, Sherwood Medical Industries). Furthermore, injections were performed slowly and steadily so as to minimize hemolysis and trauma to the fish. If these precautions were taken, the fish always remained perfectly still. In chronic cannulation experiments, where large volumes of blood were taken, the red blood cells were resuspended after plasma collection in an appropriate volume of heparinized-saline (10 U/ml). This mixture was immediately returned to the fish, the cannula was refilled with heparinized saline and plugged. Any significant drop in haematocrit due to repeated blood samplings was thus prevented (Hickman, 1968).

The blood samples were usually transferred to 6 ml sterile, polystyrene disposable culture tubes and immediately centrifuged for 5 minutes in a standard laboratory clinical centrifuge. Using glass pasteur pipettes, the plasma was separated off into clean culture tubes with caps. The plasma was stored on ice if measurements were to be made the same day, or frozen on dry ice and stored at $-12^{o}C$ if measurements were to be performed at a later date.

When large blood samples (30-50 ml) were taken from salmon, the blood was transferred to 50 ml polycarbonate centrifuge tubes and spun at 1200 x g for 5 minutes on an HN-S Centrifuge (International Equipment Co. U.S.A.).

Injection of substances into the dorsal aorta could be performed immediately following the control blood sample. Care was taken to exclude bubbles from the injectate and to keep the volume injected small (less than 0.3 ml for a 200 g trout). The

injectate was followed first by the heparin-saline and blood mixture and then by a clean 0.4 ml heparin-saline volume to fill the cannula and needle dead space. The time of injection was calculated from the moment the injectate entered the fish.

d) Caudal Vein Sampling

Terminal blood samples were obtained from the fish using the caudal vein sampling technique. The fish was usually tapped on the head at the beginning of the procedure and held securely wrapped in netting. To ensure that the sampled blood was well oxygenated, the gills were perfused with aerated water. The trout was laid flat on its side on the operating table and a heparinized plastic 1 ml syringe with a 21-G 1½ inch needle was inserted through the skin of the mid-ventral aspect of the caudal peduncle, approximately 2 cm posterior to the anal fin. The needle was directed forward between the haemal spines into the haemal canal. Using this technique, blood was withdrawn into the syringe from the tail circulation with little injury to the fish. If more than 1 ml of blood was required, the needle was left in place and a second heparinized 1 ml syringe was quickly inserted into the needle. Thus terminal blood samples could be withdrawn quickly and easily from fish in approximately 1 minute.

For salmon, the technique was identical, except that a larger syringe with an 18-G 1½ inch needle was used. In the field, the salmon were restrained by placing them ventral side up in a large V-shaped apparatus constructed of plywood.

e) Caudal Vein Cannulation

The caudal vein of the salmon was cannulated using the basic technique developed by C. P. Hickman (Hoar and Hickman, 1967). This technique was similar to the caudal vein sampling procedure except that an 18-G 3½ inch thin wall needle (for use with plastic tubing B-D Yale Luer-Lok No. 1295), through which PE 50 Intramedic tubing (Clay-Adams) could be passed, was used. The salmon was first anesthetized, placed on its side on the operating table and perfused with 1:15,000 solution of MS-222. Holding the caudal peduncle in the left hand, the 18-G needle attached to a 2.5 ml plastic syringe containing heparinized-saline, was inserted from the lateral-ventral aspect of the caudal peduncle. The needle was inserted into the caudal vein, Figure 1b, pg. 19, and successful entry was signalled by a flow of blood into the syringe. With the needle held securely in the caudal vein, the syringe was carefully and quickly removed and a 50 cm length of PE 50 cannula (filled with heparinized-saline) was threaded down the needle and into the caudal vein for a distance of 15 cm. Proper insertion of the cannula was indicated by the easy withdrawal and injection of blood through the PE 50 cannula using a 1 ml syringe and 25-G needle filled with heparinized-saline. The 18-G needle was then extracted from the puncture site, taking care not to dislodge the cannula, and slipped back down the length of the cannula. After filling the cannula with heparinized-saline and pinching it off with the thumb and forefinger, the 1 ml syringe and 25-G needle were detached from the cannula, the 18-G needle was removed and the cannula was plugged with a stainless steel pin.

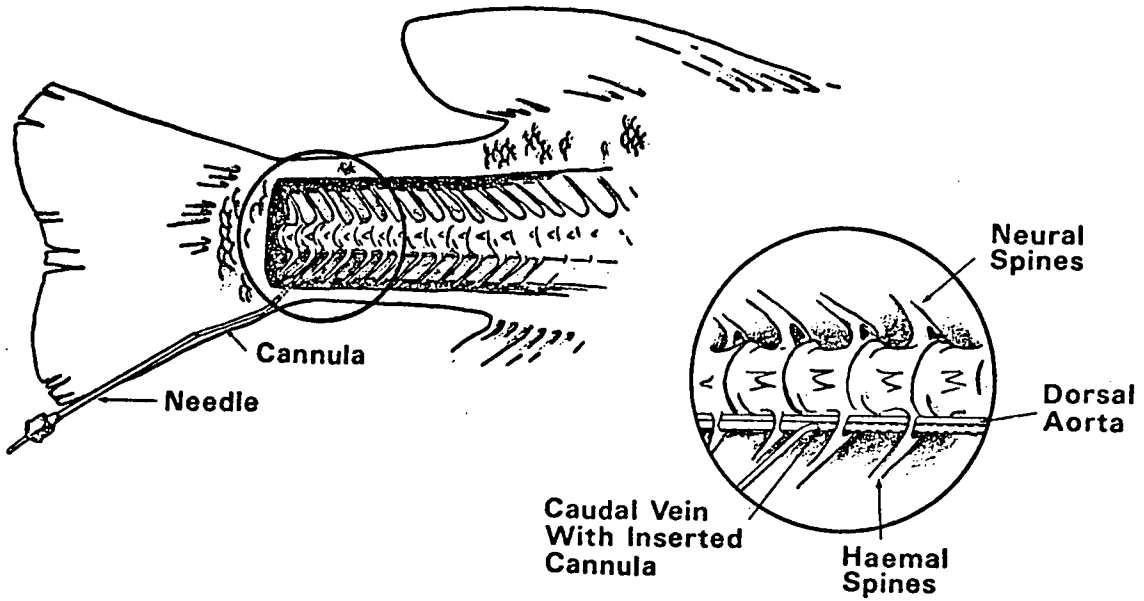

Figure 1b. Caudal vein cannulation technique. Diagram from Hoar and Hickman (1967).

Pressure was exerted on the puncture site to minimize the bleeding and the cannula was tied securely, 2 cm below the lateral line, using 2 stitches of black braided surgical silk.

This type of cannulation could only be used on fish over 500 g (hence on the salmon in this study) and always involved a small amount of blood loss from the puncture site (about 0.5 ml). Blood sampling from the caudal cannula was similar to that of the dorsal aorta outlined previously. Using the two cannulations, a free-swimming, quiet fish could be infused and blood sampled simultaneously.

f) Urinary Catheterization

The salmon was anesthetized and placed ventral side up on the operating table. Throughout the procedure, the mouth was perfused with a 1:15,000 solution of MS-222.

Catheterization was performed by opening the aperture of the urogenital papilla with a pair of fine curved forceps and inserting the tip of a rubber pediatric catheter of size French 8 or 10 (Ingram and Bell Ltd.) dorsally into the urinary orifice. The catheter was passed along the urinary duct into the urinary bladder (3-6 cm) and successful catheterization was indicated by urine flowing from the catheter when allowed to hang beneath the fish. The catheter was then secured in place by firmly tying off the urinary papilla around the catheter using surgical cotton thread. The urinary aperture was then closed off around the catheter and over the papilla by a purse string ligature. These procedures anchored the catheter in place and insured that no leakage

occurred. The catheter was further secured in position by a long stitch to the caudal peduncle using black surgical silk. The catheterized fish was then quickly transferred to the urine collection box (Figure 2, pg. 22) to recover in fresh running water.

The urine collection box used in this study was the same as that described by Smith and Bell (1967). The partitions were adjusted to the size of the fish and the urinary catheter was passed out through a rubber gasket-sealed aperture to a fraction collector (Instrumentation Specialties) for collection of hourly samples. The urine was allowed to flow by gravity and blockage of the catheter rarely occurred. The fish were free-swimming but restricted and urine was collected continuously. The box was constructed of black acrylic plastic to shield the fish from outside disturbances and under these conditions, the salmon stayed almost motionless for hours. These precautions were necessary due to the phenomenon of "laboratory diuresis" which normally occurs following any kind of disturbance or handling procedure (Holmes, 1961; Klontz and Smith, 1968; Hammond, 1969; Hunn and Willford, 1970). Therefore, the fish were allowed to recover after the operation for 15-24 hours before any experiment was conducted.

4. Analytical Procedures

a) Physical Measurements

Total body weights of the trout were measured using a standard laboratory one-arm balance. The salmon were weighed on a larger one-arm balance (Ohaus Scale Corp., cap. 6 kg). A Mettler

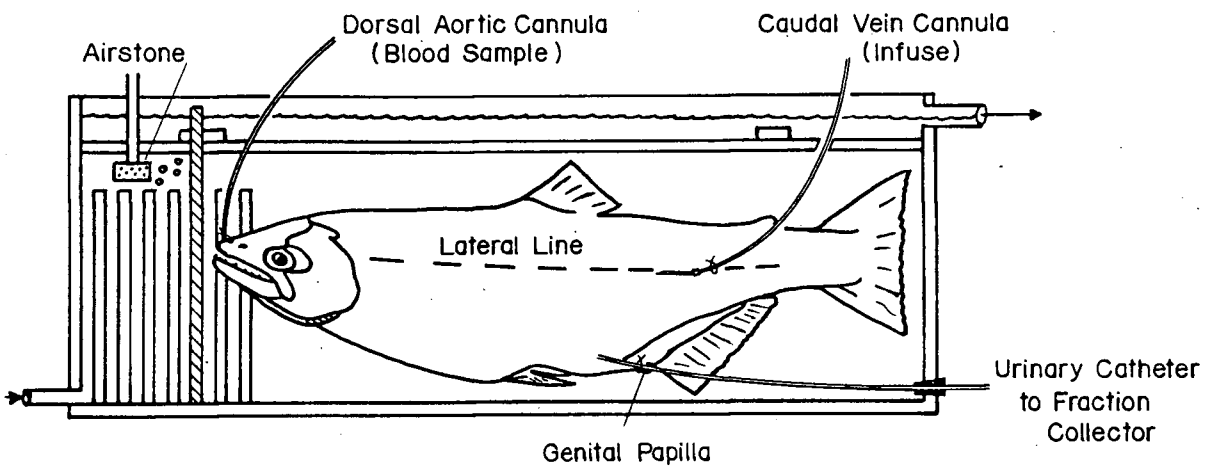

Figure 2. Blood and urine sampling technique in salmon

balance (Type H16, cap. 80g)was used for accurate weighing of chemicals, ash weights and small organs.

From the total weight (g) and the weight of both gonads (g), the gonad-somatic index (GSI) was calculated from the following equation:

$$\text{Gonad-Somatic Index (GSI)} = \frac{\text{gonad weight (g)}}{\text{total body wt (g)}} \times 100$$

This "index of maturity" was used as a measure of the degree of sexual maturation of the fish. Thus, the higher the index, the more advanced was the degree of sexual maturity (Vladykov, 1951).

Fork lengths of individual fish were also recorded. The fork length is the distance (in cm) from the tip of the snout to the fork in the caudal fin.

b) Haematocrits

Blood for haematocrit estimation was introduced into heparinized capillary tubes (Donlab, Ingram and Bell Ltd.), capped with Critocaps (Sherwood Medical Industries Inc.) and spun in a Micro-Capillary Centrifuge (International Equipment Co. - Model MB) for 4 minutes. The haematocrits were read immediately on a Critocap Micro-Haematocrit Tube Reader. The plasma was then separated from the red cells and used for measurement of percent water or calcium.

c) Plasma Water and Total Solids

Plasma water and total solids were measured using a TS-Meter (Total Solids Meter, American Optical Instrument Co. Model 10400). Using this instrument and the accompanying conversion tables,

estimates of the total solids percentage composition by weight (TS%), the percent water (% H_2O) by weight (g per 100 g at 20°C) and protein concentration (C_{PR}; g per 100 ml at 20°C) were determined on 10 μl of plasma.

d) Preparation of Tissues for Electrolyte Analysis

i. Drying and Ashing Procedures

Hard Tissues

The hard tissues taken for electrolyte analysis included vertebrae, ribs, premaxillae and scales. The tissues were first roughly dissected from the fish and stored frozen at -12°C in air-tight plastic bags.

The vertebrae, ribs and premaxillae were thawed, freed of soft tissue and samples of approximately equal weight were dried in porcelain crucibles (Coors Labware) to a constant weight (oven temperature 100°C). The dry weight was then calculated and the samples were ashed at 575°C overnight. The samples were again weighed (ash weight).

Before drying, the scales were rinsed in deionized water and wiped with tissue paper to remove any mucous or seawater.

Soft Tissues

Samples of muscle, skin and gonads were dissected and stored similar to the hard tissue. Since a large error due to evaporation was involved with the small samples of muscle and skin, the wet weight for the soft tissue was recorded only for the gonads. The muscle and skin samples were placed in porcelain crucibles and

dried in an oven at 100°C to a constant weight. The large gonads were similarly dried to a constant weight in 600 ml pyrex beakers. The dry weight was calculated. Fat-extraction was then carried out on the dried tissues with a 1:1 mixture of absolute ethanol and anhydrous ethyl ether (Analytical Reagent, Mallinckrodt Chemical Works). These samples were further dried to a constant weight at 100°C, and reweighed to obtain the fat-free dry weight (FFDW). The fat-free tissues were then ashed overnight and the ash weight calculated. Due to the large size and biochemical composition of the gonads, it was necessary to ash these samples for several days.

ii. Dilutions of Ash

The ashed tissue was weighed accurately, dissolved in 6 N hydrochloric acid, and evaporated to near dryness on a hot plate. The sample was then redissolved in 0.1 N hydrochloric acid and transferred quantitatively to the appropriate volumetric flask. A further dilution was made using a 0.5% (w/v) lanthanum chloride solution ($LaCl_3 \cdot 7H_2O$) and analyzed for calcium by atomic absorption spectrophotometry. Phosphate was measured colorimetrically (Alexander, 1968) after making the proper dilution with deionized water. The results were expressed as mg or g of calcium or phosphorus per 100 g dry weight, fat-free dry weight or ash weight.

e) Electrolyte Analysis

The glassware used for electrolyte measurements was acid-washed and free of contaminating ions. Small samples (less than 1.0 ml) were dispensed using Oxford Micro-Pipette Samplers (Oxford

Laboratories).

Lab-Trol and Patho-Trol Chemistry Reference Serums (Dade, Division American Hospital Supply Corp.) were used in all electrolyte analyses as quality control checks. The simultaneous use of the normal and abnormal controls provided a check of the standards and instrument linearity.

i. Calcium

Two methods of calcium analysis were employed in this study.

When only small amounts of plasma or solution were available, calcium was analyzed fluorometrically using a modification of the Technicon Auto Analyzer Method N-31 P (Newsome, 1969). This method could be used to measure extremely small amounts of sample (e.g. 10 μl) and was found to be both rapid and reproducible.

Calcium was also determined by atomic absorption spectrophotometry (Jarrell-Ash, Model 280 Atomsorb) at a wave length of 4227 Å and using 0.5% lanthanum chloride as the diluent. The addition of lanthanum chloride suppressed interference from sulfur and phosphorus (Trudeau and Freier, 1966). The readings were recorded on a strip chart recorder (Sargent Recorder, Model SR).

ii. Magnesium

Magnesium was determined on the same atomic absorption spectrophotometer at a wave length of 2852 Å. Samples and standards were again diluted with 0.5% lanthanum chloride.

iii. Sodium and Potassium

Sodium and potassium, in the plasma and urine, were analyzed

by flame photometry (Instrumentation Laboratory Inc., Model 143). A standard lithium solution (15 mEq Li per litre) was used as diluent. Samples were diluted and dispensed with an automatic dilutor (Fisher Dilutor, Model 240).

iv. Inorganic Phosphorus

Plasma, urine, and tissue phosphorus were measured using a modification of the Technicon Auto Analyzer N-4c Method (Alexander, 1968). This method is based on the formation of phosphomolybdic acid, which is then reduced by stannous chloride-hydrazine.

f) Serum Ionic Calciums

i. Collection of Blood Samples

The blood was sampled by caudal vein puncture using a 12 ml plastic syringe and 18-G 1½ inch needle. The syringe, including needle dead space, was previously filled with 2 ml of mineral oil (Nujol, Plough Ltd.) which had been cooled on ice. It should be noted that throughout the entire procedure, the blood and serum were kept anaerobic and on ice. All mineral oil, syringes and test tubes were cooled on ice before use.

The blood sample was taken with the syringe in a vertical position i.e. needle down, to ensure that a layer of oil remained above the blood. After discarding the first few drops of blood, the sample was immediately ejected under the mineral oil in a glass centrifuge tube. The blood was then allowed to clot for 2 hours (under oil, on ice) and spun for 2 minutes in a clinical centrifuge. To ensure that the serum did not contact air during

transfer, a layer of oil was first drawn up in a pasteur pipette. The serum was then siphoned into the pipette followed by a second layer of oil. The sample was ejected into cooled plastic tubes containing 2 ml of oil, capped and stored on ice until measurement. The temperature of the water in the fish tank was carefully noted.

ii. Measurement of Serum Ionic Calcium

Equipment

Serum ionic calcium activity (Ca^{++}) was measured potentiometrically using a Calcium Activity Flow-Thru System (Orion Research Inc., Model 99-20) attached to a digital research pH/mV meter (Corning Scientific Instruments, Digital 112 Model). The pH meter was connected to a strip chart recorder (Sargent Recorder, Model SRG) as shown in Plate 3, pg. 29. To minimize external electrical interference, a Faraday cage was constructed out of galvanized wire mesh (6 mm square) and properly grounded. The strip chart recorder, pH meter, electrode, and syringe pumps were all grounded to the Faraday cage. Two water jackets were constructed out of steel (2 mm thickness) to contain both the electrodes and the syringe (Plate 4, pg. 30).

Directly after determination of serum calcium activity, the serum pH was measured using an Ultra-Micro pH/Blood Gas Analyzer (Instrumentation Lab. Inc., Model 113-S1) with a Constant Temperature Control Module (IL, Model 127).

All glassware used in the procedure was either acid-washed or disposable to minimize contamination of specimens with calcium. Solutions were prepared using deionized water.

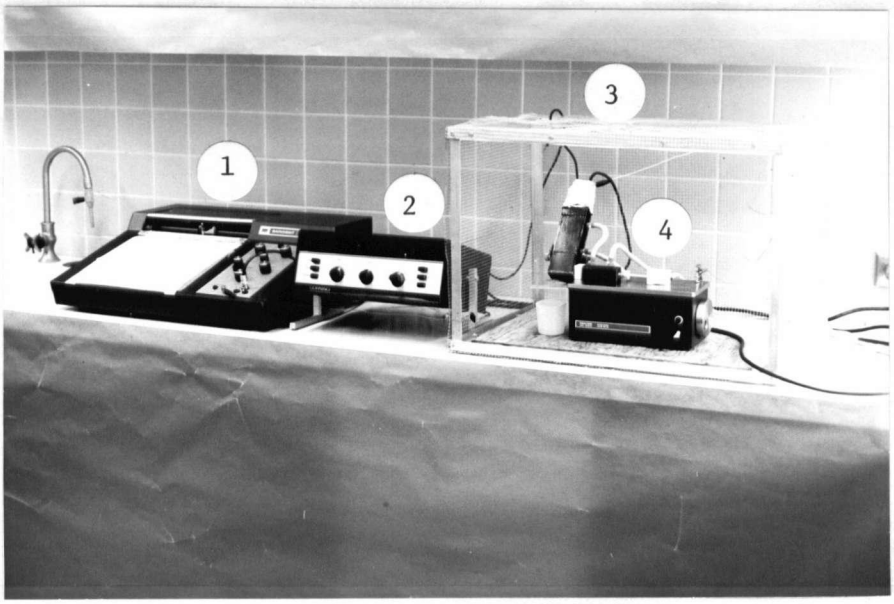

PLATE 3. Calcium-Activity Flow-Thru System

1. Strip chart recorder
2. Digital pH/mV meter
3. Faraday cage
4. Infusion pump and electrode assembly

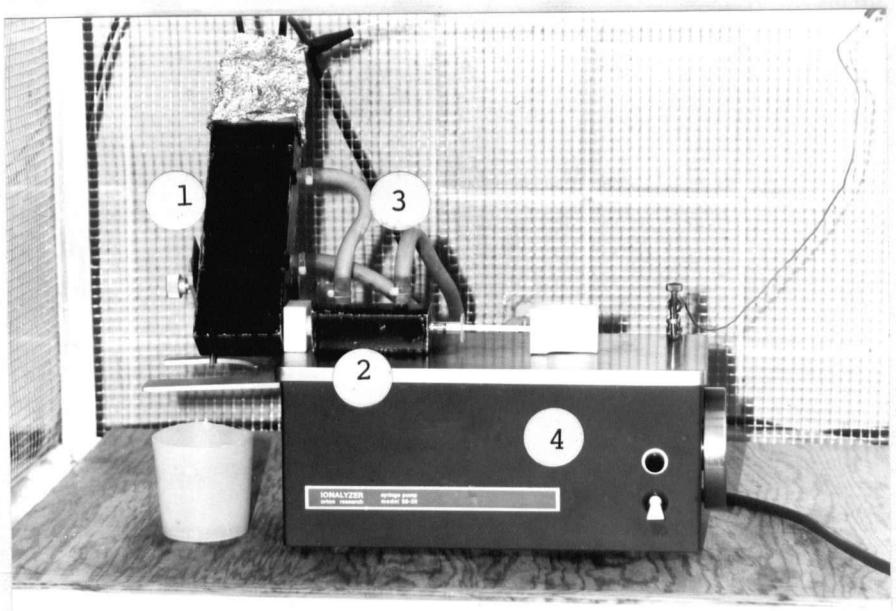

PLATE 4. Infusion Pump and Electrodes with Water Jacket

1. Electrode water jacket (in black)
2. Syringe water jacket (in black)
3. Inlet and outlet tubings for water
4. Infusion pump

Procedure

Use of the Flow-Thru System enabled ionic calcium to be measured on serum samples of 0.2 ml under anaerobic conditions.

Calcium standards were prepared fresh daily by suitable dilution of a stock solution of $CaCO_3$ in 150 mM NaCl. The standards, corresponding to Orion standards A, B and C, contained 2.00, 4.00 and 8.00 mg/100 ml respectively. These were checked against reference standards using atomic absorption spectrophotometry. Three drops of 0.1 M triethanolamine and 0.006 g of trypsin (Trypsin: 2 x crystalline, salt free, Nutritional Biochemicals Corps.) were added to each 10 ml of pure standard. The standards, samples, syringes and electrodes, were all cooled in running tap water to the same temperature for several hours before measurement.

The "B" Standard was run through the system for 20-30 minutes at the beginning of each day to condition the electrodes and remove any accumulated ion exchanger. Separation between the standards was always $\pm$ 7.5 mV. The standards were repeated several times at the beginning and end of each set of analyses. When the system was stable and the drift was less than $\pm$ 0.2 mV, the serum samples were introduced. The "B" standard was run between each sample and each serum sample was measured in duplicate. The "C" standard was introduced periodically in order to check the standard curve reproducibility. All serum samples were removed from under the mineral oil using a 1 ml plastic tuberculin syringe and a 26-G ½ inch needle. Care was taken to exclude air bubbles and oil from both the sample and the electrodes. The serum was removed

immediately before measurement and a new syringe used for each sample. The standards and samples were run for at least 3 minutes or until the reading stabilized on the recorder. Immediately following the ionic calcium activity reading, serum pH was measured using 30 µl of the same sample, at the same temperature. Trial tests indicated that serum samples could be stored anaerobically at 4^{0}C for 3 days without any significant change in serum ionic calcium activity. Therefore, all ionic calcium measurements were performed as soon as possible after sample collection and always within the 3 day limit.

Total serum calcium was measured fluorometrically (Newsome, 1969) on duplicate 20 µl samples, directly following the ionic calcium activity and pH readings. This allowed the calculation of percent ionic calcium.

$$\text{Percent ionic calcium } (\%\ Ca^{++}) = \frac{\text{ionic } Ca^{++}(mg/100ml)}{\text{total } Ca^{++}(mg/100ml)} \times 100$$

Calculations

The calcium flow-thru electrode developed a potential proportional to the logarithm of the calcium ion activity in the sample. Potentials became increasingly positive in more concentrated solutions, and increasingly negative in more dilute solutions. Using 2-cycle, semilogarithmic graph paper, the mean potential developed in each standard (linear axis) was plotted against the concentration value of the standard (log axis). The calcium concentration of the unknowns was then determined from this calibration curve.

A computer program was devised on a desk-top computer (Olivetti Underwood, Programma 101 Model) which computed the slope

of the standard curve and calculated the ionic and total calcium (in mg per 100 ml) and the percent ionized calcium for each unknown sample.

g) Bioassay of Calcitonin in Ultimobranchial Glands

i. Rats

All calcitonin bioassays were performed on 3-4 week old, 80 g, black hooded male rats of the Long-Evans strain (Blue Spruce Farms, N.Y., U.S.A.). They were housed in metabolic cages (5 per cage) and maintained on a natural photo-period. The rats were kept on Purina rat chow and tap water ad libitum for several days before use and starved 24 hours before assay.

ii. Collection and Preparation of Ultimobranchial Glands

The fish were sacrificed by severing the spinal cord and the head excised from the body slightly posterior to the operculum. The liver, gonads and esophagous were removed and the transverse septum containing the ultimobranchial gland exposed. Using a fine pair of scissors, the gland was carefully cut out. It was weighed immediately (wet weight), quickly frozen on dry ice and stored at $-12^{o}C$ in capped auto analyzer cups. The UB gland appeared quite distinct in the trout (Plate 5, pg. 39) and just the area around this "moustache-shaped" gland was cut out. However, the salmon UB gland was more diffuse (Plate 6, pg. 40) and hence necessitated removal of the majority of the transverse septum.

Since the transverse septum of fish contains little fat, it was found unnecessary to fat-extract the ultimobranchial tissue.

The glands were homogenized using a tissue homogenizer (Tri-R Instruments Inc.) in a vehicle of 0.1 N HCl and 0.1% glycine (pH = 4.3). Fibrous material was removed by filtering the homogenate through a piece of sterile gauze. Care was taken to keep the gland and homogenate on ice at all times.

iii. Bioassay Technique

All bioassays were performed using a modification of the method developed by Kumar et al (1965). The rats were starved overnight and injected intravenously via the tail vein with a dose of 0.3 ml per 80g body weight. Five rats were used for each point and the control group received vehicle alone (0.1N HCl + 0.1% glycine, pH 4.3). The injection was performed under ether anesthesia and the rats were bled from the tail 60 minutes after the injection. Blood was collected directly into heparinized capillary tubes (0.2 ml blood) and spun for 10 minutes in a micro-capillary centrifuge. Duplicate plasma calciums were measured fluorometrically using the Technicon Auto-Analyzer Method (Newsome, 1969).

A rough assay was first performed to determine the correct dilutions for each gland. Once the dilutions which gave the proper curve were obtained, a fine assay was performed. The difference between the mean plasma calcium level in mg per 100 ml of the control group and the plasma calcium level of each experimental blood sample was calculated and called the response. A log dose-response curve was constructed and the amount of calcitonin in m Units/mg wet weight of gland was calculated from the standard curve of MRC Research Standard B (Calcitonin, Porcine Thyroid).

The concentration of calcitonin per body weight (mU/kg body wt) was also estimated for each gland. A house standard of purified salmon calcitonin (MRC, Mill Hill, England) and checked against the MRC Research Standard B, was injected in assays to ensure the consistency of the rats' hypocalcaemic response. It should be noted that, due to the diffuse nature of the ultimobranchial gland in the salmon and the inherent errors of the bioassay, the calcitonin contents of the UB glands represent only an estimate. The method could detect from 2 to 10 mU per 0.3 ml per 80 g rat and had an index of precision (λ) below 0.2.

h) Radioimmunoassay of Plasma Calcitonin

Plasma calcitonin levels were measured using a very sensitive and specific radioimmunoassay for salmon calcitonin developed by Dr. Len Deftos, Endocrine Section, Department of Medicine, University of California, San Diego, U.S.A. (Deftos et al, in press).

Plasma samples for calcitonin assay were frozen on dry ice immediately after collection and stored at -12°C in sterile polypropylene culture tubes. These samples were then packed in dry ice and shipped to Dr. Deftos by air.

All plasma calcitonin measurements reported in this thesis were performed by Dr. Len Deftos. Under optimal conditions, the assay could detect 50-100 pg of calcitonin per millimeter of salmon plasma.

i. Statistical Analysis

All measurements within each group were expressed as the mean $\pm$ standard error about the mean (SE). Group comparisons were made using the "Student's" t-test calculated with the aid of a desk-top computer (Olivetti Underwood Co., Programma 101). Probability values were obtained from standard tables.

I. ULTIMOBRANCHIAL GLAND CALCITONIN CONCENTRATIONS

Introduction

The ultimobranchial gland first appears phylogenetically in fish. Van Bemmelen, in 1885, originally described the gland in elasmobranchs. He named the two small epithelial masses which were found caudal to the last pair of branchial clefts, the "suprapericardial bodies" and considered that they represented a rudimentary seventh pair of branchial pouches. De Meuron (1886) gave a brief account of the suprapericardial body in selachians and amphibians and homologized the body in these forms with the "accessory thyreoid" of reptiles, birds and mammals. The term "ultimobranchial body" (ultimobranchialen Körper) was introduced by Greil in 1905 and more correctly describes the embryonic origin and location in most vertebrates. Watzka reported that the gland was present in all orders of vertebrates except the cyclostomes (Watzka, 1933).

In elasmobranchs, Camp found that the ultimobranchial (UB) gland consisted of large distended vesicles the majority of which intercommunicated (Camp, 1917).

Van Bemmelen did not find the suprapericardial bodies in teleosts, but Supino (1907) described postbranchial bodies in leptocephalus lying between the pharynx and the pericardial wall. Giacomini (1909) found them not only in leptocephalus but also in adult Anguilla sp. The ultimobranchial gland has since been described in many species of fish (Giacomini, 1912; Nusbaum-Hilarowicz, 1916; Giacomini, 1936 and Krawarik, 1936).

Camp (1917) found that only the left suprapericardial body

persisted in the adult selachian. This condition is known to occur in other lower vertebrates. In contrast, the ultimobranchial gland of most teleosts is bilateral and imbedded in the transverse septum between the abdominal cavity and sinus venosus (Krawarik, 1936). In the rainbow trout, it takes the appearance of a small "moustache-shaped" band of white tissue and lies immediately ventro-lateral to the esophagus (Plate 5, pg. 39). The salmon UB gland is located in a similar position, except that the gland appears to be much more diffuse (Copp and Parkes, 1968b)(Plate 6,pg. 40).

The gland in the dogfish shark, Squalus acanthias, lies imbedded in the connective tissue of the pharyngo-pericardial wall, between the ceratobranchial cartilage laterally and the cardiobranchial cartilage and coracobranchial muscle medially.

The ultimobranchial gland has a follicular appearance in sharks (Camp, 1917; Copp, 1969a). A portion of the adult selachian gland was found to be secondarily connected to the pharynx by a true duct, giving the gland the appearance of a cross between an endocrine and an exocrine gland (Camp, 1917). The teleost UB gland normally has a follicular structure (Rasquin and Rosenbloom, 1954; Sehe, 1960; Robertson, 1967, 1969; Lopez et al, 1968; Copp, 1969a; Deville and Lopez, 1970). Some authors have noted this gland appears as cords of cells (Eggert, 1938; Copp, 1969a;Pang, 1971b;which take on a follicular aspect when the gland becomes hypertrophied. Since the ultimobranchial tissues possess a well-developed vascular and nervous supply, Watzka (1933) believed that the gland, at least in birds and reptiles, might possibly have an endocrine function.

Robertson (1969) has demonstrated by electron microscopy,

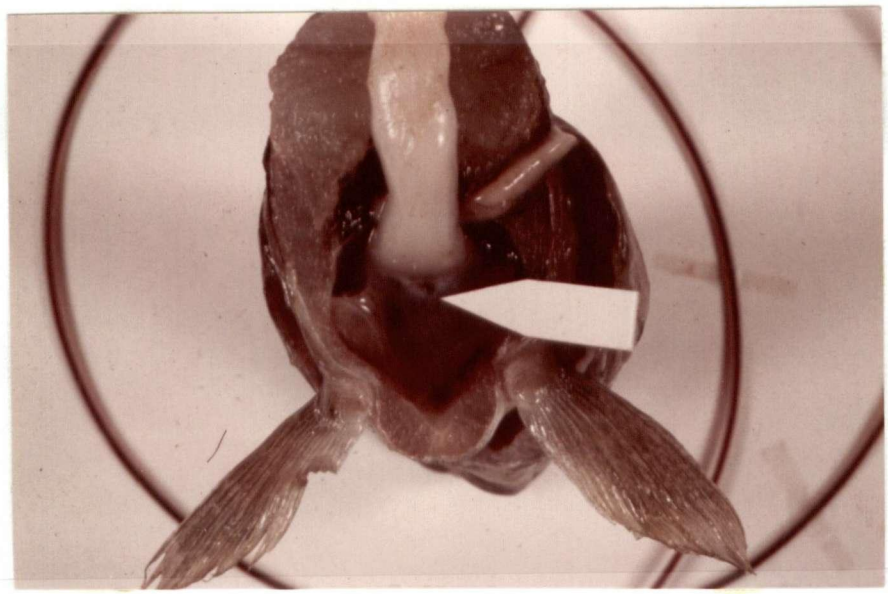

PLATE 5. Trout Ultimobranchial Gland

Midline cross-section of a male trout 1 cm posterior to pectoral fins. Triangular-shaped ultimobranchial gland (arrow) lies immediately below esophagus within the transverse septum.

PLATE 6. Salmon Ultimobranchial Gland

Midline cross-section of a seawater female sockeye salmon 1 cm posterior to pectoral fins. Diffuse ultimobranchial gland lies below esophagus within the transverse septum. Kidney tissue lies above the esophagus and liver tissue below.

that the UB gland of the rainbow trout consists of epithelial components (columnar and a few goblet cells) surrounding a simple follicular structure with a ductless central cavity. The presence of an active Golgi apparatus in many cells and the accumulation of membrane-bound cytoplasmic granules, suggested to him a possible endocrine secretory function. The membrane-bound, osmiophilic granules, seen in the teleost UB gland, resemble those seen in the ultimobranchial secretory cell in all jawed vertebrates.

Although it had been known for many years that the mammalian ultimobranchial gland cells become incorported into the thyroid gland (Baderstscher, 1918; Kingsbury, 1935a, 1935b; Gorbman, 1947), the function of this curious pharyngeal gland derivative was unknown. In 1932, Nonidez described large epithelial cells with argyrophile granules in the thyroid of the dog. He named them "parafollicular cells" since they lay in the interstitial spaces adjacent to the follicles and were readily distinguished from the follicular epithelium. Godwin (1937) demonstrated that the "parafollicular cells" of the thyroid were really of ultimobranchial origin.

Foster et al (1964) hypothesized that the parafollicular or mitochondria-rich cells may be responsible for the secretion of calcitonin. This led to the discovery by Pearse in 1966, that the parafollicular, or "C cells" as he named them, were probably the source of calcitonin. This finding has since been confirmed (Bussolati and Pearse, 1967; Kalina et al, 1970). More recently, it has been shown that the calcitonin-secreting cells are derived from the neural crest (Le Dourain and Le Lievre, 1970, 1972; Pearse and

Polak, 1971, 1972).

In fish, amphibians, reptiles and birds, the ultimobranchial gland remains separate from the thyroid (Copp, 1967). The first evidence indicating a relationship between the ultimobranchial gland and calcium regulation in fish, was presented by Rasquin and Rosenbloom (1954). These authors showed that when the Mexican cavefish, Astyanax mexicanus, was raised in the dark, the UB gland underwent hypertrophy and tissue hyperplasia. Associated with this change were fibrosis and lesions of the skeleton and extensive degeneration and calcification of the kidneys. They postulated that these pathological changes were due to over-secretion of the parathyroid-like ultimobranchial gland.

Copp et al (1967a) were the first to demonstrate in fish, that the ultimobranchial gland did not have a parathyroid function but in contrast, was a rich source of calcitonin. Calcitonin was first extracted from the dogfish shark, Squalus suckleyi and chickens, Gallus domestica (Copp et al, 1967a, b) and later from the salmon, genus Oncorhynchus (Copp and Parkes, 1968a, b). The fact that calcitonin was not detectable in the thyroids of these animals provided proof that it was an ultimobranchial rather than a thyroid hormone. The ultimobranchial origin of calcitonin was confirmed by Tauber (1967) in the chicken and by Moseley et al (1968) in lizards, pigeons and chickens.

Calcitonin has since been extracted from the UB glands of the blue shark, Prionace glauca, and horn shark, Heterodontus francisci (Urist, 1967), the gray cod, Gadus macrocephalus (Copp and Parkes, 1968a), lungfishes, Neoceratodus forsteri and Lepidosiren pardoxa, the killifish, Fundulus heteroclitus and the codfish, Gadus

morhua (Pang et al, 1971), the eel, Anguilla japonica (Orimo et al, 1872a), the lingcod, Ophiodon elongatus and the rainbow trout, Salmo gairdneri (Copp et al, 1972a). Calcitonin activity was not detectable in extracts of the thyroids of catfish, Ictalurus melas (Louw et al, 1967) or of school sharks, Galeorhinus galeus (Louw et al, 1969).

The amino acid composition of salmon calcitonin was determined in 1969 by O'Dor et al and the complete amino acid sequence of salmon calcitonin was determined by Niall et al in the same year. Synthesis of the salmon calcitonin molecule by Guttmann et al (1969) made it the first non-mammalian hormone to be fully characterized. The salmon molecule consists of 32 amino acids but it differs considerably from that of porcine, bovine and human calcitonins, the four hormones being homologous in only 9 out of 32 positions. Curiously, the salmon sequence is more homologous to the human structure (16 out of 32 positions are similar) than it is to either the porcine or the bovine sequence (Niall et al, 1969).

The high specific biological activity of pure salmon calcitonin (5000 MRC U/mg, O'Dor et al, 1969b) is coupled with its more hypocalcaemic and prolonged action in mammals (Brooks et al, 1969; Copp et al, 1970; Guttman et al, 1970). In the standard rat bioassay,the biological potency of purified salmon calcitonin is at least 20 times greater than porcine, ovine or bovine calcitonin (O'Dor et al, 1969b; Keutmann et al, 1972). The high activity of the salmon hormone may be due in part, to its greater stability and is characteristic of the three different forms of salmon calcitonin which have been isolated (Keutmann et al, 1972). Thus, the ultimobranchial glands of fish have been shown to be a rich source

of calcitonin and the study of the function of calcitonin in fish must begin with an investigation of these glands. With so much information known about the salmon calcitonin molecule, it seemed reasonable to characterize the UB gland calcitonin concentrations in salmon and trout. The purpose of this chapter is to present measurements of calcitonin in the ultimobranchial glands of trout and salmon in order to determine the relationship of calcitonin to growth, sexual maturation and osmoregulation.

Materials and Methods

Trout

The fish were all held under laboratory conditions (seasonal photoperiod) and were acclimated to these conditions at least one week before the experiment. Except for the fingerling trout which were fed chopped beef liver, all fish were fed commercial trout pellets and starved one week before sacrifice. Samples were collected over the period from January 16 to September 11, 1970 and the water temperature and physical measurements (General Materials and Methods, pg. 21) were recorded for each fish. Blood was taken by caudal vein sampling and plasma calcium and inorganic phosphorus (Pi) were measured for each fish. In the case of the fingerling trout, blood was collected from the caudal vein directly into heparinized tubes after severance of the caudal peduncle.

The ultimobranchial glands, taken in an identical manner from each fish, were weighed and immediately frozen on dry ice. The glands were then stored at -12^{0}C until bioassay (General Materials and Methods, p. 33).

Five groups of trout were sampled:

a) Fingerling trout

b) Adult immature trout

c) Adult mature trout

d) Smolting trout

e) Sea-water acclimated trout.

The fingerling trout were approximately 7 to 8 months old and their sexes were indistinguishable at this stage. Since only a small amount of plasma was available, plasma Pi levels of the fingerling trout were measured using a micro-phosphorus method (Goldenberg and Fernandez, 1966).

The other four groups of trout were approximately 2 - 3 years old and the sexes of the individual fish were recorded.

The adult immature trout were normal, sexually-immature fish and were sampled to obtain control levels of ultimobranchial calcitonin concentrations.

A group of sexually-maturing trout were studied, since it was noted that the ultimobranchial gland became more distinctly outlined at this stage. The glands were bioassayed for calcitonin content to determine whether there was increased UB gland activity during spawning.

The UB gland also appeared more active in smolting trout. Smolting is a stage in the life history of a trout or salmon characterized by morphological, physiological, behavioural and hormonal changes which prepare the fish for its seaward migration.

The final group of samples were obtained from trout that had been adapted to sea-water conditions at the Vancouver Public Aquarium. This group of trout were first held for one week in a 55 gallon tank

of fresh running water, gradually adapted to full strength sea-water (salinity, 26.5-28.4 parts per thousand) over a period of 4 days, and maintained in the running sea-water for 33 days. It was not possible to obtain the wet weights of their ultimobranchial glands and since these may have dehydrated somewhat during storage at -12^{o}C, the calcitonin activity of the sea-water acclimated trout may only be meaningful when calculated on the basis of Units per kilogram body weight of fish. The glands were weighed prior to each bioassay test. All trout in this group were sexually immature thus eliminating sexual maturation complications. It should be noted that a problem was encountered in segregating immature from mature trout. Part of the difficulty was due to the fact that the trout mature at different rates and times. By nature, rainbow trout spawn in the spring (Leitritz, 1969), but fish inter-breeding practices have developed trout that spawn in spring and fall. An arbitrary decision was made, therefore, to designate trout with gonad weights under 1.0 g as immature.

Salmon

Two species of salmon were obtained from the State of Washington, U.S.A., through the co-operation of the Washington State Department of Fisheries. Both species, captured in freshwater, were in peak spawning condition.

The chinook salmon (Oncorhynchus tshawytscha) were obtained on October 21, 1970 from the Deschutes River Holding Ponds near Olympia, Washington, U.S.A. These fish were about 1 mile from the sea (Puget Sound) and had been in freshwater for approximately 2 weeks. The normal spawning season for these chinook salmon extends from

the end of September to November 10th (C.H. Ellis, Chief Hatchery Management, Washington State Department of Fisheries, personal communication). These fish were hatchery raised and had an average age of 4 years.

The coho salmon (Oncorhynchus kisutch) were taken on November 30, 1970 from the Samish River Holding Pond approximately 10 miles from the sea (Puget Sound). The journey from sea to river takes the fish about a day and the spawning season extends from the end of October to the middle of November. The coho were, on the average, 3 years old and had been detained in the holding pond for a period of 2 weeks.

Both the chinook and coho salmon were captured by hand-net, stunned by a blow on the skull and immediately blood sampled. Blood was collected from the caudal vein as outlined in General Materials and Methods, pg. 17). Measurements on each fish included fork length, total fish weight and gonad weight. The female coho salmon were extremely ripe (sexually mature), making it impossible to obtain an accurate gonad weight. The head of the salmon was excised and the ultimobranchial gland dissected and stored on dry ice. The UB gland weights were recorded upon return to the laboratory. In salmon, the ultimobranchial gland is quite diffuse, so the entire transverse septum was uniformly cut out. The glands were stored at $-12^{o}C$ until assayed and all assays were performed within 2 months of the date of collection. The procedure for collection, homogenization and bioassay of the glands is described in detail in General Materials and Methods page 33 .

Results

Calcitonin activity and other parameters for the 5 groups of trout and 2 salmon groups are summarized in Tables I and II, pages 49 and 50. Plasma calcium and inorganic phosphorus (mEq/l), and calcitonin activity (mU per mg gland; Units per kg fish) are illustrated in histogram form in Figures 3, 4, 5, pages 51, 53 and 54 respectively.

Plasma calcium and inorganic phosphorus levels showed a wide variation among the groups. The highest plasma calcium values for the trout were recorded for the mature females. Smolt plasma calciums were also somewhat elevated over immature adult trout levels. It is interesting to note that the seawater trout plasma calciums were within the normal range, despite the high environmental calcium concentration. The coho salmon exhibited the highest plasma calcium values of all the groups and the mean plasma calcium level for the chinook females was slightly higher than that of the mature female trout. The high plasma calcium levels in the salmon were probably a reflection of their advanced stage of gonad development. From Figure 3 it can be seen that the females for each group display higher plasma calciums than the males, although the difference is only significant in the case of the chinook salmon ($p < 0.05$).

The plasma inorganic phosphorus levels were extremely variable. Individual group plasma Pi measurements also displayed a wide range, as evidenced by the size of the SE bars (Figure 3).

Table I. Physical Measurements, Plasma Electrolytes and Calcitonin Activities of Rainbow Trout (Mean ± SE)

Fish Group	Sex	n	Total Wt (g)	GSI	Plasma		UB Gland Fresh Wt (mg)	Calcitonin Activity		
					Ca mEq/l	Pi mEq/l		mU/mg Fresh gland	U/gland	U/kg fish
Fingerling Trout 25 Feb./70 5.5°C	m&f	8	12.8 ± 1.09	-	4.84 ± 0.17	7.66 ± 0.40	5.96 ± 0.80	91.2 ± 21.38	0.5 ± 0.09	35.5 ± 4.36
Adult Immature trout 11 Sept./70 14°C	m	6	196.6 ± 10.00	0.19 ± 0.05	4.17 ± 0.12	5.00 ± 0.18	34.96 ± 3.04	833.9 ± 124.41	29.3 ± 4.87	148.1 ± 21.99
	f	7	184.5 ± 9.20	0.26 ± 0.03	4.50 ± 0.19	4.68 ± 0.18	35.24 ± 3.17	302.1[a] ± 42.35	10.7[a] ± 1.92	58.6[a] ± 9.97
Adult Mature trout 16 Jan./70 6°C	m	7	236.8 ± 15.20	1.55 ± 0.28	5.00 ± 0.49	5.13 ± 0.19	38.80 ± 3.81	368.7 ± 126.36	15.7 ± 6.48	62.3 ± 22.55
	f	7	219.1 ± 8.30	6.76 ± 2.74	5.65 ± 0.24	5.10 ± 0.39	47.67 ± 2.05	565.1 ± 173.40	25.9 ± 7.40	114.9 ± 29.18
Immature Smolt Trout 18 Mar./70 6°C	m	5	145.2 ± 14.82	0.03 ± 0.03	5.38 ± 0.26	7.16 ± 0.62	31.68 ± 3.45	474.8 ± 186.35	13.1 ± 4.43	92.2 ± 29.01
	f	4	144.5 ± 5.67	0.19[b] ± 0.04	5.51 ± 0.25	7.63 ± 0.68	27.77 ± 2.44	691.7 ± 199.22	20.2 ± 6.84	145.1 ± 53.91
Immature Seawater Trout 2 June/70 10.5°C	m	2	200.0 ± 7.00	0.07 ± 0.02	4.55 ± 0.25	5.75 ± 0.29	75.90 ± 26.10	190.9 ± 28.34	15.3 ± 7.14	75.1 ± 33.10
	f	5	207.4 ± 11.90	0.32[b] ± 0.05	4.83 ± 0.26	6.31 ± 0.47	52.66 ± 13.36	327.3 ± 72.92	13.9 ± 0.63	67.4 ± 2.20

t-test probability male vs. female. a. $p < 0.005$ b. $p < 0.01$

Table II. Physical Measurements, Plasma Electrolytes and Calcitonin Activities of Coho and Chinook Salmon (Mean ± SE)

Fish Group	Sex	n	Total Wt (Kg)	GSI	Plasma		UB Gland Fresh Wt (g)	Calcitonin Activity		
					Ca mEq/l	Pi mEq/l		mU/mg Fresh gland	U/Gland	U/Kg Fish
Coho Salmon 30 Nov./70 4.5°C	m	7	5.0 ± 0.20	4.72 ± 0.10	6.69 ± 0.23	8.14 ± 0.48	1.84 ± 0.09	171.3 ± 32.67	317.2 ± 66.47	62.7 ± 12.59
	f	7	4.5 ± 0.21	-	6.90 ± 0.50	6.78[b] ± 0.36	1.38[a] ± 0.09	166.7 ± 45.72	243.1 ± 80.17	52.3 ± 16.18
Chinook Salmon 21 Oct./70 6.0°C	m	8	8.4 ± 0.49	4.63 ± 0.70	5.21 ± 0.14	5.56 ± 0.33	2.48 ± 0.26	105.7 ± 24.80	241.0 ± 45.01	28.2 ± 4.51
	f	5	8.1 ± 0.47	26.74 ± 1.43	5.98 ± 0.38	7.04[b] ± 0.48	2.47 ± 0.17	203.5 ± 86.65	482.3 ± 195.71	63.6 ± 29.81

t-test probability male vs. female a. $p < 0.005$ b. $p < 0.05$

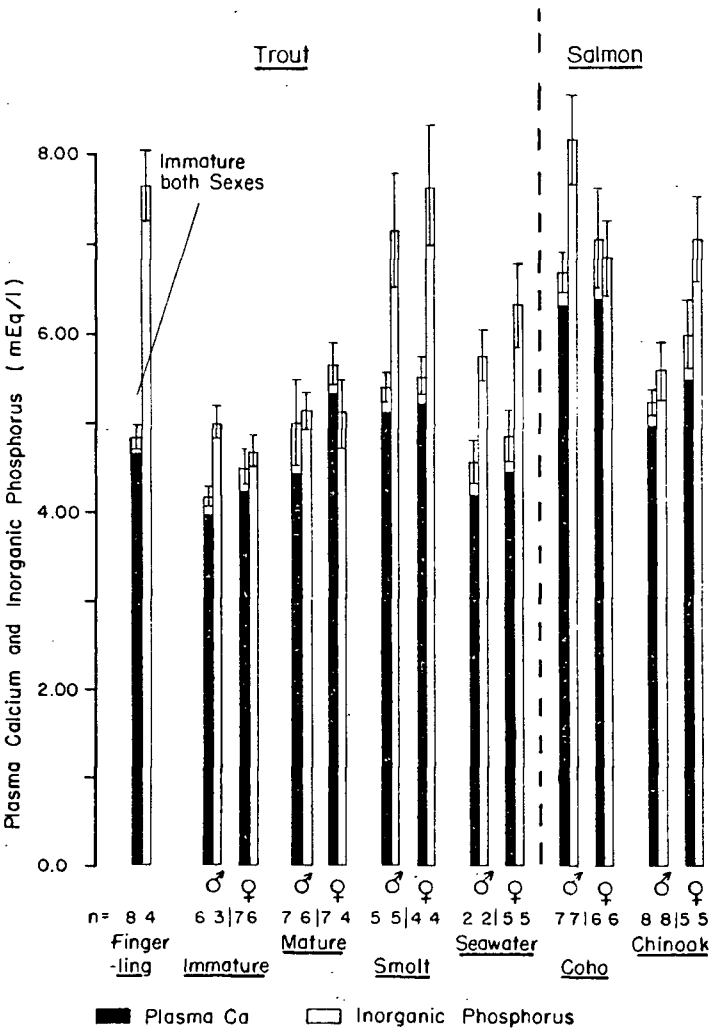

Figure 3. Plasma calcium and inorganic phosphorus levels in trout and salmon

In the trout, the highest plasma Pi values were recorded for the fingerlings and smolts. The coho males displayed the highest plasma Pi levels of the salmon, while the chinook males were the lowest. In contrast to plasma calciums, plasma Pi levels showed no consistent sex difference. For example, the coho male mean plasma Pi was significantly higher ($p<0.05$) than the female level, whereas in the chinook salmon, the female mean plasma Pi was markedly higher ($p<0.05$) than the male level. In all cases except the mature female trout and the female coho, the mean plasma inorganic phosphorus was higher than the mean plasma calcium level.

The data as illustrated in Figures 4 & 5 on pages 53 and 54 shows that the calcitonin activity of the ultimobranchial glands exhibited an extremely wide range of values. The individual calcitonin activity variation is indicated by the large SE values for each group of fish. The lowest CT activity was found in the fingerling trout (91.2 ± 21.38 mU/mg gland) and the highest activity (833.9 ± 124.41 mU/mg gland) in the immature male trout (Figure 4, pg. 53). The seawater trout glands contained fairly low CT concentrations. This may in part reflect the fact that the glands weighed slightly more than those of the other adult trout. However the wide variation of calcitonin activities in the control group of immature adults, makes a valid comparison difficult.

Low levels of calcitonin activity were found in the two groups of salmon.

Except for the immature and seawater trout, no significant sex difference in the calcitonin levels of the UB glands was observed.

Estimated on a U per kg fish basis (Figure 5, pg. 54), the fingerling trout again exhibit the lowest level of calcitonin activity

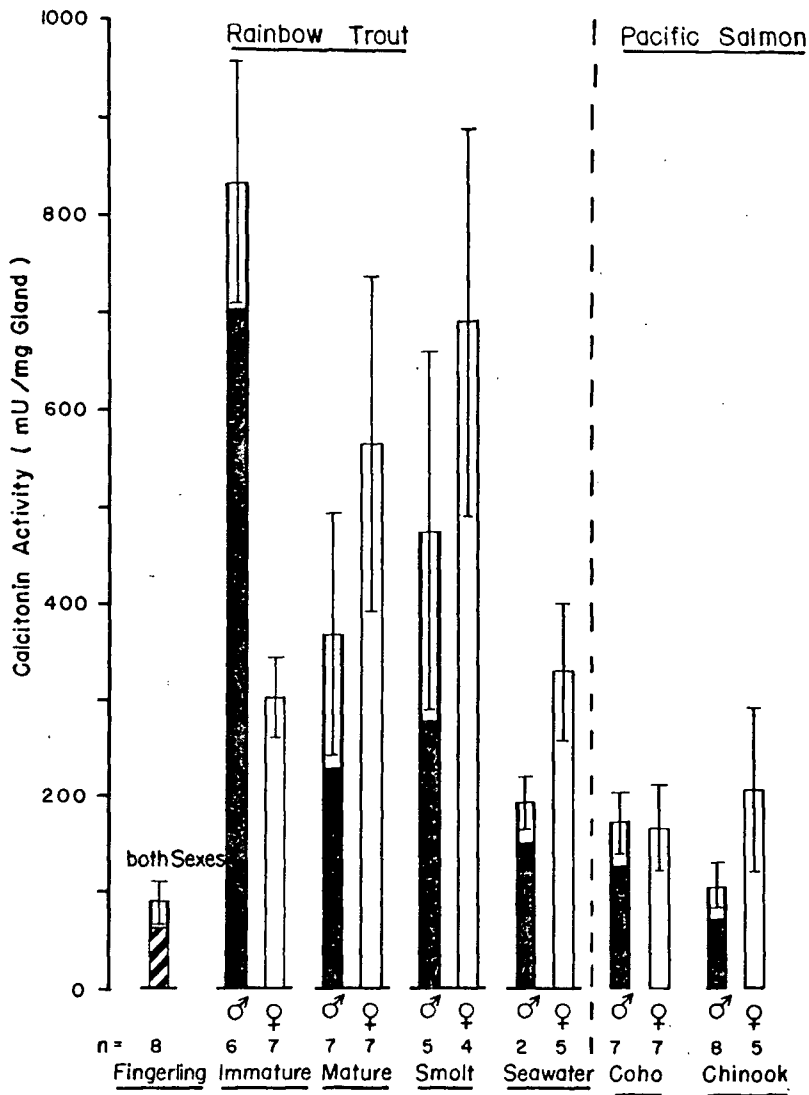

Figure 4. Ultimobranchial gland calcitonin concentrations (mU/mg gland) in trout and salmon

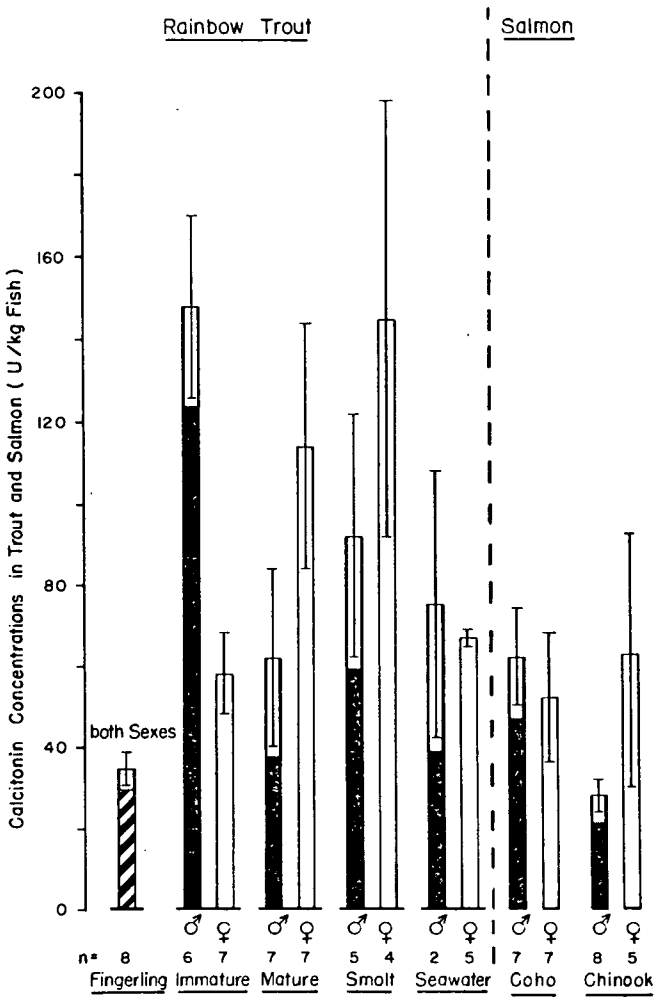

Figure 5. Ultimobranchial gland calcitonin concentrations (U/kg fish) in trout and salmon

among all the groups of trout. The chinook male salmon also have low levels of calcitonin activity (U per kg fish). The immature male trout calcitonin activity (U per kg fish) is higher than all other groups with the exception of the mature females, the smolt females and the smolt males.

Discussion

Although it gives little information concerning secretion rate, measurement of the calcitonin activity of the ultimobranchial gland provides data on the storage of the hormone. Calcitonin content of the UB gland could be expected to be influenced by many factors such as age, diet, sex,species, secretion rate, bone diseases and other hormones. Thus, Robertson (1968a,b) has noted hyperplasia and cellular hypertrophy of the UB gland in hypercalcaemic frogs. He also presented histological evidence that this was due to increased production and release of calcitonin. In chickens, Bélanger (1971) has described the hypertrophy and hyperplasia of the UB gland parenchymal cells in response to hypercalcaemia and the decrease in secretory activity during prolonged hypocalcaemia. Other workers have confirmed the fact that hypercalcaemia in birds stimulates calcitonin release from the ultimobranchial gland (Ziegler et al, 1969; Bates et al, 1969; Copp et al, 1970; Care and Bates, 1972). Some vertebrates such as the goose, possess relatively small stores of calcitonin (2 U per gram fresh gland weight) and in contrast to the pig and sheep, must rely on increased biosynthesis in order to increase secretion (Care and Bates, 1972). Finally, calcitonin concentrations have been found to be elevated in the peripheral blood and thyroid gland of

humans afflicted with the condition of medullary carcinoma of the thyroid (Clark et al, 1969; Deftos and Potts, 1970; Deftos et al 1971a; Deftos et al, 1971b). Another factor affecting the calcitonin content of the UB gland, is that the hormone may be stored at the tissue level as an inactive precursor and later converted to the active principle only on the appropriate release stimuli.

In order to assess measurements of rat thyroid gland calcitonin content in terms of synthesis and release, Gittes et al (1968) have made the following postulates:

1. A net decrease in calcium lowering activity per gland, represents an excess of release over synthesis of calcitonin.
2. A net increase of calcium lowering activity per gland, represents a greater synthesis than release of calcitonin.

These authors found that persistent hypercalcaemia invariably caused a decrease in the calcitonin content of the rat thyroid glands. Further, they attributed an increased calcitonin content in chronically hypocalcaemic rats to a continuous synthesis of the hormone in the absence of any release stimulus. Thus, the interpretation of static UB gland calcitonin content measurements is difficult, and experiments designed to investigate this parameter must be rigidly controlled.

To be able to compare different sets of results, the technique of extraction and measurement of calcitonin activity should be the same. Parsons and Reynolds (1968) point this out in a statement emphasizing, "the necessity for estimates of biological potency of calcitonins [to] be accompanied by a statement of the assay method and of the standard used."

Few workers have investigated the UB gland calcitonin content changes during development. On the premise that calcitonin might be more active in the early stages of growth when there is a high bone turnover rate, Dent et al (1969) measured the UB gland CT content in developing male chickens (Ghostley strain of non-inbred White Leghorn chickens). These authors found that the calcitonin content of the UB gland increased from 83 mU/mg wet wt gland in the 18 day embryo to a maximum of 408 mU/mg wet wt gland in the 3 day old chick. From this stage until 70 weeks of age, despite considerable variation, there appeared to be no major changes associated with age. Calcitonin content, expressed as U per kg body wt, was shown to decrease with increasing age and no difference was noted between the UB gland CT contents of 70 week old males and females. These authors concluded that calcitonin did not play a major role in the development and maintenance of the bony skeleton of chickens.

Although the present study investigated only two ages of fish, the fingerling trout (age 7 - 8 months) showed considerably lower values of calcitonin content than any of the other 4 groups of trout (age 2 - 3 years). The calcitonin content (expressed as mU per mg wet wt gland and U per kg body wt) of the salmon (age 3 - 5 years) was also very low. However this may be due to the large size of the salmon, the spawning condition, species differences or the method of dissection of the UB gland (see Methods).

In contrast to Dent et al (1969), Wittermann et al (1969) showed that the calcitonin content of the UB glands of 3 week, 3 month and 9 month old female chickens (white Plymouth Rock strain) did not change with age (mU per mg wet wt gland). Calculated as U per kg body wt however, the CT content of the 3 week old chickens

was 50 percent of that found in the 3 month and 9 month old chickens. These conflicting results could be explained by nutrition, sex or species differences.

Although it may be superfluous to compare the calcitonin content of fish UB gland with rat thyroid gland, it has been shown that 5 and 15 day old rats have significantly lower thyroidal calcitonin contents (mU per mg fresh thyroid) than older age groups (Frankel and Yasumura, 1970). These authors also noted that there was no significant difference between the thyroid gland calcitonin contents in male and female rats of the same age group. They postulated that the low levels for immature rats could be attributed to either a low rate of biosynthesis or a high rate of CT secretion. This explanation could also account for the low levels of UB gland calcitonin contents of fingerling trout found in the present study. Certainly, the plasma calcium level (the signal for calcitonin release in mammals) of the fingerling trout, is not excessively high.

The higher plasma calciums in the mature versus immature adult trout, is due to sexual maturation and has been noted in other fish by many others (Miescher, 1897; Hess et al, 1928; Pora, 1935, 1936; Booke, 1964; Oguri and Takada, 1967; Urist and Van de Putte, 1967 and Woodhead, 1968). Although the plasma calcium levels in the present study are higher in the mature trout, the UB gland CT content is lower in the mature versus the immature male trout and higher in the mature versus the immature female trout. It is possible that the arbitrary division of the trout into immature and mature groups, could account for the variability of the data.

Smolting salmonids are characterized by their silver coloration (Hitching and Falco, 1944) an alteration of body proportions

(Hoar, 1939) and the development of a salinity preference (Bagger-man, 1960). The smolting process is also known to be accompanied by an increase in the adrenocortical volume (Olivereau, 1962) and an elevation of 17-hydroxycorticosteroid plasma levels (Fontaine and Hatey, 1954). Glomerular filtration and urine flow have also been shown to decrease considerably in smolting steelhead trout (Holmes and Stainer, 1966). Thus it can be seen that the physiological, morphological and behavioural changes occurring in smolting salmonids are extremely complex and appear to prepare the fish for its seaward migration (Hoar, 1951).

In the present study, the smolt plasma calcium levels were elevated over those of the immature trout yet were not significantly different from the mature trout levels. Plasma inorganic phosphorus levels were extremely high in both male and female smolts. The smolting trout as a group appear to have high UB gland calcitonin contents. These high values are not correlated with either the plasma calcium or Pi levels.

The seawater acclimated trout show plasma calcium levels only slightly elevated over those of the immature control trout, while plasma Pi levels were markedly higher. As a group, the seawater trout have the lowest UB gland CT contents of the adult trout. The low calcitonin contents observed in the seawater trout may reflect the fact that the wet weights of their UB glands were higher than those of the other trout (Table I , pg. 49). However, expressed on a U per kg body weight basis, the seawater trout UB gland CT contents still do not exceed the values for the mature and smolting trout. This finding is quite interesting, in view of the fact that

the environmental calcium concentration of the seawater (15 mEq/litre) was considerably higher than the freshwater calcium concentration (less than 0.5 mEq/litre) of the other groups of trout. These results are in marked contrast to those of Orimo et al (1972a) who found that UB gland calcitonin contents (U/gland) of eels, Anguilla japonica, kept in seawater, were significantly greater than the freshwater controls. This finding was paralleled by increased plasma calcitonin and serum calcium levels in the seawater eels. However, the fact that the eel is a catadromous fish (living in freshwater, spawning in seawater) and the rainbow trout is a euryhaline (living and spawning in freshwater) may account for these contrasting results. It should also be noted that Orimo et al (1972a) did not report the age and stage of sexual maturation of the eels.

The present study is supported by the work of Pang (1971b) who found that the ultimobranchial glands of the killifish (Fundulus heteroclitus) were more active (histological evidence) in freshwater than in seawater. He also noted that the ultimobranchial body activity was independent of serum calcium levels, and postulated that the function of the gland might be related to osmoregulation rather than calcium metabolism.

The UB gland calcitonin content (mU/mg gland) of the coho salmon was very similar to the chinook and no sex difference was detected. The slightly lower values compared to the trout could possibly be due to the fact that the salmon ultimobranchial gland is more diffuse and hence a larger area of salmon transverse septal tissue was dissected out. This inclusion of excess tissue would therefore, lower the calcitonin activity when calculated as mU per mg gland. Expressed on a U per kg basis, the salmon UB gland CT

contents were higher (except for the male chinooks) and approximately the same as the immature female control trout. It is interesting to note that the salmon, which were a different species, migrating, fasting and extremely sexually mature (note the salmon GSI, Table II, pg. 50) displayed UB gland calcitonin contents that were not very different from those found in the trout.

It may be relevant to mention that Keutmann et al (1972) have isolated and characterized 3 forms or components of salmon calcitonin. The 3 components, designated calcitonin I, II, III, have been isolated from 4 species of salmon and their distribution is shown in Table III.

Table III. Distribution of Calcitonins Among Salmon Species.*

Species	Calcitonin Component		
	I	II	III
Sockeye (O. nerka)	+++	+	
Chum (O. keta)	+++	+	
Pink (O. gorbuscha)	+++	+	
Coho (O. kisutch)	+++		+

*Data from Keutmann et al (1972)

The specific biological activities of the 3 salmon calcitonin components is compared to the mammalian calcitonins in Table IV, pg. 62.

Table IV. Specific Biological Activities of Calcitonins from Various Species.*

Preparation	Mean Specific Activity MRC Units/mg**
Porcine	120
Bovine	60
Ovine	70
Human	70
Salmon I	2,700
Salmon II	2,400
Salmon III	600

*Data from Keutmann et al (1972)

**All assays carried out on lyophilized preparations of pure hormones using the method of Parsons and Reynolds (1968).

It can be seen that only the coho salmon UB gland contains component III which has the lowest specific biological activity (600 U/mg) of the 3 salmon components. Although the salmon were not segregated by sex and trout and chinook salmon have not been examined, these findings have profound implications.

As in the case of the trout, the plasma calcium and inorganic phosphorus levels of the salmon appear to bear no consistent relationship to their UB gland calcitonin contents.

Data in the present study confirm the original observations reported by Copp et al (1967a) that the fish ultimobranchial gland is a rich source of calcitonin. In fact, the fish UB gland calcitonin contents reported in this thesis (range 90 - 830 mU/mg gland,

Kumar assay, MRC B Std) are very similar to the values found in chickens (range 83 - 408 mU/mg gland, Cooper assay, MRC B Std) by Dent et al (1969).

The higher values of UB gland calcitonin contents (mU/mg fresh gland) for trout and salmon in this study than obtained by Copp et al (1968b) for chum salmon, grey cod, and dogfish, may be due to species differences, dissection technique, and/or extraction and assay methods.

It would appear that Orimo et al (1972a,b), cannot claim to have the highest calcitonin activity per kg body weight (Anguilla japonica, 40 U per kg body weight) since the majority of the trout and salmon groups in the present study greatly exceeded this value (Figure 5 , pg. 54). The UB gland calitonin content in the adult trout (range 10.7 - 25.9 U/gland) was also significantly higher than those of the seawater adapted eels (4.3 U/gland) reported by Orimo et al (1972a). The difference may lie in the fact that Orimo used the Cooper assay while the trout glands were measured by the Kumar assay. It should be noted that Orimo did not report the MRC standard used to evaluate the assay data.

In summary, the low UB gland calcitonin contents found in fingerling trout, as compared to the adults, may indicate a relationship between calcitonin and age. The study confirmed the fact that the fish UB gland contains large quantities of calcitonin. No consistent correlation of the UB gland calcitonin contents with sex, sexual maturation, smolting, changes in environmental calcium levels or species differences was found. The wide range of calcitonin contents found in birds and mammals (Dent et al, 1969; Frankel and

Yasumura, 1970; Copp et al, 1972a), was also observed in this study on fish. This data therefore, does not provide a firm basis on which to outline the physiological role of calcitonin in fish.

II. BIOLOGICAL HALF-LIFE OF SALMON CALCITONIN IN TROUT AND SALMON

Introduction

The endogenous circulating plasma level of calcitonin depends on the secretion rate from the ultimobranchial gland and the clearance rate from the plasma. In the first chapter, it was demonstrated that the ultimobranchial gland of trout and salmon contains high concentrations of calcitonin and in succeeding chapters, evidence will be given that these fish maintain high circulating plasma levels of calcitonin as well. A knowledge of the disappearance of the hormone *in vivo* might explain the high circulating levels and give information on the normal secretion rate of calcitonin.

The more powerful and prolonged hypocalcaemic effect of salmon calcitonin (SCT) in mammals has led some workers to investigate its biological half-life (T½) in mammals (Habener *et al*, 1971a,b; 1972a,b; Newsome *et al*, 1973). The rate of disappearance from plasma may account for the rapid response of the animal to calcitonin injection (Copp *et al*, 1968a; Sturtridge and Kumar, 1968; Mills *et al*, 1972) and the prompt release of calcitonin in response to hypercalcaemic challenge (Lee *et al*, 1969; Gray and Munson, 1969; Arnaud *et al*, 1970; Cooper *et al*, 1971; Care and Bates, 1972).

The purpose of experiments in this chapter was to determine the biological half-life of salmon calcitonin (a "fish" calcitonin) in trout and salmon.

Materials and Methods

The biological half-life of salmon calcitonin in trout and salmon was measured using a modification of the bioassay method of Kumar et al, 1965 as outlined in General Materials and Methods. This technique was employed since it avoided the radiation damage and non-specific redistribution of radioactive label in plasma caused by using labelled hormone. A disadvantage of the bioassay was that it required rather large blood samples (1.0 ml) and hence made serial sampling on small fish difficult.

Trout

The calcitonin biological half-life experiment was performed in the Vancouver Public Aquarium research facilities on May 13, 1970. Eight rainbow trout were cannulated, placed in darkened 5½ gallon aquaria, in running water ($T=8^{o}C$) and allowed to recover for 24 hours. The fish had been starved for 5 days previous to the experiment.

Purified salmon calcitonin (UBC 5, 4.5 U/mg) at a dose of 3.78 Units per 0.25 ml (vehicle 1.0% sodium acetate, 0.1% glycine) was injected intravenously into each fish at time zero. Davis (1970) has shown that the circulation time in trout was 64.1 $\pm$ 16.4 seconds, therefore injection was carried out over 30 seconds to facilitate adequate mixing of the hormone in the blood. A further 5 minutes was allowed before the initial sample to ensure homogeneous distribution of the hormone in the circulation. Bleeding times were 5, 30, and 65 minutes from injection for the first 2 fish and 5, 30 and 90 minutes for the last 5 fish. One ml of blood

was obtained at each sample point, and centrifuged immediately for 2 minutes. Plasma samples were frozen on dry ice directly following separation and stored at -12°C. The bioassays were completed within 3 weeks of collection. Plasma calcium, hematocrit and percent water were measured for each sample to determine the effect of blood sampling on these parameters. The fish were sacrificed on conclusion of the experiment and all physical measurements recorded.

Salmon

Two male sockeye salmon (Great Central Lake race) were cannulated, placed in 50 gallon fibreglass tanks of running water (T=8°C) and allowed to recover for 24 hours. The sockeye were not fed in the laboratory.

Purified salmon calcitonin (37.8 U in 0.55 ml vehicle per fish) was injected intravenously at minus 10 minutes. This ten minute interval permitted even distribution of the hormone in the circulation. Four blood samples of 3.0 ml each, were collected at 0, 22, 50 and 79 minutes for fish H and 0, 27, 55 and 91 minutes for fish R. Plasma was separated and stored as previously described. In order to maintain the hematocrit, the red blood cells were re-suspended in the appropriate volume of heparinized Cortland saline and returned to the fish. Plasma sodium, potassium and magnesium, as well as the percent water and haematocrit, were determined for each sample. Results were plotted directly onto semi-logarithm paper with the plasma calcitonin concentration (mU/ml plasma) on the ordinate and the sample time on the abscissa.

Results

Trout

Physical measurements of the trout are presented in Table V, pg. 69. From this data, it is noted that all of the fish were large, sexually immature trout with the possible exception of trout C, a male in the early stages of sexual maturity.

Since the trout were quite different in size and the same dose of calcitonin was given to each, initial plasma calcitonin levels displayed a wide variation (Table VI, pg. 70). Figure 6, pg. 71, shows the graph of the individual calcitonin disappearance curves used to calculate the half-lives. The mean biological half-life of salmon calcitonin in the trout was estimated to be 27.6 ± 2.90 minutes.

The mean percent calcitonin activity remaining with time was calculated for each sample, assuming the plasma CT level at 5 min. to be 100 percent (Table VII, pg. 72).

Table VIII, pg. 73, shows the plasma calcium, percent water and haematocrit of each fish at each sample point. The zero sample was a 0.2 ml blood specimen taken immediately prior to calcitonin injection.

Table V. Trout Physical Measurements

Fish #	Sex	Total Wt (g)	Fork Length (cm)	Gonad Wt (g)	GSI
B	f	248.2	28.0	1.1	0.44
C	m	215.5	25.5	3.4	1.58
D	f	255.0	30.0	0.3	0.12
E	f	261.0	30.0	0.7	0.27
F	m	260.0	29.0	0.1	0.04
G	f	236.0	29.0	1.3	0.55
H	m	263.0	29.0	0.1	0.04
n =		7	7	7	7
Mean =		248.4	28.6	1.0	0.43
SD =		15.97	1.43	1.07	0.50
SE =		6.52	0.58	0.43	0.21

Table VI. Plasma Calcitonin Levels and Biological Half-Lives of Salmon Calcitonin in Trout

		Plasma Calcitonin mU/ml				Half-Life
Fish #	0 min.	5 min.	30 min.	65 min.	90 min.	Min.
B	Calcitonin	120.96	60.48	21.17	-	30.0
C	Injection	163.30	99.79	43.85	-	38.3
D	↓	71.82	42.34	-	11.34	37.0
E		187.49	77.11	-	15.12	24.3
F		120.96	45.36	-	19.66	23.0
G		104.33	38.56	-	9.98	22.0
H		102.82	29.48	-	9.45	18.5
n =		7	7	2	5	7
Mean =		124.53	56.16	32.51	13.11	27.58
SD =		36.15	22.96	11.34	3.83	7.11
SE =		14.76	9.37	11.34	1.91	2.90

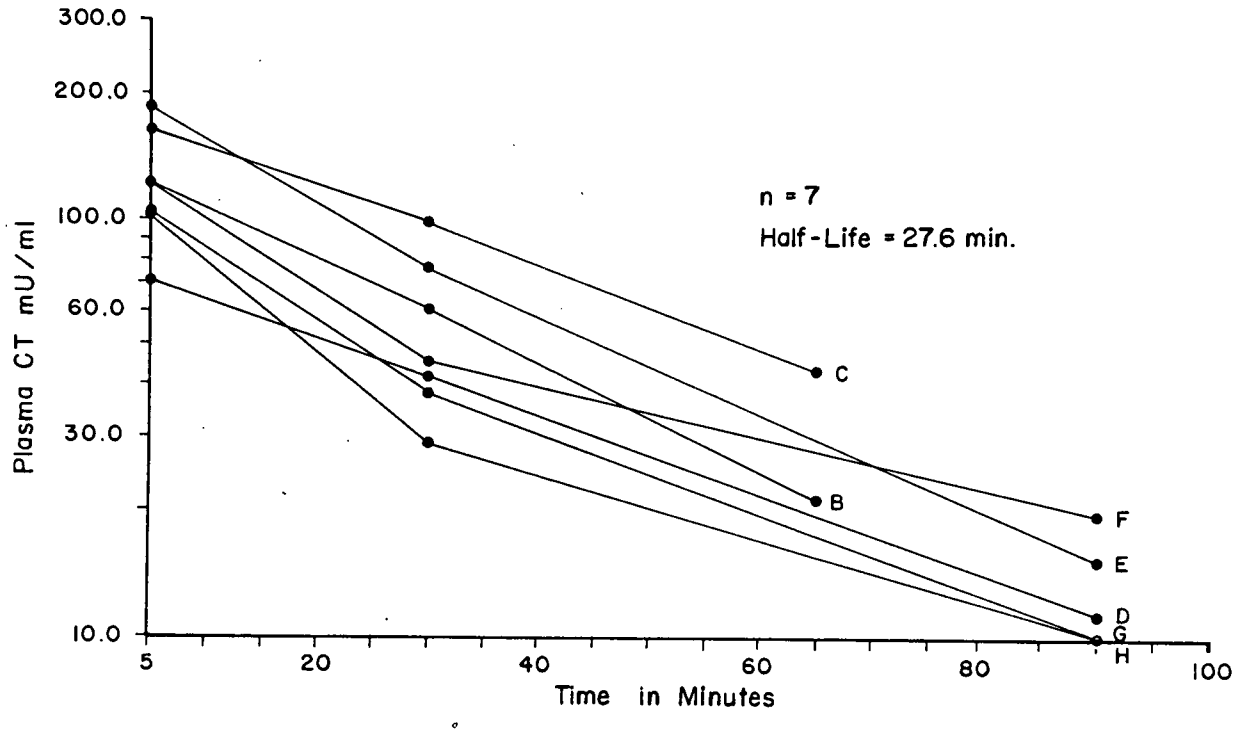

Figure 6. Biological half-life of salmon calcitonin in rainbow trout

Table VII. Percent Calcitonin Activity Remaining with Time

	Sample Time			
Fish #	5 min.	30 min.	65 min.	90 min.
B	100%	50.0%	17.5%	-
C	100	61.2	26.8	-
D	100	59.0	-	15.8%
E	100	51.0	-	8.1
F	100	37.6	-	16.3
G	100	37.0	-	9.8
H	100	28.6	-	9.2
n =	7	7	2	5
Mean =	100.0	46.3	22.2	11.9
SD =		11.30	4.64	3.48
SE =		4.61	4.64	1.74

Table VIII. Plasma Calcium, Percent Water and Haematocrit Changes in Trout

Fish #	Measurement	Sample Time: Pre-Injection sample 0 min.	5 min.	30 min.	65 min.	90 min.
B	Plasma-Ca mEq/l	4.45	4.60	4.30	5.00	-
	% Water	94.9	95.6	95.7	96.0	-
	Haematocrit	22	26	16	14	-
C	Plasma-Ca mEq/l	4.90	4.70	4.60	5.40	-
	% Water	93.8	94.2	94.6	95.0	-
	Haematocrit	33	33	26	27	-
D	Plasma-Ca mEq/l	4.75	4.70	4.65	-	4.45
	% Water	94.7	-	94.9	-	95.4
	Haematocrit	32	34	35	-	20
E	Plasma-ca mEq/l	4.75	4.50	4.70	-	4.55
	% Water	95.3	95.6	95.5	-	95.9
	Haematocrit	26	25	28	-	17
F	Plasma-Ca mEq/l	4.70	4.65	4.75	-	4.60
	% Water	94.9	95.4	95.2	-	95.5
	Haematocrit	28	27	26	-	21
G	Plasma-Ca mEq/l	4.65	4.60	4.50	-	4.80
	% Water	94.5	94.9	94.9	-	95.5
	Haematocrit	24	26	22	-	18
H	Plasma-Ca mEq/l	4.50	4.45	4.35	-	4.30
	% Water	95.2	95.7	95.7	-	96.1
	Haematocrit	26	27	28	-	19

T-test Comparison of 0 min. versus 65 and 90 min. Samples

	T	degrees of freedom	p
Ca mEq/l	0.435	6	NSD
% Water	9.66	6	$p < .001$
Haematocrit	11.1	6	$p < .001$

Salmon

The physical measurements of the two male sockeye salmon are presented in Table IX.

Table IX. Salmon Physical Measurements

Fish #	Total Wt (g)	Fork Length (cm)	Gonad Wt (g)	GSI
H	2028	63.5	58	2.86
R	2800	66.0	73	2.61

The sampling intervals and the plasma measurements at each point are shown in Table X, pg. 75.

Figure 7, pg. 76, is a graph illustrating the disappearance curves of CT in the two salmon. The biological half-life of salmon calcitonin in the male sockeye salmon was 46 minutes for salmon H and 50 minutes for salmon R.

No significant change in plasma levels of sodium, potassium, or magnesium were detected due to the injection (Table X).

In order to compare the disappearance curves in trout and salmon, the percent of calcitonin activity remaining was plotted against time (Figure 8, pg. 77). The trout appeared to have a slightly faster initial disappearance time than the salmon.

Table X. Plasma Measurements in Two Male Sockeye Salmon

Fish #	Sample Time (min)	Calcitonin mU/ml plasma	Percent Calcitonin Remaining	Plasma Percent Water	Hct	Plasma Ions mEq/l		
						Na^+	K^+	Mg^{++}
H	0	982.8	100.00%	94.6	11	153	2.8	1.30
	22	899.6	91.53	94.8	11	145	2.5	1.39
	50	418.8	42.61	94.9	11	148	2.7	1.47
	79	79.4	8.07	95.1	10	153	2.8	1.23
R	0	831.2	100.00	93.4	28	145	2.6	1.66
	27	717.8	86.35	93.6	26	145	2.7	1.39
	55	320.9	38.60	93.6	26	147	2.7	1.56
	91	86.9	10.45	93.8	26	146	2.8	1.50

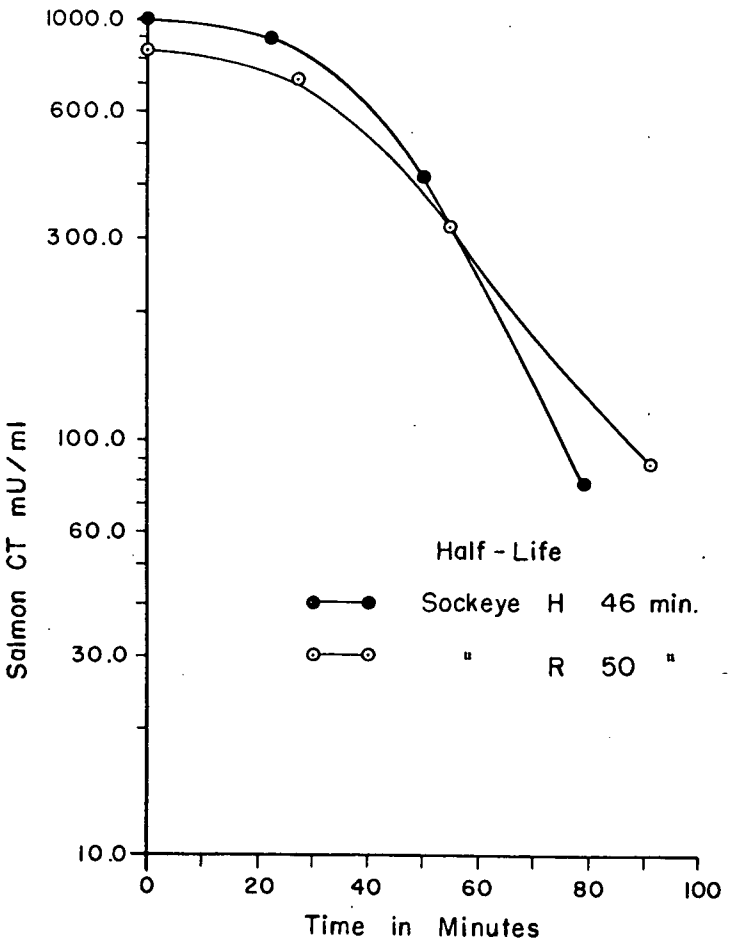

Figure 7. Biological half-life of salmon calcitonin in two male sockeye salmon

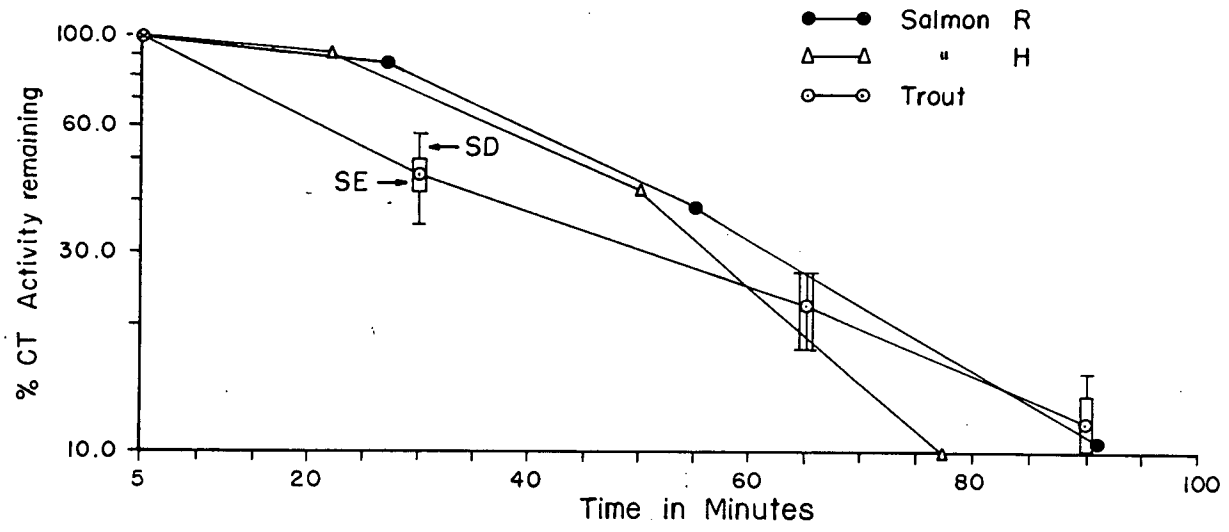

Figure 8. Disappearance of salmon calcitonin in trout and salmon

Discussion

Polypeptide hormones are known to be rapidly cleared from the blood after intravenous injection into mammals. The half-lives of these hormones are in the order of minutes, for example; T½ = 8.1 minutes for gastrin in humans (Ganguli et al, 1971), and T½ = 20 minutes for parathyroid hormone in the cow (Sherwood et al, 1968).

The biological half-life of any hormone is influenced by many factors, among which are:

a) the level of circulating endogenous hormone already present,

b) the secretion rate of endogenous hormone,

c) the degree of binding of the hormone to plasma proteins,

d) the binding of the hormone to receptor sites in the target and other organs,

e) the destruction of the hormone in the target and other tissues,

f) the inactivation of the hormone by plasma enzymes,

g) the renal excretion of the hormone,

h) the age, sex, physiological condition and species of the animal used in the test.

Lee et al (1969) showed there was a rapid turnover of endogenous calcitonin in the rabbit and that the half-life for porcine calcitonin (PCT) in this mammal followed first order kinetics. The disappearance of PCT in the pig was shown to follow two exponential components, the first component had a T½ = 4-5 minutes and the second component had a T½ = 35-40 minutes (West et al, 1969).

The division of the disappearance curve into two components has been shown by other workers, the first steep segment representing the distribution and mixing of the hormone in the fluid compartments and the second, less steep segment the actual rate of inactivation of the hormone (West et al, 1969).

Foster et al (1972a) reported that the initial disappearance of human calcitonin in the dog ($T\frac{1}{2}$ = 3 minutes) was largely due to kidney inactivation. This was demonstrated by measuring arteriovenous differences in plasma CT concentration across both the liver and the kidney. Blood samples during a calcitonin infusion, were collected simultaneously from indwelling catheters in the aorta, hepatic and renal veins of anesthetized dogs and the CT concentration measured by radioimmunoassay. The liver appeared to remove calcitonin from the circulation only at levels above 90 ng per ml, while the kidneys consistently removed 30% of the arterial level of calcitonin. On removal of the kidneys, the first rapid component of the disappearance curve was abolished and higher levels of plasma CT were measured. The slower disappearance of salmon calcitonin in nephrectomized versus normal rats, was also shown by Newsome et al (1973). Hepatectomy, in one dog, did not affect the disappearance curve and Foster et al (1972a) concluded that the liver plays an insignificant role in the inactivation of human calcitonin in the dog. Since only 0.3% of an infused dose of calcitonin was detected in the urine, they reasoned that the rapid phase of the disappearance curve was due to renal uptake and/or destruction and not due to renal excretion. The role of renal excretion in determining the half-life disappearance curve was also considered

unimportant by Habener et al (1972a), who found that the metabolic clearance of porcine calcitonin greatly exceeded the GFR in the dog. The binding of the hormone to plasma proteins, besides providing protection from plasma enzymes, would also preserve it from renal excretion. Foster et al (1972a) also found that the slow component of the disappearance curve of human calcitonin in the dog had a T½ = 40 minutes and they postulated that it was due primarily to protein binding since it did not change with nephrectomy or hepatectomy. This view has been supported by Habener et al (1971a,b; 1972a) who demonstrated using gel filtration, that the slow component of the disappearance curve was due to protein-bound calcitonin, whereas the free calcitonin disappeared rapidly.

Injection of ^{125}I porcine calcitonin into rats showed that the major site of accumulation of radioactivity was the liver (de Luise et al, 1970) and these authors concluded that the liver played a role in the early phase of the disappearance curve. Since the accumulation of labelled CT in the liver could be prevented by simultaneous injection of unlabelled CT and since the authors knew of no known effect of calcitonin on the liver, they postulated that the hepatic uptake may be related to hormone catabolism. It is interesting to note that at 10 minutes, 13.9% of the injected dose of ^{125}I PCT was found in the liver, 2.6% in the kidney, 13.0% in the skeletal muscle, 4.4% in the bone and 6.5% in the blood.

More recent work by de Luise et al (1972) has indicated that, whereas, an injection of ^{125}I PCT accumulated mainly in the liver of the rat, both human and salmon calcitonin were primarily taken up by the kidney. Salmon calcitonin resisted enzymatic

breakdown by homogenates of all rat tissues except the kidney.

Salmon calcitonin and human calcitonin have also proven to be very stable in *in vitro* studies. The incubation of SCT in rat plasma at 37°C showed a T½ of approximately 6 hours, whereas incubation of SCT in salmon plasma showed a T½ of 15 hours (O'Dor *et al*, 1971). Habener *et al* (1972b) also found that over 90% of initial SCT activity remained after 24 hours of incubation at 25°C in salmon and human plasmas. In contrast, the porcine, bovine, and ovine calcitonins are much more rapidly inactivated than either the salmon or human calcitonins (T½ less than 3 hours). In dog plasma at 37°C, PCT showed a T½ of 96 minutes whereas SCT remained stable for over 48 hours (Habener *et al*, 1971b). Thus salmon and human calcitonin appear to resist enzymatic inactivation *in vitro* more successfully than the other mammalian calcitonins.

Further work demonstrating the superior stability of SCT has come from *in vivo* experiments as well. Habener *et al* (1971a), using specific radioimmunoassays in the dog, demonstrated fast and slow components of porcine and salmon calcitonin (PCT 2.5 and 80 min.; SCT 20 and 80 min.). Salmon calcitonin, as measured by bioassay, was shown to have an initial half-life of 10 - 15 minutes in normal rats, whereas porcine calcitonin showed a very rapid initial disappearance of 2 minutes (Newsome *et al*, 1973).

The long half-life of SCT, both *in vivo* and *in vitro*, may explain the greater biological activity of salmon calcitonin (Habener *et al*, 1972a, b). The stability of the salmon hormone is undoubtedly a reflection of some peculiarity of its structure. Comparison of the results of *in vitro* versus *in vivo* experiments,

would seem to indicate that enzymatic inactivation plays a minor role in the disappearance of calcitonin.

The half-life for salmon calcitonin of 27.6 $\pm$ 2.90 minutes in trout and 48.0 minutes in salmon, indicates that the salmon hormone has a relatively long half-life in fish. Although single injections of hormones do not equilibrate evenly in the fluid compartments of the body and tend to distort the disappearance curves, these results are in substantial agreement with the half-times found for SCT in mammals. The fact that the second sample was taken at 30 minutes makes it possible that these results reflect the measurement of the second, slower component of the disappearance curve. Even if these findings represent the first, rapid component of the curve, the half-life of SCT in fish is still slower than the initial half-life of SCT in the dog (20 min.) found by Habener et al (1971a). These measurements agree quite well with those of Bass (1970) who demonstrated that the disappearance curve of synthetic SCT in rainbow trout showed two components ($T_{a½}$ = 12.5 min. and $T_{b½}$ = 59 min.), as measured by radioimmunoassay. Caution must be exercised when comparing the results of bioassay and radioimmunoassay, since loss of immunological activity may not coincide with loss of biological activity (Lequin et al, 1969; Cooper et al, 1971).

The shape of the disappearance curve for SCT in the salmon is interesting and may reflect the fact that a longer interval was left following injection of the hormone (mixing time: salmon 10 min., trout 5 min.) before collection of the first sample. Consequently, there may have been a more homogeneous concentration

of the hormone in the fluid compartments than was observed in the trout.

The longer half-life of SCT in the salmon compared to the trout, could be due to many factors. Since the salmon were in the spawning condition, the level of plasma binding proteins may have been higher than in the trout. This increased protein binding of the hormone in the plasma would prolong its half-life. In the sexually mature skate, Raja radiata, Fletcher et al (1969) have shown that the sex hormone-binding-protein binds the appropriate steroids quite strongly. They demonstrated that the metabolic clearance rates (MCR) of testosterone from skate plasma were considerably lower than those reported for humans. Furthermore, the metabolic clearance rates for the females were consistently higher than for the males.

On the other hand, a significant increase in the cortisol MCR was observed in the sexual maturation of the sockeye salmon (Donaldson and Fagerlund, 1968, 1970, 1972). This greater MCR was correlated with an increased apparent volume of cortisol distribution. Thus, they concluded that the elevated cortisol levels in maturing and spawning salmon were not due to a low MCR but to a rise in cortisol secretion. The half-life of cortisol, however, did increase during maturation and spawning.

It appears that the situation in the spawning salmon is quite complex and experiments on the MCR and secretion rate of calcitonin in immature and mature salmon may help to explain the prolonged half-life in this fish. The fact that fish are poikilothermic animals means that they have a lower basal metabolic rate

than mammals, and this undoubtedly contributes to a more prolonged clearance rate.

Nothing has been done on the distribution of labelled SCT in fish so the role played by the kidney, liver and other organs in the removal and inactivation of circulating calcitonin is not known.

It may be significant to note that although Habener et al (1971b) claim that the prolonged half-life of salmon calcitonin may explain its increased potency in mammals, the long half-life of SCT in the fish was not accompanied by a hypocalcaemic effect. It would be informative from a structure-function-stability point of view to examine the plasma electrolyte effects and biological half-lives of the mammalian calcitonins in fish.

III. PLASMA AND RENAL EFFECTS OF SALMON CALCITONIN

Introduction

In mammals, calcitonin has been shown to exert a rapid hypocalcaemic and hypophosphatemic response. The evidence is conclusive, from both *in vitro* and *in vivo* studies that the primary target organ for calcitonin is bone. The reduction of plasma calcium and phosphate is achieved through an inhibition of bone resorption (Copp, 1969a, b; Copp, 1970a; Behrens and Grinnan, 1969; Rasmussen and Pechet, 1970. A more marked hypocalcaemic response has been demonstrated in young developing animals (Copp and Kuczerpa, 1967; Phillippo and Hinde, 1968; Sturtridge and Kumar, 1968; Sorenson *et al*, 1970) and this effect is likely due to the higher rate of bone turnover associated with periods of rapid growth (Frankel and Yasumura, 1970; Copp, 1970a). Further evidence to support the fact that the hypocalcaemic action of calcitonin is mediated by its effect on bone, came from experiments which demonstrated that this action could be produced in nephrectomized and eviscerated rats (Webster and Frazer, 1967; Copp, 1970a).

Salmon calcitonin, the first non-mammalian calcitonin to be characterized, has been shown to possess an extremely high specific biological activity (O'Dor *et al*, 1969a; O'Dor *et al*, 1969b; Keutmann *et al*, 1970; Keutmann *et al*, 1972) and to exert an extremely potent and long-lasting hypocalcaemic effect in a variety of mammals (Copp *et al*, 1970; Brooks *et al*, 1969; Singer *et al*, 1970; Galante *et al*,

1971; Barlet et al, 1971; Barlet, 1972). Minkin et al (1971) have shown salmon calcitonin (4 mU/ml) to be more effective than greater concentrations of mammalian calcitonins in preventing calcium release from newborn mouse calvaria.

Calcitonin has been shown to exert a variable effect on renal electrolyte excretion in mammals (Hirsch and Munson, 1969; Copp, 1970a; Foster et al, 1972b). In general, porcine calcitonin in the rat increases the excretion of phosphate, calcium, sodium and potassium and decreases the excretion of magnesium. Salmon calcitonin, in addition to causing hypercalciuria (large doses), hyperphosphaturia and marked hypomagnesuria in rats, has been shown to be one of the most potent natriuretic agents known (Aldred et al, 1970; Keeler et al, 1970). These results have recently been confirmed using synthetic salmon calcitonin in the rat (Williams et al, 1972). Long term (96 hour) infusion of synthetic SCT into male lambs resulted in significant increases in urinary excretion of calcium, inorganic phosphorus and sodium and a marked depression of Mg^{++} excretion (Barlet,1972).

Up until 1968, only three workers had reported on the effect of injection of mammalian calcitonin into fish with inconsistent results (Pang and Pickford, 1967; Louw et al, 1967; Chan et al, 1968). Following the discovery of the ultimobranchial origin of calcitonin in 1967 (Copp et al, 1967a; Copp et al, 1968b), salmon calcitonin became available in purified form. Since this hormone had not been tested in fish, a study of the plasma and renal electrolyte effects of salmon calcitonin in rainbow trout and salmon was performed.

Materials and Methods

Trout

Purified salmon calcitonin was injected into 2 groups of trout, fingerlings and cannulated adult trout. In all experiments, calcitonin was weighed out the day of the experiment and the activity confirmed by bioassay.

(i) Fingerling trout

Since calcitonin had been shown to be more effective in young mammals, fingerling trout (age 7-8 months) were used to determine the effect of salmon calcitonin on plasma calcium and inorganic phosphorus levels.

A group of 150 fingerling trout were tagged behind the dorsal fin with a small length of coloured thread and randomly divided into three groups of 50 fish. All fish were weighed (in a beaker of water) and measured during the tagging procedure, taking care to return them to the water as quickly as possible. A 3-week period prior to the experiment was then allowed for recovery and for acclimation to laboratory conditions. Food consisted of daily rations of finely-chopped beef liver and fish that were not actively feeding were removed. Before the experiment, the fish were starved for 2 days.

The experimental procedure was as follows:
Salmon calcitonin (62.5 mU in 0.1 ml vehicle per fish) was injected intraperitoneally (gills immersed under water) into the fish in Group I at time zero. Group II received vehicle alone (0.1 ml of vehicle, 1.0 percent sodium acetate + 0.1 percent glycine, pH = 4.3). Group III, the control group, was not injected. Samples were taken at

intervals of 1½, 3½, 7½ and 25½ hours after injection. The fish were caught by dip net and quickly dried with tissue paper. Blood samples (0.5 ml per fish) were collected from the caudal vein directly into heparinized capillary tubes, after severance of the caudal peduncle. Using this technique, blood could be collected in 20 seconds without anesthetic. From 5 to 10 fish were sampled and weighed at each time period. Plasma calcium was measured fluorometrically (Newsome, 1969) and plasma inorganic phosphorus was determined colorimetrically using the micro-method of Goldenberg and Fernandez (1966).

(ii) Cannulated trout

Twenty-four adult immature trout (mean total wt = 205 $\pm$ 7.3 g) were divided into 4 groups of 6 fish each. Each trout was cannulated and held separately in 5½ gallon darkened aquaria (water temperature 8^{o}C) as outlined in General Materials and Methods (pg. 12). The trout were starved 2 weeks prior to the experiment.

The four groups of trout consisted of a control group (no injection), a vehicle group (0.1 ml vehicle per 100 g), and two calcitonin groups (CT_1 = 125 mU per 0.1 ml vehicle per 100 g fish; CT_2 = 500 mU per 0.1 ml vehicle per 100 g fish). Injection and sampling procedures have been previously described (General Materials and Methods, pg. 13). Three control blood samples (0.15 ml each) were collected at -2, -1 and 0 hours. The trout were injected intravenously at time zero and post-injection samples taken at +1, +3, +5 and + 24 hours. Haematocrit, percent water and plasma calcium were measured at each sample point. On completion of the experiment,

the fish were sacrificed and physical measurements recorded.

Salmon

The effect of salmon calcitonin infusion on plasma electrolyte levels and urine electrolyte excretion was tested in sockeye salmon. Techniques used for cannulation and catheterization were outlined in General Materials and Methods, pg. 12 - 21. The urine box used to restrain the salmon in these experiments is illustrated in Figure 2, pg. 22.

Three sockeye salmon (Great Central race) were cannulated, their urinary bladders were catheterized and they were allowed to recover for 24 hours before the experiment. All 3 fish were sexually-ripe females.

The experimental procedure was as follows. Salmon calcitonin (2.0 Units/100 g fish in 1.0 ml) was infused (Harvard Apparatus, Infusion Withdrawal Pump) into the 3 fish for a period of 30 min. The infusate was kept cool by surrounding the syringe with a plastic bag filled with ice. Two pre-injection blood samples (1.2 ml) were taken and post-injection samples were collected at 1½, 3½ and 5½ hours. On the following day, vehicle (vol. = 1.0 ml) was infused for the same time period (30 minutes) and blood samples collected at the same time intervals. Hourly urine samples were collected by fraction collector in preweighed 15 ml tubes and the urine volume (by weight) recorded. The samples were immediately frozen on dry ice and stored at $-12^{o}C$.

Haematocrit, percent water, and plasma electrolytes were measured for each fish. Urinary calcium, magnesium, phosphorus, potassium, and sodium were also determined. On termination of the

experiment, the fish were sacrificed and physical measurements recorded. The experiments were conducted December 5 - 18, 1970 and the water temperature during this period ranged from 6.0 - 6.5^{o}C.

Results

Trout

(i) Fingerling trout

Results of intraperitoneal injection of salmon calcitonin into fingerling trout are presented in Table XI, pg. 92 . Graphs illustrating the plasma calcium and inorganic phosphorus changes are found on Figure 9 pg. 93, and Figure 10, pg. 94 respectively.

It is apparent from the data that plasma electrolyte levels displayed a wide range of values. In fact, although the plasma calcium and Pi levels of the vehicle group did not differ significantly from the calcitonin-injected group at any of the sample times, the control plasma calcium and Pi levels were elevated well above the other two groups at 3½ and 7½ hours. Control plasma electrolyte levels differed significantly from those of the vehicle group (Ca 3½ hr. $p < 0.025$; Pi 3½ hr. $p < 0.001$ and 7½ hr. $p < 0.005$). By 25½ hr. the control levels had returned to normal.

A comparison of the vehicle versus calcitonin-injected groups does not reveal any significant hypocalcaemic or hypophosphatemic effect of calcitonin.

(ii) Cannulated trout

Physical measurements and individual plasma calcium values of the 4 groups of trout are tabulated in Table XII, pg. 96. Mean total body weight of the 24 trout used in this experiment was 205.0 ± 7.3 g. Evidence that these fish were sexually immature is demonstrated by the low GSI values. Each group contained approximately equal numbers

Table XI. Effects of Salmon Calcitonin on Plasma Electrolytes in Fingerling Rainbow Trout.

Sample Time (After Injection)	Group	n	Total Wt (g) Mean ± SE	Plasma Ions (mg per 100 ml) Total Calcium Mean ± SE (n)	Inorganic Phosphorus Mean ± SE (n)
1½ hour	Control	6	10.6 ± 1.50	9.4 0.39 (5)	11.8 0.25 (3)
	Vehicle	8	10.0 ± 1.75	9.8 0.27 (8)	11.7 0.65 (6)
	Calcitonin	8	12.0 ± 1.30	9.0 0.35 (8)	12.4 0.52 (8)
3½ hour	Control	10	12.7 ± 0.62	10.9 0.21 (9)	15.3 0.36 (10)
	Vehicle	10	12.0 ± 1.07	9.9 0.31 (10)	10.9 0.50 (10)
	Calcitonin	10	12.2 ± 0.62	9.7 0.29 (9)	12.1 0.67 (9)
7½ hour	Control	10	11.2 ± 0.82	10.9 0.38 (10)	15.0 0.48 (8)
	Vehicle	10	12.9 ± 0.97	10.0 0.34 (10)	12.3 0.65 (8)
	Calcitonin	10	12.2 ± 0.99	10.3 0.27 (10)	12.0 0.90 (5)
25½ hour	Control	5	12.5 ± 2.15	9.7 0.43 (4)	12.7 0.69 (5)
	Vehicle	10	11.2 ± 1.16	9.5 0.28 (10)	12.3 0.40 (10)
	Calcitonin	10	10.2 ± 0.84	9.7 0.28 (10)	12.9 0.50 (8)

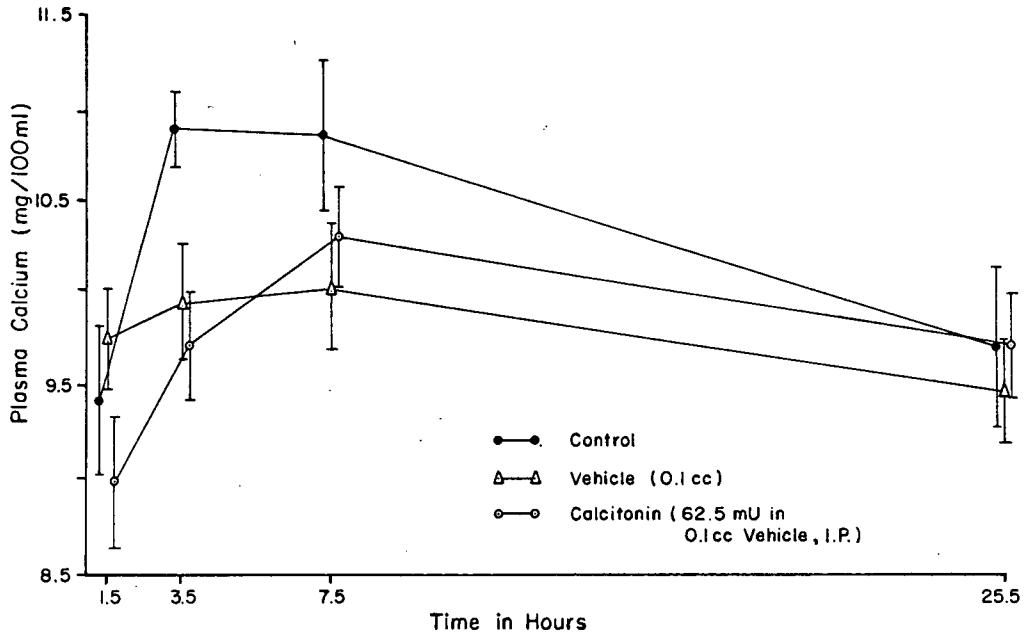

Figure 9. Plasma calcium changes in fingerling trout - effect of salmon calcitonin. Injection at time 0.

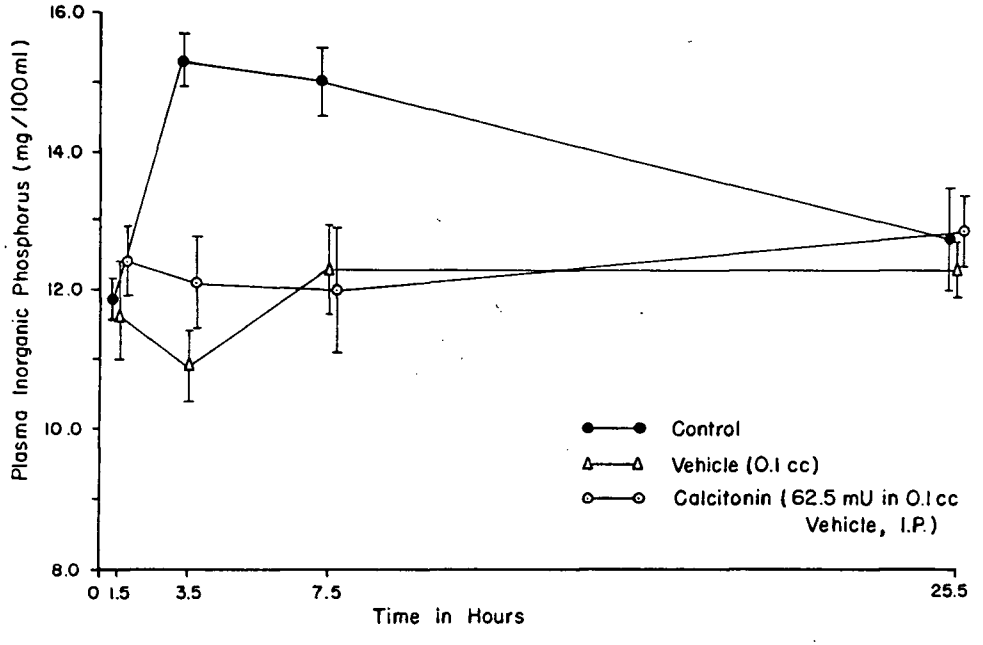

Figure 10. Plasma inorganic phosphorus changes in fingerling trout - effect of salmon calcitonin. Injection at time 0

of males and females (non-spawning trout are difficult to sex).

The data, as presented in Table XII was difficult to analyze statistically due to the variation in individual plasma calciums and was therefore, recalculated (Table XIII, pg. 97). The 3 pre-injection plasma calcium levels for each fish were averaged (Table XIII, column 2). This mean was then arbitrarily adjusted to 10.0 mg per 100 ml. Column 5 contains the number added to each individual mean plasma calcium level to equal 10.0 mg per 100 ml. This number was then added to each actual sample time plasma calcium (Table XIII) for the appropriate fish. Calculated in this manner, it is possible to obtain an average of the plasma calcium levels for each group and to compare the results at each time period. The data of Table XIII is presented graphically in Figure 11, pg. 98. Figure 12, pg. 99, illustrates the actual plasma calcium changes in 3 fish taken from the control, vehicle and calcitonin groups. Haematocrits decreased from an average of 25 to 18 percent and plasma percent water increased from 94.8 to 95.2 percent over the 24 hour period experiment.

Salmon

Physical measurements of the 3 female salmon used in this experiment are given in Table XIV, pg. 100. The mean total weight was 1137 $\pm$ 59.3 g. All 3 salmon were very sexually mature.

Results of calcitonin and vehicle infusions on the plasma electrolyte levels of each fish are shown in Figures 13, 14, 15, pages 101, 102, 103, respectively. No consistent effect of calcitonin was demonstrated on any of the plasma electrolytes at 1½, 3½ and 5½ hours

Table XII. Physical Measurements and Individual Plasma Calcium Levels of Cannulated Trout.

Group	#	Sex	Total Wt. (g)	GSI	Fork Length (Cm)	Plasma Calcium (mg per 100 ml.)						
						-2 hr	-1 hr	0	+ 1 hr	+ 3 hr	+ 5 hr	+ 24 hr
Control	1	M	104.5	0.48	22.3	9.5	9.2	9.4	9.3	8.9	8.6	8.6
	2	M	207.5	0.05	27.0	8.1	8.2	7.8	7.9	7.5	7.3	7.9
	3	M	218.0	0.18	26.6	8.8	8.2	8.6	8.7	8.4	8.5	8.2
	4	F	238.0	0.21	28.9	9.0	8.5	8.9	8.6	8.6	8.6	8.1
	5	F	249.0	0.40	28.7	8.3	8.1	8.4	8.3	8.3	8.5	8.4
	6	M	209.0	0.05	28.2	8.8	8.3	8.0	8.4	8.2	8.5	8.6
n =	6											
mean =			204.0	0.23	27.0	8.8	8.4	8.5	8.5	8.3	8.3	8.3
SD =			47.1	0.16	2.24	0.46	0.36	0.52	0.42	0.42	0.45	0.24
SE =			21.0	0.07	1.00	0.20	0.14	0.22	0.17	0.17	0.20	0.10
Vehicle	1	M	215.0	0.05	26.7	8.0	8.1	8.0	7.8	7.9	7.5	7.5
	2	F	204.5	0.10	28.0	7.9	7.4	7.5	7.5	7.5	7.5	7.3
	3	F	232.5	0.34	28.0	7.6	7.4	7.6	7.5	7.8	7.9	7.9
	4	F	220.0	0.45	26.8	8.2	7.8	8.5	8.0	8.0	8.2	8.2
	5	F	196.0	0.10	26.6	8.3	7.7	7.2	7.2	7.2	7.1	8.1
	6	M	134.0	0.15	23.3	8.1	8.0	8.1	8.1	7.8	7.9	8.4
n =	6											
mean =			200.0	0.20	26.6	8.0	7.7	7.8	7.7	7.7	7.7	7.9
SD =			31.8	0.14	1.57	0.23	0.26	0.42	0.30	0.26	0.34	0.38
SE =			14.2	0.06	0.70	0.10	0.10	0.17	0.10	0.10	0.14	0.17
Calcitonin 125 mU/100 g fish	1	M	228.0	0.09	28.7	8.5	8.7	8.3	8.2	8.6	8.2	8.1
	2	M	248.0	0.04	29.0	9.2	9.0	9.0	9.0	9.5	8.5	8.8
	3	M	204.0	0.10	26.2	6.8	6.6	6.7	6.4	7.1	6.1	6.9
	4	F	218.0	0.23	27.3	8.0	7.8	7.5	7.7	8.0	7.4	7.8
	5	F	210.0	0.38	28.3	8.3	7.6	8.4	7.8	8.4	8.1	8.3
	6	M	226.0	0.04	28.6	9.1	8.8	9.2	8.9	9.1	9.4	8.9
n =	6											
mean =			220.0	0.15	28.0	8.3	8.1	8.2	8.0	8.5	8.0	8.1
SD =			14.2	0.12	0.96	0.80	0.83	0.85	0.86	0.76	1.01	0.66
SE =			6.3	0.05	0.42	0.36	0.37	0.37	0.38	0.33	0.44	0.28
Calcitonin 500 mU/100 g fish	1	F	205.0	0.34	26.7	7.4	8.0	8.4	7.8	7.9	7.2	7.8
	2	F	164.0	0.06	25.3	8.2	7.9	7.7	7.6	7.5	7.4	7.6
	3	F	215.5	0.32	28.0	8.9	8.9	8.4	8.7	8.5	8.5	8.5
	4	M	238.0	0.04	29.3	8.4	8.6	8.4	8.8	8.4	8.6	8.8
	5	M	149.0	0.07	24.9	7.3	7.3	6.9	7.3	7.2	7.4	7.8
	6	M	182.5	0.05	26.5	5.8	5.7	5.7	5.8	5.7	5.7	5.8
n =	6											
mean =			192.0	0.15	26.8	7.7	7.7	7.6	7.7	7.5	7.5	7.7
SD =			30.4	0.13	1.50	1.00	1.03	1.00	0.99	0.93	0.95	0.95
SE =			13.6	0.05	0.67	0.45	0.45	0.44	0.43	0.41	0.42	0.42

Table XIII. Effect of Salmon Calcitonin on Plasma Calcium Levels in Cannulated Trout.

Group	#	Mean Control Plasma Calcium* (mg/100 ml) mean	SD	SE	Number added to mean	Plasma Calcium Zero Time** (mg/100 ml)	Plasma Calcium Change from Zero Time (mg/100 ml) + 1 hr	+ 3 hr	+ 5 hr	+ 24 hr
Control	1	9.3 ±	0.00	0.00	0.7	10.0	10.0	9.6	9.3	9.3
	2	8.0	0.14	0.10	2.0		9.9	9.5	9.3	9.9
	3	8.6	0.24	0.14	1.4		10.1	9.8	9.9	9.6
	4	8.9	0.22	0.10	1.1		9.7	9.7	9.7	9.2
	5	8.4	0.17	0.10	1.6		9.9	9.9	10.1	10.0
	6	8.4	0.31	0.22	1.6		10.0	9.8	10.1	10.2
	n =	6					6	6	6	6
	mean =	8.60					9.93	9.72	9.73	9.70
	SD =	0.41					0.12	0.13	0.33	0.36
	SE =	0.17					0.05	0.05	0.15	0.16
Vehicle	1	8.0 ±	0.00	0.00	2.0	10.0	9.8	9.9	9.5	9.5
	2	7.7	0.24	0.14	2.3		9.8	9.8	9.8	9.6
	3	7.7	0.20	0.10	2.3		9.8	10.1	10.2	10.2
	4	8.2	0.26	0.14	1.8		9.8	9.8	10.0	10.0
	5	7.7	0.48	0.34	2.3		9.5	9.5	9.4	10.4
	6	8.1	0.00	0.00	1.9		10.0	9.7	9.8	10.3
	n =	6					6	6	6	6
	mean =	7.90					9.78	9.80	9.78	10.00
	SD =	0.20					0.14	0.18	0.27	0.34
	SE =	0.00					0.06	0.08	0.12	0.15
Calcitonin 125 mU/100 g fish	1	8.6 ±	0.22	0.10	1.4	10.0	9.6	10.0	9.6	9.5
	2	9.1	0.00	0.00	0.9		9.9	10.4	9.4	9.7
	3	6.7	0.00	0.00	3.3		9.7	10.4	9.4	10.2
	4	7.8	0.17	0.10	2.2		9.9	10.2	9.6	10.0
	5	8.2	0.31	0.17	1.8		9.6	10.2	9.9	10.1
	6	9.1	0.14	0.00	0.9		9.8	10.0	10.3	9.8
	n =	6					6	6	6	6
	mean =	8.25					9.75	10.20	9.70	9.88
	SD =	0.83					0.12	0.16	0.32	0.24
	SE =	0.36					0.05	0.07	0.14	0.10
Calcitonin 500 mU/100 g fish	1	7.9 ±	0.40	0.28	2.1	10.0	9.9	10.0	9.3	9.9
	2	7.9	0.17	0.10	2.1		9.7	9.6	9.5	9.7
	3	8.7	0.22	0.14	1.3		10.0	9.8	9.8	9.8
	4	8.5	0.00	0.00	1.5		10.3	9.9	10.1	10.3
	5	7.2	0.17	0.10	2.8		10.1	10.10	10.2	10.6
	6	5.7	0.00	0.00	4.3		10.1	10.10	10.0	10.1
	n =	6					6	6	6	6
	mean =	7.65					10.02	9.88	9.82	10.07
	SD =	0.99					0.18	0.14	0.32	0.31
	SE =	0.43					0.08	0.06	0.14	0.14

*mean of 3 control plasma calcium levels (-2, -1, 0 hour samples)

**mean plasma calcium (column 2) of each fish taken as 10.0 (mg/100 ml)

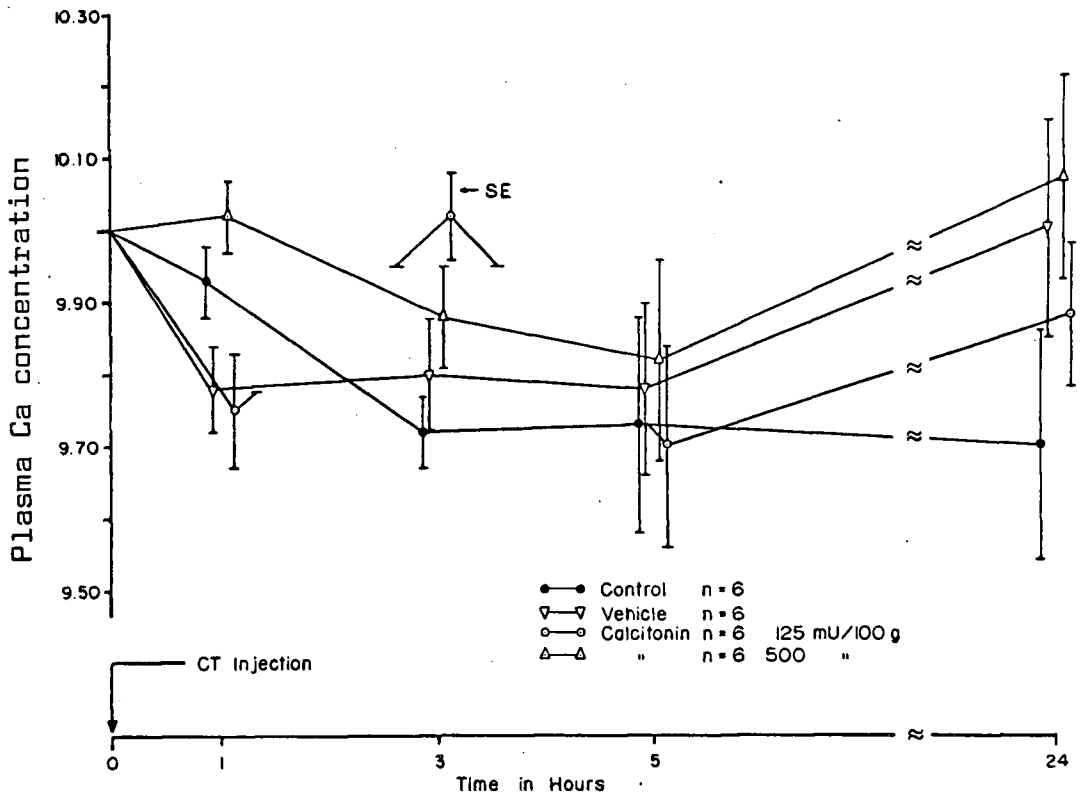

Figure 11. Mean plasma calcium changes in adult cannulated trout - effect of salmon calcitonin.

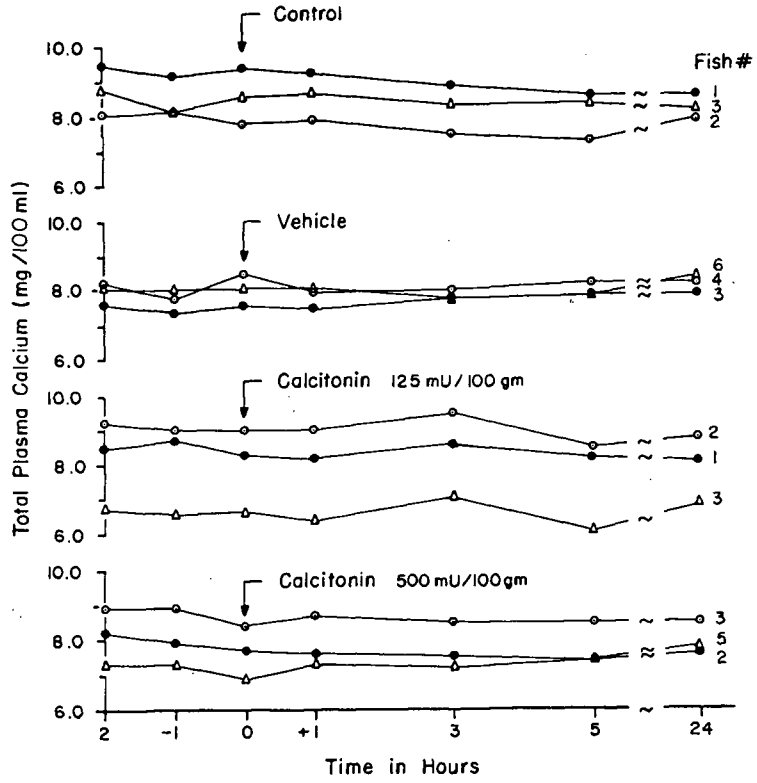

Figure 12. Individual plasma calcium changes in cannulated adult trout - effect of salmon calcitonin.

Table XIV . Salmon Physical Measurements.

Salmon	Sex	Total Wt. (g)	Gonad Wt. (g)	GSI	Fork Length (cm)
V	female	1255	205	16.33	50.2
W	female	1085	250	23.04	46.6
Z	female	1070	265	24.77	47.0
n =	3	3	3	3	3
mean =		1137.	240.	21.38	47.9
SD =		83.8	25.4	3.63	1.60
SE =		59.3	18.0	2.57	1.13

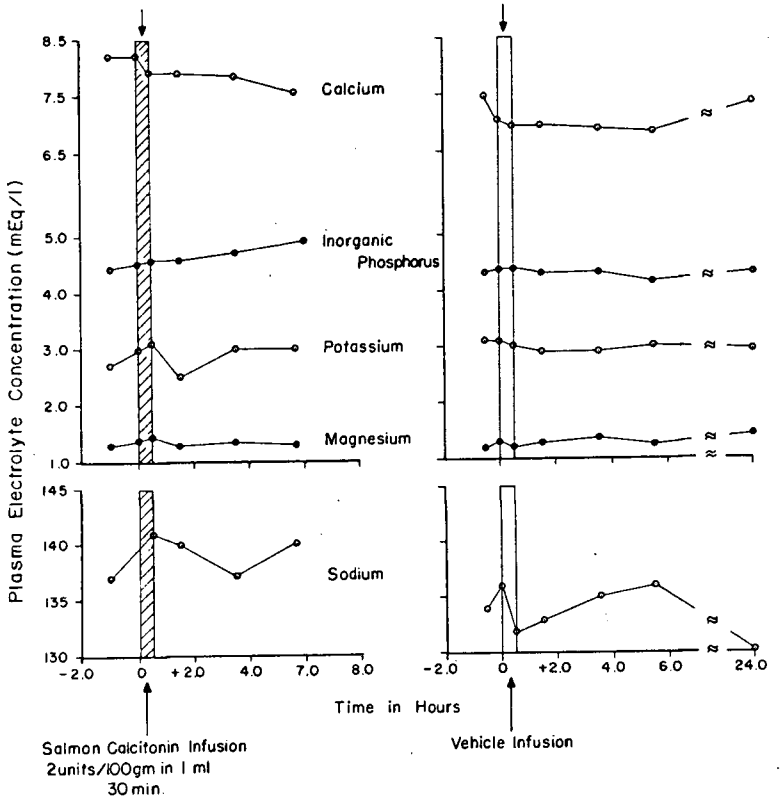

Figure 13. Plasma electrolyte changes in a sockeye salmon - effect of salmon calcitonin infusion. Female sockeye V.

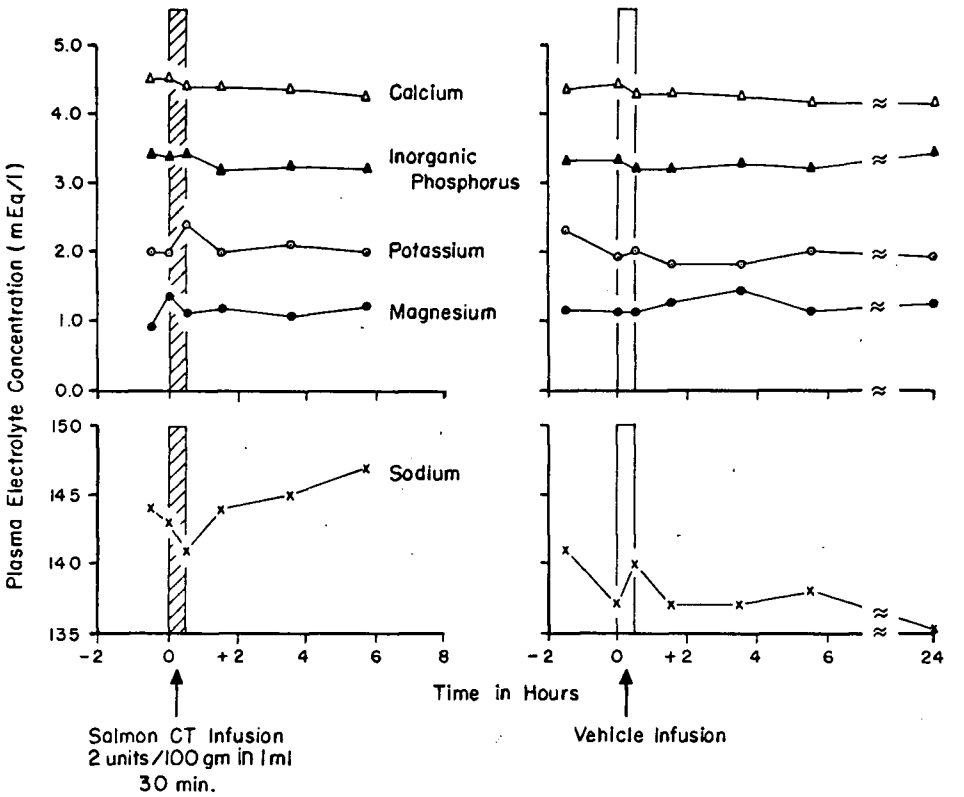

Figure 14. Plasma electrolyte changes in a sockeye salmon - effect of salmon calcitonin infusion. Female sockeye W.

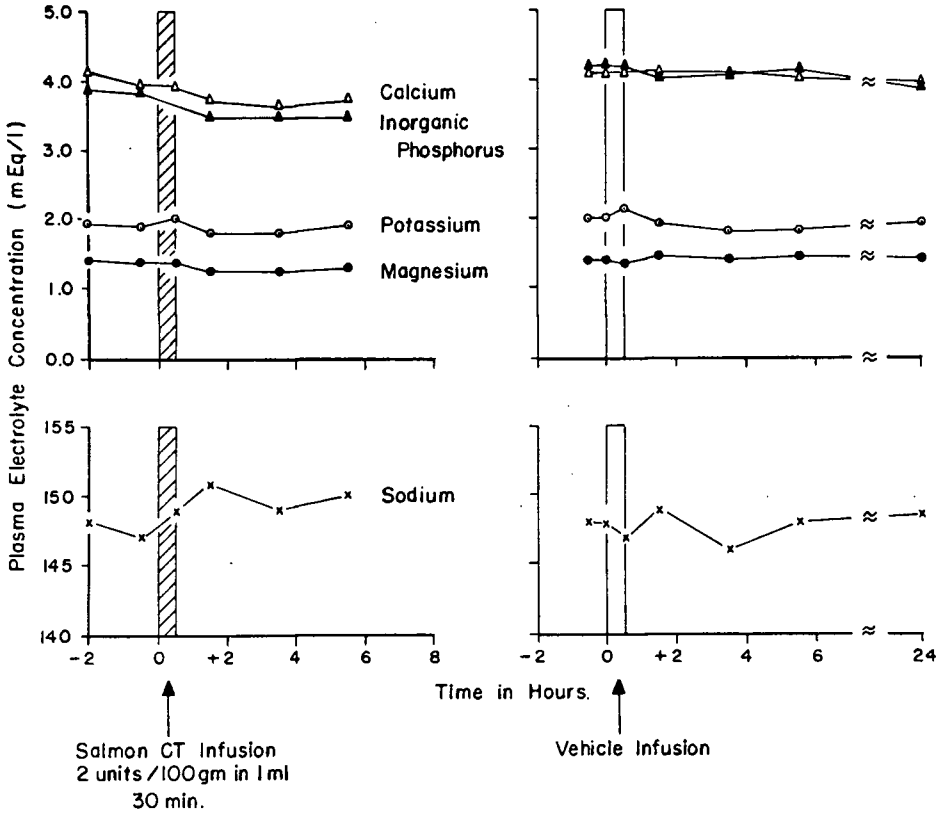

Figure 15. Plasma electrolyte changes in a sockeye salmon - effect of salmon calcitonin infusion. Female sockeye Z.

post-injection. Plasma magnesiums were particularly stable while plasma sodiums showed wider fluctuations.

The effect of calcitonin infusion on renal electrolyte excretion and urine flow rates of the 3 salmon are summarized in Figure 16, pg.105. Both vehicle and calcitonin infusions appeared to cause a slight diuresis but the infusion procedure itself may have caused "laboratory diuresis" (Forster and Berglund, 1956). The control period electrolyte excretion and urine flow rates displayed little variation from the calcitonin and vehicle infusion experiments. Calcitonin infusion caused a slightly greater increase in sodium output compared to the vehicle infusion (not significantly different) but in both cases the sodium output was back to control values within 3 hours. Magnesium output also increased slightly due to the infusion of both calcitonin and vehicle. Calcium excretion was least affected. No evidence of phosphaturia due to the calcitonin infusion was observed. Urinary potassium output (not shown) was very stable. Urine flows of the 3 salmon were remarkably similar to control collections ranging from approximately 3.0 - 4.5 ml per hour.

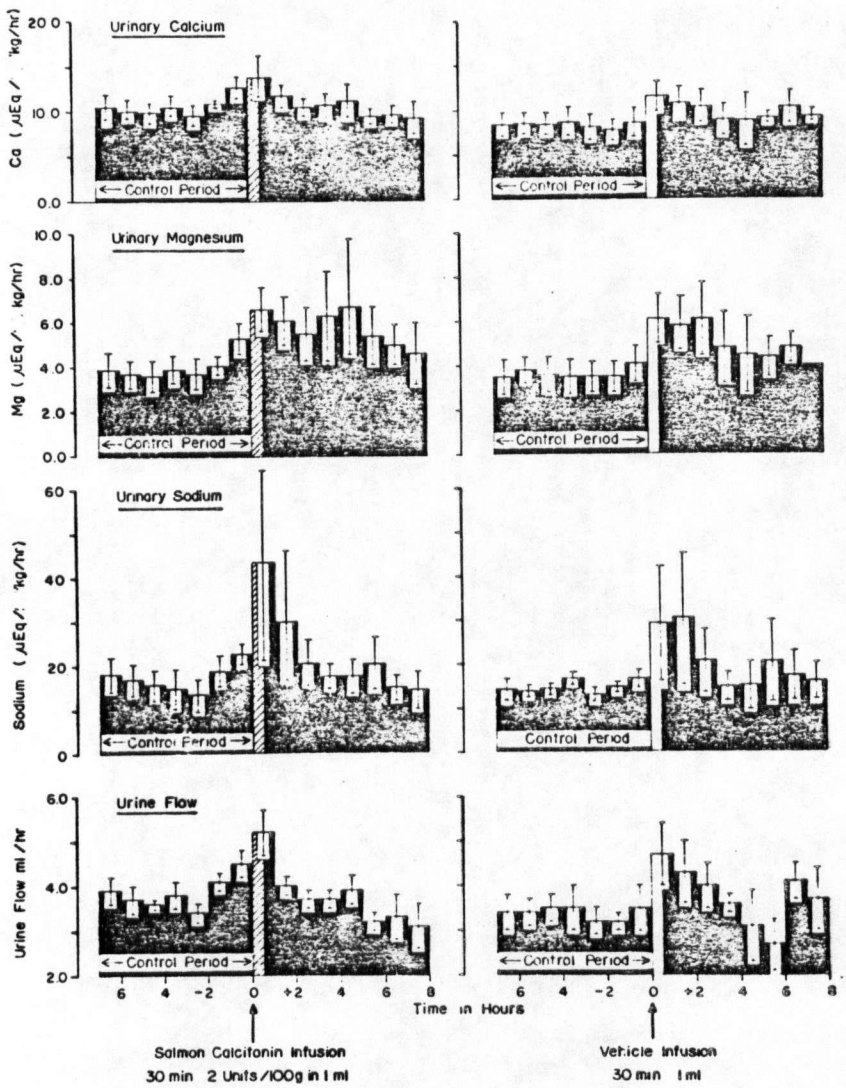

Figure 16. Urinary electrolyte excretion and urine flow in 3 sockeye salmon - effect of salmon calcitonin infusion.

Discussion

Plasma Effects of Salmon Calcitonin

Injection of calcitonin into fish has led to inconsistent results. The first report, published by Pang and Pickford (1967), revealed that intravenous and intraperitoneal injections of partially purified hog calcitonin (2 - 4 units per gram fish) produced no change in the serum calcium levels of male killifish, Fundulus heteroclitus, at 1, 2 and 4 hours post-injection. The hormone was also ineffective in intact and hypophysectomized fish maintained in freshwater and seawater.

Louw et al (1967) reported a hypocalcaemic and hypophosphatemic effect of partially purified porcine calcitonin in the catfish, Ictaluras melas, at 60 minutes post-injection. These results, however, have since been retracted (Kenny, 1972) since the crude thyroid extracts used in the above study were found to be contaminated with histamine and other unidentified pharmacologically active substances.

Chan et al (1968) demonstrated a hypocalcaemic and hyperphosphatemic effect with intravenous injection of partially purified porcine calcitonin (10 mU/100 g and 50 mU/100 g) into intact, immature freshwater European eels (Anguilla anguilla L.). The response lasted several hours depending on the dose and the maximal peak response of the 50 mU/100 g dose occurred at 6 hours. The hyperphosphatemia caused by calcitonin is interesting in view of the fact that in mammals, calcitonin lowers plasma phosphate as a consequence of the inhibition of bone resorption.

Injection of porcine calcitonin (50 mU/100 g) into

stanniectomized eels (their corpuscles of Stannius had been removed one week prior to the experiment) had no effect on plasma calcium levels, while a slight hyperphosphatemia occurred. Removal of the corpuscles of Stannius in the eel is known to significantly elevate plasma and muscle calcium levels and to lower plasma inorganic phosphorus one week after the operation (Chan et al, 1967; Chan, 1972). The changes in calcium returned to normal within 4 to 6 weeks, possibly due to increased calcitonin secretion from the ultimobranchial gland (Chan, 1969; Henderson et al, 1970). The lack of effect of calcitonin in stanniectomized eels might be related to the almost total disappearance of osteoclasts observed 4 weeks after corpuscle removal (Lopez, 1970a). It may also be that the eel was insensitive to exogenous calcitonin administration since the receptor sites were fully saturated from the high plasma levels of calcitonin (Chan, 1972).

Chan (1969) also demonstrated that ultimobranchialectomy of the Asian eel, Anguilla japonica, resulted in a small but significant increase of plasma calcium while plasma phosphate remained unchanged (samples collected 4 weeks after operation). More recent work (Chan, 1972) has shown that ultimobranchialectomy in Anguilla japonica resulted in a slight drop ($P < 0.05$) in plasma total calcium (haemodilution) at 4 weeks while plasma ionic calcium was unaltered (2 weeks post-operation).

Thyroidectomy of rats has no effect on plasma calcium levels (Talmage et al, 1965; Sturtridge and Kumar, 1967; Cooper et al, 1970; Sorensen, 1970). Recently, Milhaud et al (1972) have shown that thyroidectomy of rats will raise plasma calcium and phosphate levels only if the operation is performed during the dark night

period when they were feeding.

Pang (1971b) obtained a hypocalcaemic and hyperphosphatemic response following injection of salmon calcitonin into the fresh-water American eel, Anguilla rostrata. However, the same experiment, repeated on seawater-adapted eels, produced no effect. The hyperphosphatemic effect found by Pang and Chan has also been observed in the heart-lung preparation (bone-and kidney-free) of the dog by Stahl et al (1968). This suggests an extra-skeletal and extra-renal mechanism of action for calcitonin.

Ma (1972) produced a marked hypocalcaemic and hypokalaemic response in the Asian eel, Anguilla japonica, on injection of salmon calcitonin at a dose as low as 100 mU/100 g body weight. This response was elicited only after pre-treatment of the eels with L-thyroxine (10 µg/100 g body weight) and was time-and dose-dependent. A slight but significant elevation of serum inorganic phosphorus was also noted.

On the other hand, Pang (1971b) has shown that injections of salmon calcitonin, codfish ultimobranchial extract and porcine calcitonin had no significant effect on any of the serum electrolytes tested in the killifish, Fundulus heteroclitus, in various experiments. Injection of shark ultimobranchial extracts into the blue shark, Prionace glauca, and the horn shark, Heterodontus francisci, failed to elicit a hypocalcaemic response (Urist, 1967). Porcine calcitonin (10-20 U/kg, IP) extract produced no significant changes in serum calcium or inorganic phosphorus levels in the lazy shark, Poroderma africanum (Louw et al, 1969). Copp et al (1970) were also unable to detect any change in plasma calcium levels in dogfish

sharks, Squalus suckleyi, injected intravenously with 10 units of salmon calcitonin.

In contrast to the above results, Orimo et al (1972a) claim that injection of eel calcitonin (5 MRC Units) caused a significant increase in serum calcium in freshwater Anguilla japonica. These authors also detected a significant hyponatremia and hypochloremia ($p < 0.05$) following injection of eel calcitonin into a similar group of freshwater-adapted eels.

Recently Urist et al (1972), reported that injection of purified and synthetic salmon calcitonin (500 MRC U/kg) into the female South American lungfish, Lepsidosiren paradoxa, had no effect on plasma calcium at 1 and 4 hours post-injection. Porcine calcitonin, at doses of 44, 88 and 176 MRC U/kg, also failed to suppress the plasma calcium level 1 hour post injection. These authors also reported that the skeleton of Lepidosiren, which contained perichondral bone, apatite mineral, and osteocytes but no osteoclasts, was unresponsive to vitamin D, parathyroid extract and calcitonin.

The variability of the fingerling trout control group plasma electrolyte levels in the present study, was probably due to stress. The absence of an effect of salmon calcitonin on the fingerling trout plasma calcium and inorganic phosphorus levels may indicate that in young, growing fish, bone turnover is not as rapid as in mammals. In fact, some authors (Moss, 1962; Norris et al, 1963; Nelson, 1967; Simmons et al, 1970; Simmons, 1971) have shown that fish bone has a very low rate of turnover.

Unlike mammals, fish are able to obtain adequate amounts

of calcium and phosphorus directly from the water via their gills, oral epithelia and fins (Simmons, 1971). The transport of calcium across the gills also appears to be more efficient in freshwater fish. These points are mentioned to suggest that the action of calcitonin in fish may not be on bone but on other target sites such as the gill. Certainly the type of bone (acellular versus cellular) found in fish does not appear to influence the hypocalcaemic response to calcitonin since the eel, salmon and trout all have cellular bone whereas the killifish has acellular bone.

The results obtained on the fingerling trout were confirmed by Pang (1971b) who did not detect any hypocalcaemic effect in juvenile channel catfish, Ictaluras melas, due to injection of salmon or porcine calcitonin.

Sex differences would not appear to explain the negative effect of salmon calcitonin (SCT) in the trout since the hormone was equally ineffective in males and females (Table XII, pg. 96). This contrasts with the results of Hinde and Phillippo (1967) who observed a marked difference in the response of rats to calcitonin injection, the males of a given age being more sensitive than the females.

The original report on the negative effect of salmon calcitonin (125 and 500 mU/100 g fish) on plasma calcium levels in the trout (Watts et al, 1970) was supported by Pang (1971b) who was also unable to produce a hypocalcaemic response by injection of salmon calcitonin into freshwater coho salmon, Oncorhynchus kisutch. Pang (1971b), however, does not report the dosages of calcitonin used in any of his experiments and this undoubtedly is

a critical factor.

With the sockeye salmon in the present study, the effect of salmon calcitonin infusion might have been obscured by the hormonal changes of sexual maturation. Other endocrine factors such as thyroxine and the corpuscles of Stannius may have compensated for the infusion of calcitonin.

One explanation for the negative result of salmon calcitonin (SCT) in the salmon would appear to reside in the finding that spawning female salmon have elevated levels of plasma calcitonin (Chapter IV). Measurement of the circulating level of plasma calcitonin at 24 hours post-infusion of vehicle revealed calcitonin levels of 6775 $\pm$ 1042 pg/ml plasma and 14,700 $\pm$ 1731 pg/ml plasma for salmon V and W, respectively. The high circulating calcitonin levels in these salmon may indicate that the receptor sites for this hormone were already saturated. Infusion of SCT therefore, might better be tested in male or gonadectomized salmon but unfortunately these fish were not available for this study.

It would be interesting to test the hypocalcaemic effect of SCT in ultimobranchialectomized trout since Talmage and Kennedy (1969) have demonstrated that thyroidectomized rats were more sensitive than intact rats to calcitonin treatment. The effect of different levels of calcium in the food and water on the response of trout to calcitonin has not been fully investigated.

It may be significant that a hypocalcaemic response to calcitonin injection has been demonstrated only in the eel. This may be explained by a species difference or by the fact that the eel is an extraordinarily stable experimental fish. While the salmon is an anadromous fish (lives in seawater, spawns in fresh-

water), the eel is catadromous (lives in freshwater, spawns in seawater) and thus their osmoregulatory problems at similar stages in the life-cycle are quite different. Since Dacke (Chan, 1972, discussion) was unable to confirm Chan's hypocalcaemic effect using salmon and porcine CT in eels, physiological condition and sexual development may be crucial factors.

Renal Effects of Salmon Calcitonin

Since no effect on plasma electrolyte levels was observed, it is not too surprising to find that infusion of salmon calcitonin did not alter renal electrolyte excretion. It is possible that the dose required to produce renal effects in salmon might be extremely large, owing to the fact that spawning salmon have such high circulating calcitonin levels. However, the dose used in the present study (2 U/100 g body wt) was the same as that which produced a 2- to 3-fold increase in urine volume and a 3- to 5-fold increase in sodium extretion in saline-loaded rats (Keeler et al, 1970; Aldred et al, 1970).

Aldred et al (1970) demonstrated that urine volume, sodium, phosphorus and magnesium excretion levels showed significant changes only at the 3rd hour after treatment. In this study, however, urinary parameters returned to the normal range at approximately the 3rd hour following infusion.

The renal effects of calcitonin in mammals seems dependent upon the species and dose of calcitonin and the experimental animal. For example, Williams et al (1972) have shown that although synthetic salmon calcitonin causes a profound natriuresis in rats,

no effect on sodium excretion was observed with synthetic human calcitonin. These same authors showed that SCT produced no effect on phosphate excretion whereas other workers have noted a phosphaturia under similar circumstances (Keeler et al, 1970; Aldred et al, 1970).

It is possible that the urinary effects of SCT in the salmon were extremely rapid and obscured by the hourly collection of urine samples. Salako et al (1971) have shown this to be true in rabbits where intra-aortic injections of porcine calcitonin caused an immediate increase in urine flow within 3 minutes of injection.

Two other studies on the renal effects of calcitonin on fish have yielded negative results. Goncharevskaya et al (1971) reported that bovine calcitonin intramuscular injection (150 units/ 100 g body wt) had no effect on serum calcium and inorganic phosphorus levels in the sea scorpion, Myoxocephalus scorpius (L.). There was also no urinary diuresis or change in urinary bladder ionic composition. Hayslett et al (1972) observed no change in the fractional excretion of calcium or urea, the GFR or urine volume on injection of salmon calcitonin (4.4 U/kg body wt) into the elasmobranch, Squalus acanthias. A slight decrease in fractional excretion of sodium and potassium was noted but no hypocalcaemic effect was observed.

With regard to the natriuretic effect of salmon calcitonin in mammals, it is of interest to note that purified dogfish calcitonin like the salmon hormone, is extremely natriuretic in the rat (MacIntyre et al, 1972).

Chan (1972) reported that ultimobranchialectomy of Anguilla

japonica caused an increase in calcium excretion which returned to normal levels in 4 weeks. No change in urine flow rate was observed.

Since the major osmoregulatory problem in freshwater fish is the conservation of electrolytes and excretion of water, it would be physiologically inexpedient to excrete electolytes in freshwater. It might prove informative to test the effect of salmon calcitonin in a seawater salmon.

Besides the positive hypocalcaemic effect of calcitonin in eels, Lopez et al (1971) have provided evidence of a possible role of calcitonin in rainbow trout. Porcine calcitonin injection (50 mU every 2 days for 3 weeks) into immature trout, prevented bone demineralization caused by calcium-free water and thyroxine. These authors believe therefore, that bone is the target organ for calcitonin and that the ultimobranchial gland, by secreting calcitonin, plays an important role in calcium homeostasis in fish.

Lopez and Deville (1972) have also investigated the effect of salmon calcitonin on vertebral bone morphology and ultimobranchial activity in the mature female eel, Anguilla anguilla L. Immature silver eels were made experimentally mature by intraperitoneal injection of carp pituitary extract (1 mg per 100 g body weight, twice per week until maturation). Development of the gonads in sexual maturation is accompanied by a marked hypercalcaemia. Two groups of these mature eels were submitted to prolonged treatment with salmon calcitonin. The first group, after receiving pituitary injections for 8 weeks, were injected with SCT (300 mU per body weight, IP, daily for 43 days). The second group, which had also received the pituitary injections and had spawned, were injected

with the same dose of SCT daily for 11 days.

Salmon calcitonin, in the first group, did not alter the hypercalcaemia but prevented halastasic demineralization (reduction of mineralization of intercellular substance without histological modifications of the organic matrix). Osteoclastic and osteolytic resorption were reduced and the UB gland was still highly stimulated. In the second group, SCT reduced the hypercalcaemia by 50 percent and decreased the osteoclastic and osteolytic resorption. The UB gland appeared inactive.

These authors conclude that calcitonin in the eel acts on bone to prevent bone resorption as it does in mammals. They attribute the negative effect of calcitonin in preventing the hypercalcaemia to the reduced responsiveness to calcitonin caused by gonadal steroids (Sorensen and Hindberg, 1971). This explanation could apply equally well to the present study.

In summary, although salmon calcitonin has negligible effects on plasma and renal electrolytes in trout and salmon, other target organs such as the gill, bone and gut must be investigated before the role of calcitonin in these fish can be elucidated.

IV. PLASMA CALCITONIN AND TISSUE MINERAL CHANGES IN MIGRATING SALMON

Introduction

There are five main species of Pacific salmon under the genus Oncorhynchus:

Scientific name		Common name
Oncorhynchus nerka (Walbaum)	-	sockeye, red
Oncorhynchus kisutch (Walbaum)	-	coho, silver
Oncorhynchus tshawytscha (Walbaum)	-	chinook, spring, king
Oncorhynchus gorbuscha (Walbaum)	-	pink, humpback
Oncorhynchus keta (Walbaum)	-	chum, keta, dog

As mentioned previously, salmon are anadromous fish, which means that they live most of their lives in the sea, but return to freshwater to spawn. The five species all have different life-histories, morphology, sizes, behaviour, feeding and spawning habits. This chapter will deal mainly with the sockeye, chinook and coho salmon.

Pacific salmon begin their life cycle in freshwater. Females dig a nest or "redd" in gravel beds of freshwater streams, rivers or lakes and deposit up to 6000 eggs, the amount dependent on the species and size of the individual fish. The eggs are fertilized by the male, covered with gravel by the female, and remain under the gravel throughout the winter. On completion of the spawning act, both male and female Pacific salmon die within a few days or weeks. The eggs develop through the sac-like alevin stage and emerge from the gravel in the spring as fry. Depending on the species

and environmental conditions, the subsequent period of freshwater residence varies from a few days to several years. All pink and chum salmon migrate to the sea directly following their emergence from the gravel. Other species, such as the sockeye, descend or ascend the tributary from the spawning grounds to the nursery lake where they spend one or two years. Here, the fry develop into smolts at which stage they migrate to the sea. Chinook salmon migrate within the first year while most coho spend one year in freshwater before their seaward migration. The timing of the downstream migration also depends on temperature, food availability and fish size (Ricker, 1966).

On reaching the sea, the smolts or fry may spend several days in the estuarine waters of the river mouth. They then move out into the offshore pelagic environment where their distribution covers most of the North Pacific Ocean and the Bering Sea (Jackson, 1963). The time spent at sea varies according to species and even within a species. Coho and pink salmon stay in seawater for one and two years respectively. Fraser River sockeye spend two or three years in the ocean while the chinook salmon commonly ocean feed for four years.

Under the influence of a homing instinct, the adult salmon approach inshore waters, heading in the direction of their natal stream. This shoreward migration occurs at a characteristic time for each species. For example, maturing Fraser River sockeye appear in coastal waters from May through October. The majority of these sockeye enter the Strait of Georgia via the Straits of Juan de Fuca with usually less than 10 percent entering through Johnstone Strait

(Ricker, 1966). The salmon delay off the mouth of the Fraser for varying periods of time (Killick, 1955). Generally, the earliest Fraser River runs go furthest upstream.

The gonads begin to mature some time before the shoreward migration and are often well-developed before the fish enter their home stream (Hanamura, 1966; Ishida, 1966; Vladykov, 1962). At this time, the salmon also cease feeding (Greene, 1904). The journey up the river is particularly arduous,often involving long distances and swift currents. Some species, such as the coho and chum, generally spawn in coastal streams and hence have very short migrations.

After reaching the particular spawning grounds, spawning occurs on a predictable date plus or minus a few days. In general, spawning of sockeye tends to coincide with water temperature. Sockeye in British Columbia spawn at temperatures of 3 - 7^{0} C. Other environmental factors such as light, water level, etc. also undoubtedly influence the time of spawning. The adults spawn and then die, and the life cycle is repeated.

During their freshwater migration, Pacific salmon undergo complex physiological changes and are subjected to a variety of internal and environmental stresses. These include:

1. osmoregulatory stress on movement from sea to freshwater
2. exhaustion, from the often long, arduous migration
3. sexual maturation and development of secondary sexual characteristics
4. starvation, often for many months, due to cessation of feeding
5. diseases, bacterial and fungal infections, parasites

6. water temperature changes.

The dramatic development of the gonads and the immense problems of osmoregulation encountered by the salmon during the spawning migration, have led many researchers to investigate the endocrine changes involved in these processes. Sexual maturation and development of secondary sex characteristics involves extensive tissue reorganization in a relatively short period of time and under fasting conditions. This chapter presents results of experiments designed to investigate the role of calcitonin in osmoregulation and/or sexual maturation and spawning, in the migrating salmon.

Materials and Methods

Three species of Pacific salmon, sockeye, coho and chinook, were investigated at various stages in their spawning migrations. The method of collection and storage of samples was outlined previously in General Materials and Methods.

This chapter is divided into three sections. In Section A, experimental material is presented on the migration of the Chilko race of sockeye which was studied during the summers of 1971 and 1972. In Section B, material collected on coho salmon under various conditions is outlined. Section C is a summary of the data from 3 species of spawning salmon: sockeye,coho and chinook.

A. Migration of Chilko Sockeye Salmon

Chilko Migration 1971

The purpose of this study was to examine the changes in plasma calcitonin and electrolyte levels in migrating sockeye salmon. The Chilko race of sockeye were chosen for several reasons. Extensive biochemical and physiological studies had already been performed on migrating sockeye salmon thus providing a good basis for further investigation (Idler and Clemens, 1959; MacLeod *et al*, 1958; Idler and Tsuyuki, 1958; Idler and Bitners, 1958; Idler *et al*, 1960). The International Pacific Salmon Fisheries Commission (Salmon Commission) supplied the necessary manpower and facilities to collect sockeyefrom this particular race both from the sea and on the spawning grounds. By means of scale analysis,

the age and race of the seawater sockeye were identified by the Salmon Commission and only Chilko sockeye were included in the study. This identification was an important consideration. By examining one discrete race of sockeye, the salmon were essentially at the same stage of sexual development when sampled at any one particular point in their migration. Also, sockeye salmon of the same age group are relatively uniform in body weight and length.

All salmon were captured and blood sampled within 5 minutes of capture. In the sea, salmon were caught by reef net and on the spawning grounds by beach seining.

Migrating salmon were sampled at 3 points along their migratory route. Phase I, seawater sockeye were sampled at Lummi Island, Washington State, U.S.A. Phase II, freshwater arrival sockeye were sampled at Chilko Lake, British Columbia, Canada. Phase III, spawning sockeye were sampled on the spawning grounds of Chilko River at the outlet from Chilko Lake. Table XV, pg.123, presents a summary of the sampling times, dates and locations. Figure 17, pg.122,shows the location of the 3 sampling points and the route travelled by the migrating salmon.

Physical measurements, plasma electrolytes (calcium, inorganic phosphorus, sodium, potassium), plasma percent water, plasma protein and haematocrit were measured for each fish. Plasma calcitonin was measured by radioimmunoassay on each individual sample. Procedures used for the above analyses were described in General Materials and Methods.

The scientists with the Salmon Commission divided the

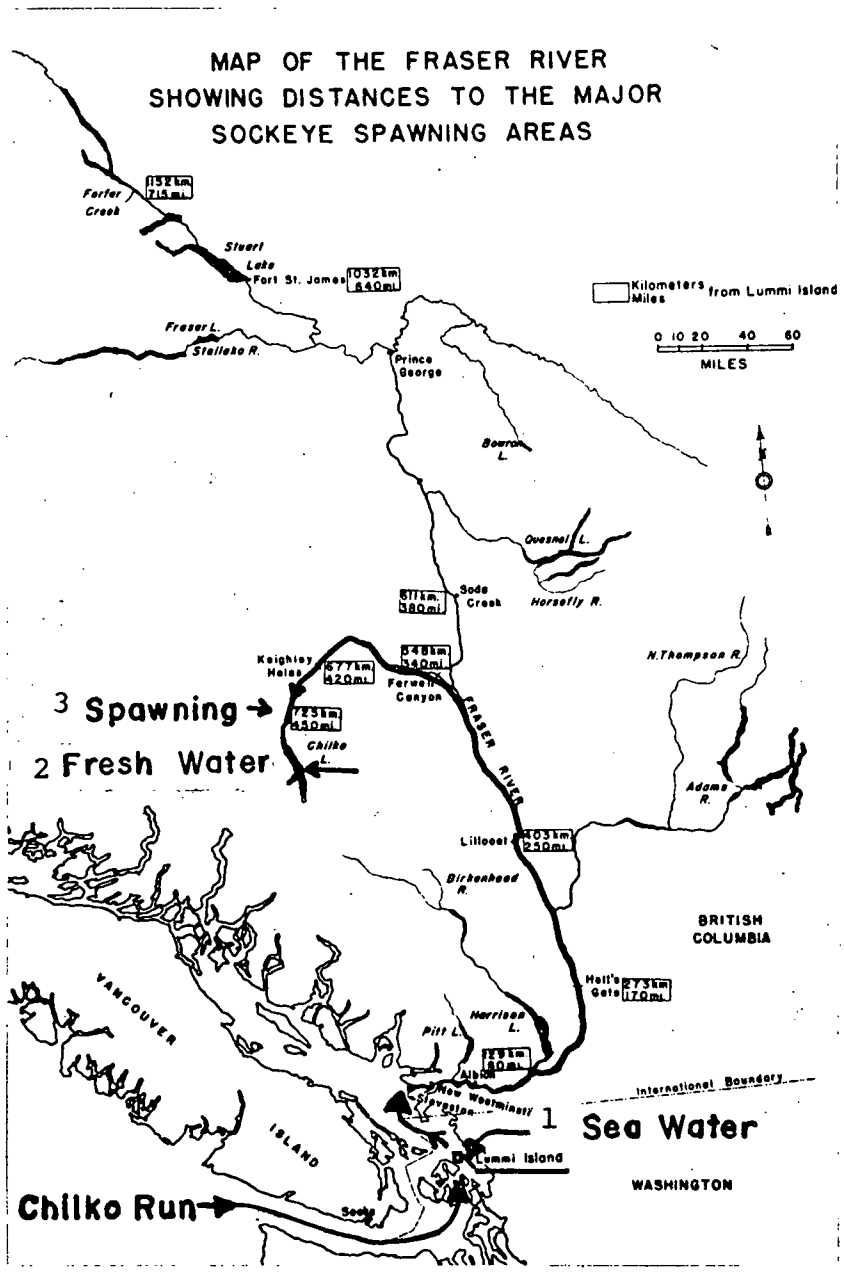

Figure 17. Map of Fraser River and British Columbia. Chilko sockeye migratory route indicated by dark line and locations of the 3 sampling points are shown by numbers.

spawning female sockeye into 3 groups: 0 percent spawned (ripe but egg case intact), 50 percent spawned (half of the eggs remaining) and 100 percent spawned or spawned out females (almost no eggs remaining). The sexually mature female Chilko sockeye contains approximately 3000 eggs. The male spawning sockeye were impossible to classify as to degree of spawning and hence were sampled as one group.

Table XV. Chilko Sockeye Migration 1971

	Phase	Sampling Date(s)	Water Temperature	Location	Number of Salmon
I	Seawater (beginning migration)	July 23 & 24/71 August 6/71	11.5°C 10.5°C	Lummi Island (seawater)	45 m 44 f
II	Freshwater Arrival (to Chilko Lake)	August 17/71 August 26 & 27/71	14.4°C 13.9°C	Chilko Lake (freshwater)	26 m 34 f
III	Spawning (spawning grounds Chilko River)	September 22/71	8.9°C	Chilko River (freshwater)	15 m 12 f (0% sp) 10 f (50% sp) 10 f (100% sp)

Chilko Migration 1972

The purpose of the second migration study was to investigate the serum ionic calcium changes in migrating salmon in an attempt to correlate these with plasma calcitonin changes. The calcium and phosphorus contents of vertebrae, premaxillae, scales, muscle, gonads and skin were also analyzed. Scales, skin, and muscle samples were taken from the dorsal aspect at the right side of the fish, 1 cm behind the posterior edge of the operculum. Scales were individually removed from the 1 inch square skin sample and rinsed 3 times in deionized water before drying. The vertebrae were also obtained from a position 1 cm behind the posterior edge of the operculum. The premaxillae were collected from the right side of the jaw only and the teeth, which had solidly fused to the premaxillae in the spawning fish, were removed as completely as possible. Both gonads were taken from each fish for analysis. Methods of analysis were outlined previously in General Materials and Methods. Due to phosphate interference, it was found necessary to employ a 1.0 percent lanthanum chloride solution in the analysis of calcium of the soft tissues whereas a 0.5 percent lanthanum chloride solution was suitable for the hard tissues.

Table XVI, pg.125, gives a summary of the dates and locations of sampling points of the 1972 Chilko Migration.

Physical measurements, plasma calcium, inorganic phosphorus, sodium, potassium, percent water, plasma protein and haematocrit were measured for each fish. The serum ionic calcium was measured in the laboratory at a temperature close to that of the water in which the fish were originally captured. Serum pH was determined on

Table XVI. Chilko Sockeye Migration 1972

	Phase	Sampling Date	Location	Number of Salmon
I	Seawater (beginning migration)	July 21/72	Lummi Island (seawater)	10 m 10 f
II	Freshwater Arrival (to Chilko Lake)	August 28/72	Chilko Lake (freshwater)	10 m 10 f
III	Spawning (spawning grounds)	Sept. 24/72	Chilko River (freshwater)	10 m 10 f (0% sp) 10 f (100% sp)

the freshwater arrival and spawning samples immediately following the ionic calcium measurements. The female spawning sockeye were divided into unspawned females (ripe but egg case intact) and spawned out females (almost no eggs remaining). The spawning males were in various stages of sexual maturation but were generally very ripe.

It should be noted that the location and method of capture of the fish and the handling of the samples, were the same for the 1971 and 1972 migrations.

B. Plasma Calcitonin Levels in Coho Salmon: Effect of Sexual Maturation and Environmental Salinity

The purpose of this study was to investigate the plasma calcitonin levels in coho salmon at different stages of development and in different environments. Table XVII, pg.127,summarizes the experimental conditions of the 3 groups of coho salmon. The first group has been described previously in Chapter I, and consisted of freshwater spawning adult male and female coho salmon. The second group were sexually immature coho salmon which had spent their entire lives in freshwater. The third group were young, very sexually immature salmon (grilse) which had been in seawater for 7 months. It should be noted that the adult spawning coho salmon in Group I had not been feeding for at least 1 month pior to sacrifice since in nature they cease to feed upon entry into fresh water. The freshwater immature coho in Group II were fed trout pellets once weekly and starved 6 days prior to sacrifice. The coho grilse in Group III were growing rapidly and being fed 3 times daily with a frozen meat diet consisting of canned salmon, beef liver and horse heart. These fish had been fed 4 hours prior to sampling.

Physical and electrolyte measurements were determed for each fish as outlined previously. Plasma calcitonin levels were again measured using the salmon calcitonin radioimmunoassay.

Table XVII. Coho Salmon Study: Summary of Sampling Data

	Group	Sampling Date	Location	Number of Salmon	History
I	Adults (ripe, spawning)	November 30/70	Samish Hatchery Washington, U.S.A. (from river) Freshwater (temp. 4.4°C)	15 m 15 f	Wild fish - migrated from seawater to freshwater (Samish River) Age 2 -3 years
II	Freshwater (immature)	July 28/71	U.B.C.Physiology (fish laboratory) Freshwater (temp. 10°C)	4 m 11 f	Hatchery-raised in freshwater Age 3 years
III	Grilse (very immature)	December 10/71	Fisheries Research Board, West Vancouver (outside tank) Seawater for 7 months (salinity range 28-30 ppt, temp. 9°C)	5 m 5 f	Hatchery-raised in freshwater until smolt stage when adapted to sea-water Age 1 year

C. Plasma Calcitonin Levels in Spawning Adult Sockeye, Coho and Chinook Salmon

This section is a summary of the plasma calcitonin levels obtained from spawning sockeye, coho and chinook salmon. Details on the dates and locations of the collection of these salmon are found in Chapter I and in Chapter IV, Sections A and B. Plasma electrolytes and physical measurements were obtained for each of the three species. Ultimobranchial gland calcitonin concentrations were measured for several coho and chinook salmon. Blood sampling and handling techniques were previously described in General Materials and Methods.

Results

A. Migration of Chilko Sockeye Salmon

Chilko Migration 1971

The dramatic changes in morphology of the migrating Chilko sockeye salmon are illustrated in Plates 7 and 8, pg.130, and Plates 9 and 10, pg. 131. The seawater sockeye (Plate 7) have olive-green backs, silver sides and white bellies. The sexes at this stage are indistinguishable upon external examination. Plate 8 shows the sockeye 3 - 4 weeks later on arrival to Chilko Lake. They have lost the silver colour from their sides and now have a reddish appearance. The secondary sexual characteristics such as the hooked snout and hump back in the male are beginning to develop. In the spawning condition, after approximately 2 months in fresh-water, the sexes are clearly distinguishable by the secondary sexual characteristics in the male (Plates 9 and 10). Both the male and female have brilliant crimson backs, black bellies and green heads and tails. The male has developed a cartilaginous hump and a hooked snout. The anterior teeth are much larger than those of the female and are now firmly attached to the jaw bones. The spawning male is generally larger in size than his mate, for the body shape of the female changes little from the seawater condition.

Table XVIII, pg.132, presents physical parameters and plasma measurements for the male and female sockeye at the 3 stages of the migration. Plasma electrolyte and calcitonin levels in these same fish are shown in Table XIX, pg. 133 .

Plate 7. Seawater Chilko sockeye

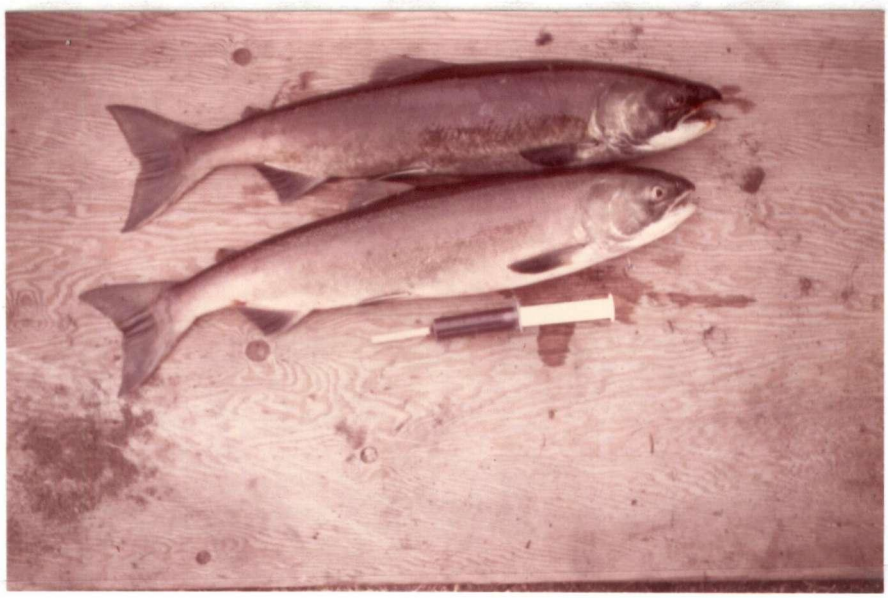

Plate 8. Freshwater arrival Chilko sockeye
(Male above, female below).

Plate 9. Spawning male Chilko sockeye. Note colouration, jaw, teeth and hump development.

Plate 10. Spawning female Chilko sockeye

Table XVIII. Physical and Plasma Measurements - Chilko Migration 1971

Parameter	Sex	Seawater Mean	±	SE	(n)	Arrival Chilko Lake Mean	±	SE	(n)		Spawning Mean	±	SE	(n)
Total Weight (g)	m	2534		52.4	(45)	2175		103.9	(26)		2780		130.1	(14)
	f	2506		67.5	(44)	1923		40.9	(34)	0%	2175		82.2	(12)
										50%	1944		115.0	(10)
										100%	1788		63.8	(10)
Fork Length (cm)	m	59.2		0.3	(45)	59.8		0.8	(26)		61.5		0.8	(14)
	f	59.0		0.4	(44)	57.6		0.3	(33)	0%	58.6		0.4	(12)
										50%	57.3		0.9	(10)
										100%	57.3		0.7	(10)
Gonad/Somatic Index	m	3.07		0.14	(45)	3.06		0.16	(26)		2.64		0.19	(14)
	f	4.01		0.13	(44)	10.14		0.26	(34)	0%	14.81		0.31	(12)
										50%	8.45		1.31	(9)
										100%	1.13		0.10	(10)
Haematocrit (vols %)	m	51		0.7	(44)	40		1.5	(26)		36		3.4	(15)
	f	49		0.8	(44)	40		0.7	(34)	0%	41		3.4	(12)
										50%	41		3.8	(10)
										100%	36		1.9	(10)
Plasma Protein (g/100 ml)	m	8.1		0.20	(44)	5.4		0.19	(26)		2.9		0.36	(15)
	f	8.7		0.27	(42)	6.6		0.12	(34)	0%	4.2		0.53	(12)
										50%	4.2		0.61	(10)
										100%	2.0		0.39	(10)
Plasma % H_2O (g/100g)	m	90.6		0.19	(44)	93.1		0.19	(26)		95.5		0.34	(15)
	f	90.0		0.26	(42)	92.0		0.11	(34)	0%	94.3		0.55	(12)
										50%	94.2		0.57	(10)
										100%	96.4		0.37	(10)

Table XIX. Plasma Electrolyte and Calcitonin Levels - Chilko Migration 1971

Plasma Measurement	Sex	Seawater Mean	± SE	(n)	Arrival Chilko Lake Mean	± SE	(n)	Spawning	Mean	± SE	(n)
Calcium mEq/l	m	6.9	0.12	(44)	5.6	0.11	(26)		4.1	0.24	(15)
	f	9.2	0.29	(44)	8.7	0.18	(34)	0%	7.3	0.84	(12)
								50%	7.0	0.81	(10)
								100%	4.0	0.51	(10)
Phosphate mEq/l	m	7.6	0.24	(44)	7.1	0.19	(26)		5.8	0.29	(15)
	f	7.8	0.27	(44)	6.8	0.20	(34)	0%	6.7	0.52	(12)
								50%	6.2	0.50	(10)
								100%	4.9	0.23	(10)
Sodium mEq/l	m	164	1.1	(43)	151	1.5	(26)		140	2.9	(15)
	f	159	0.8	(38)	151	0.7	(34)	0%	133	6.1	(12)
								50%	143	6.1	(10)
								100%	130	3.6	(10)
Potassium mEq/l	m	0.7	0.08	(43)	1.4	0.92	(26)		1.2	0.38	(15)
	f	0.8	0.10	(38)	1.9	0.40	(34)	0%	0.9	0.26	(12)
								50%	0.6	0.14	(10)
								100%	0.9	0.17	(10)
Plasma Calcitonin pg/ml	m	117	34	(45)	12	12	(25)		141	29	(14)
	f	545	136	(44)	687	112	(34)	0%	1649	240	(12)
								50%	709	330	(5)
								100%	306	105	(9)

Water samples, collected at the same location and time as the collection of the Chilko sockeye, were analysed for calcium, sodium and potassium concentration (Table XX, pg. 134). According to Reid (1961), phosphorus is a trace element in seawater where its concentration ranges from 0.0001 to 0.01 mg/100 ml, depending upon many factors. Freshwater contains 0.001 to 0.003 mg/100 ml, while even phosphate "rich" freshwater contains less than 0.03 mg/100 ml.

Table XX. Water Analysis - Chilko Migration 1971

Date	Location	Depth (feet)	Seawater Ions (mEq/l)		
			Calcium	Sodium	Potassium
July 23, 1971	Lummi Island (seawater)	15-20	15.6	350	7.65
August 6,1971	"	15-20	16.9	391	8.50
September 21, 1971	Chilko River Spawning Grounds (freshwater)	2	0.17	-	-

Plasma calcitonin changes in the sockeye at the 3 stages of migration are illustrated in Figure 18, pg. 135 . It is readily apparent that the females maintained higher circulating levels of calcitonin than the males, at all stages of the migration. The CT levels of the females increased significantly from sea to freshwater up to the 0 percent spawning stage, falling off precipitously after spawning. The male CT levels decreased to

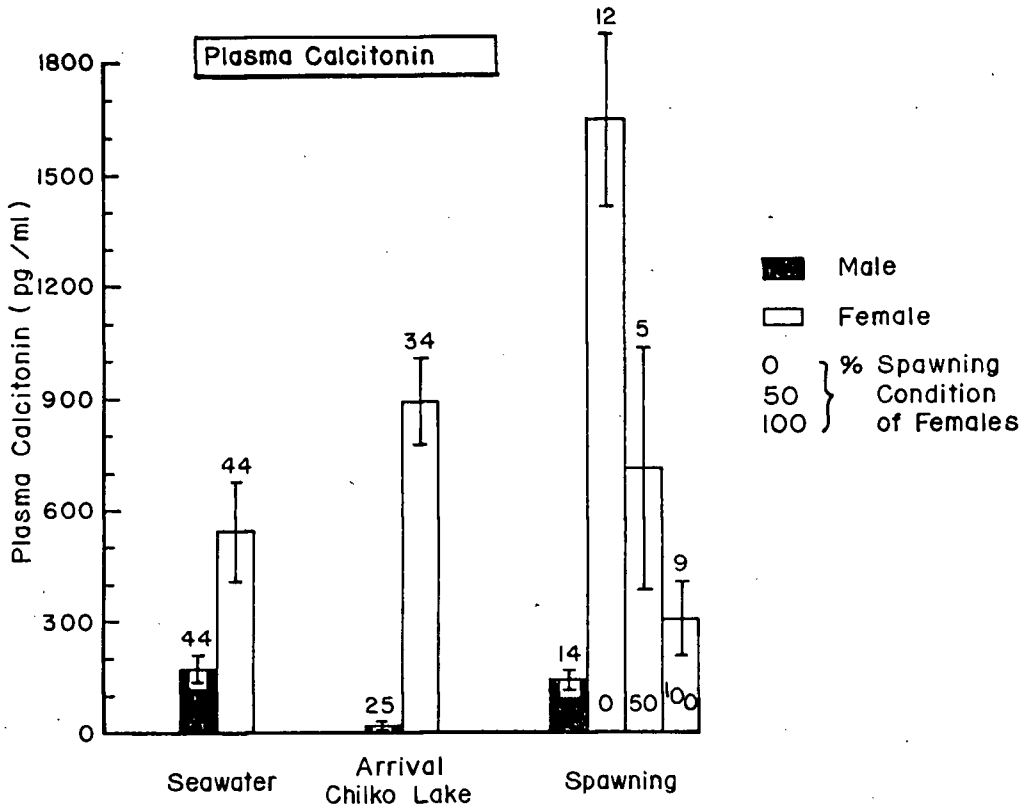

Figure 18. Plasma calcitonin changes in migrating Chilko sockeye.

extremely low levels on arrival to Chilko Lake and then increased at spawning.

In order to relate the plasma calcitonin concentrations of the sockeye with ultimobranchial gland calcitonin concentrations, the UB gland calcitonin contents of 6 seawater females were compared with those of 6 0% spawning females. Table XXI, pg. 137 summarizes the physical measurements, UB gland calcitonin contents and plasma CT levels in these 2 groups of female sockeye. The data show that there were significant increases in the GSI ($p < 0.001$) and plasma calcitonin levels ($p < 0.001$). While the UB gland calcitonin content increased from 35.81 to 55.63 Units per gland, the increase was not statistically significant due to the variation in the data.

Plasma electrolyte changes in the sockeye throughout the migration are illustrated in Figure 19, pg. 138 . The plasma sodium, phosphate and calcium levels decreased gradually throughout the migration in both sexes. The total plasma calciums in the females were significantly ($p < 0.001$) higher than the males at all stages in the migration except the spawned out females. Plasma potassium levels rose on arrival to Chilko Lake and fell with spawning.

Figure 20, pg. 139 ,shows the plasma calcium, plasma calcitonin and GSI changes throughout the migration. It can be seen that as spawning time approaches, the female gonads grow rapidly and that on spawning as the eggs are shed, the gonad-somatic index falls off. The GSI of the 0 percent spawning females was 269.3 percent higher than the seawater females. A decrease in the

Table XXI. Physical Parameters, Ultimobranchial Gland and Plasma Calcitonin Levels in Migrating Female Chilko Sockeye (1971)

Group	Location and Date	Fish #	Total Wt (g)	GSI	Calcitonin Content U/gland	Plasma Calcitonin Level pg/ml	Plasma Calcitonin Level mU/ml*
Seawater Females	Lummi Island Seawater August 6/71	94	3019	4.79	5.5	178	0.89
		96	3043	3.57	72.4	379	1.90
		100	2099	3.50	68.5	0	0
		101	2626	3.24	23.9	0	0
		103	2541	3.25	21.0	0	0
		105	2028	4.87	23.6	1090	5.45
n =		6	6	6	6	6	6
mean =			2559.33	3.87[a]	35.81	274.50[a]	1.37[a]
SD =			396.70	0.69	25.28	389.72	1.95
SE =			177.41	0.31	11.30	174.29	0.87
Spawning Females (0%)	Chilko River Spawning Grounds September 22/71	69	2391	14.88	34.9	2591	12.96
		70	2604	14.80	55.0	1466	7.33
		71	2455	13.01	55.7	1406	7.03
		72	2044	15.24	40.9	2089	10.45
		76	1947	16.05	71.0	1290	6.45
		77	1769	13.96	75.7	1433	7.17
n =		6	6	6	6	6	6
mean =			2201.66	14.66	55.53	1712.50	8.57
SD =			299.66	0.96	14.64	469.90	2.35
SE =			134.01	0.43	6.54	210.14	1.05

t-test probability seawater vs. spawning a. $p<0.001$

*Plasma CT biological activity based on salmon CT specific biological activity of 5000 MRC U/mg.

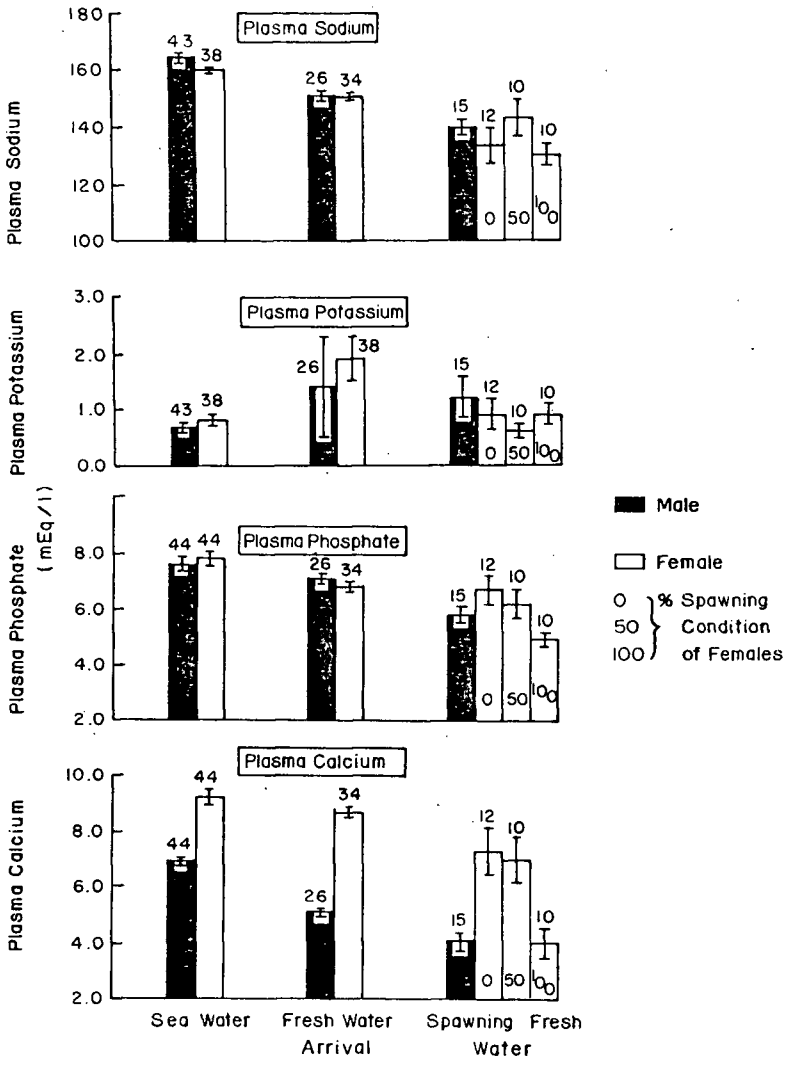

Figure 19. Plasma electrolyte changes in migrating Chilko sockeye.

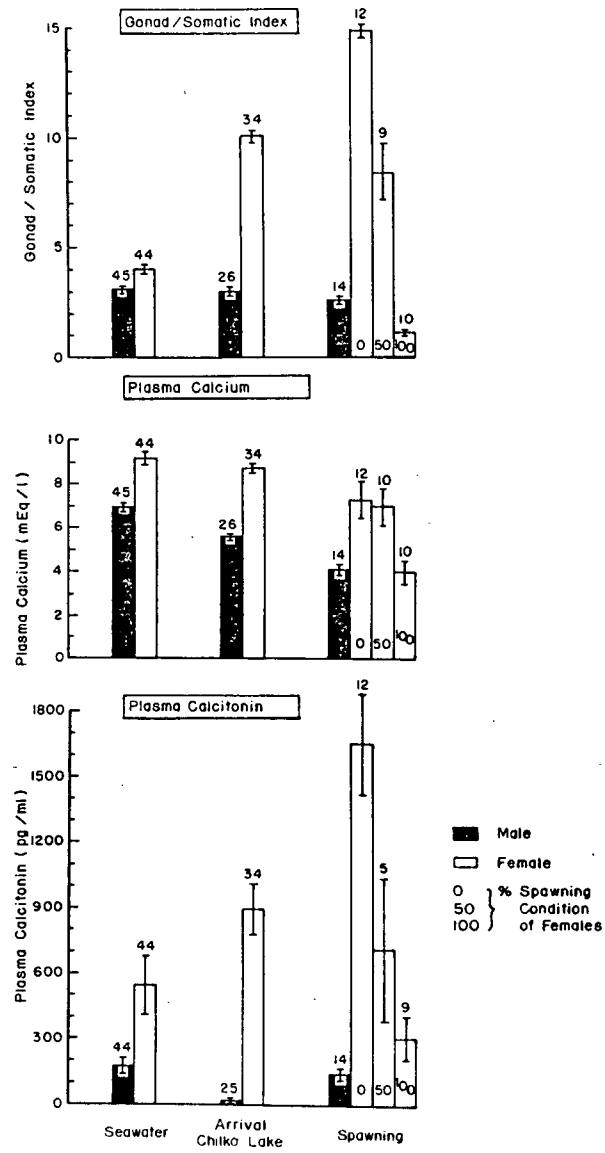

Figure 20. Plasma calcitonin, plasma calcium and gonad-somatic index changes in migrating Chilko sockeye.

male GSI of 14.0 percent from seawater to spawning, is evidence that some males had partially spawned before capture.

Changes in plasma percent water, plasma protein and haematocrit are illustrated in Figure 21, pg. 141 . The data indicate that the plasma percent water increased throughout the migration with the males having higher readings than the females, except for the spawned out females. Plasma protein levels in both sexes decreased during the migration. The females had consistently higher levels than the males except for the spawned out females. The haematocrits of both males and females decreased throughout the migration.

Chilko Migration 1972

Physical measurements, haematocrit, plasma protein and percent water for the sockeye sampled in the 1972 Chilko sockeye migration study are presented in Table XXII, pg. 142 . Plasma electrolyte levels are shown in Table XXIII, pg. 143 . There was close agreement between the data for the 1971 and 1972 migrations.

Serum total calcium, serum ionic calcium, percent ionic calcium, plasma protein and serum pH measurements are presented in Table XXIV, pg. 144 . Serum pH of the seawater sockeye was not measured. Changes in serum total and ionic calcium are illustrated in Figure 22, pg. 145 .

As stated previously, serum ionic calcium levels were measured at a water temperature close to that in which the fish were originally captured. Table XXV, pg. 146 , shows temperature readings both on location at the time of capture and of the ion electrode water jacket in the laboratory.

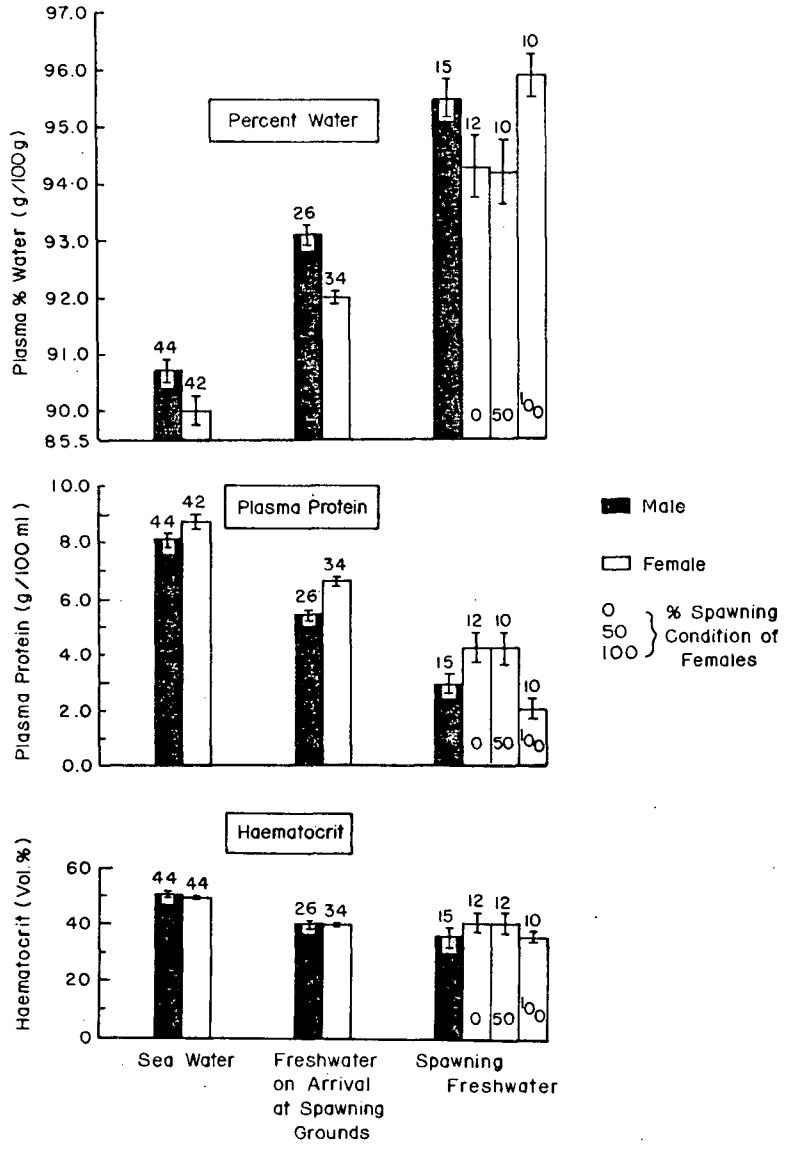

Figure 21. Haematocrit, plasma protein and plasma percent water changes in migrating Chilko sockeye.

Table XXII. Physical and Plasma Measurements - Chilko Migration 1972

Parameter	Sex	Seawater				Arrival Chilko Lake				Spawning			
		Mean	±	SE	(n)	Mean	±	SE	(n)		Mean	±	SE	(n)
Total Weight	m	2672		192.8	(10)	2667		114.5	(11)		2743		118.3	(10)
(g)	f	2433		94.5	(12)	2011		81.9	(10)	0%	2152		37.8	(10)
										100%	1853		93.6	(10)
Fork Length	m	60.7		1.8	(6)	63.3		0.6	(11)		61.9		0.7	(10)
(cm)	f	59.4		0.8	(12)	58.6		0.6	(10)	0%	58.5		0.3	(10)
										100%	59.0		0.5	(10)
Gonad/Somatic	m	2.42		0.22	(10)	2.77		0.14	(9)		1.79		0.17	(10)
Index	f	3.98		0.24	(11)	9.90		0.75	(9)	0%	15.36		0.30	(9)
										100%	-			
Haematocrit	m	50		2.7	(9)	37		1.0	(10)		36		1.5	(10)
(vols %)	f	48		2.1	(10)	40		1.2	(10)	0%	40		1.7	(10)
										100%	40		4.2	(10)
Plasma	m	7.6		0.26	(8)	5.1		0.17	(10)		3.1		0.43	(10)
Protein	f	8.6		0.35	(11)	6.9		0.17	(10)	0%	4.8		0.30	(10)
(g/100 ml)										100%	2.1		0.28	(10)
Plasma %	m	91.2		0.22	(8)	93.4		0.14	(10)		95.3		0.42	(10)
H_2O	f	90.1		0.33	(11)	91.7		0.17	(10)	0%	93.7		0.30	(10)
(g/100g)										100%	96.3		0.26	(10)

Table XXIII. Plasma Electrolyte Levels - Chilko Migration 1972

Electrolyte	Sex	Seawater Mean	±	SE	(n)	Arrival Chilko Lake Mean	±	SE	(n)		Spawning Mean	±	SE	(n)
Serum Total Calcium mEq/l	m	7.3		0.17	(10)	5.7		0.14	(11)		4.9		0.33	(10)
	f	11.2		0.36	(10)	12.7		0.30	(10)	0%	8.2		0.32	(11)
										100%	4.5		0.29	(9)
Plasma Phosphate mEq/l	m	10.1		0.48	(10)	7.0		0.26	(10)		6.1		0.17	(10)
	f	10.5		0.37	(11)	7.5		0.14	(10)	0%	6.3		0.48	(10)
										100%	5.8		0.44	(10)
Plasma Sodium mEq/l	m	165		1.9	(10)	138		8.5	(10)		140		6.3	(10)
	f	163		2.0	(11)	127		8.3	(10)	0%	151		3.4	(10)
										100%	133		8.0	(9)
Plasma Potassium mEq/l	m	0.4		0.06	(10)	2.3		0.23	(10)		1.1		0.23	(10)
	f	0.5		0.04	(11)	1.8		0.23	(10)	0%	1.3		0.24	(10)
										100%	1.3		0.26	(9)

Table XXIV. Ionic and Total Serum Calcium, Serum pH and Plasma Protein Changes in Migrating Chilko Sockeye (1972)

Sex		Seawater: Total Serum Ca mEq/l	Seawater: Ionic Serum Ca mEq/l	Seawater: % Ionic Ca	Seawater: Plasma Protein g/100 ml	Arrival Chilko Lake: Total Serum Ca mEq/l	Arrival Chilko Lake: Ionic Serum Ca mEq/l	Arrival Chilko Lake: % Ionic Ca	Arrival Chilko Lake: Plasma Protein g/100 ml	Arrival Chilko Lake: Serum pH	Spawning:	Spawning: Total Serum Ca mEq/l	Spawning: Ionic Serum Ca mEq/l	Spawning: % Ionic Ca	Spawning: Plasma Protein g/100 ml	Spawning: Serum pH
m	n =	10	10	10	8	11	11	11	10	11		10	8	8	10	10
	mean =	7.25[c]	3.06	42.2[c]	7.6[a]	5.65[c]	3.02	53.4[c]	5.1[c]	7.329		4.87	2.75	59.4	3.1	7.515
	SD =	0.51	0.30	2.07	0.70	0.50	0.18	2.56	0.51	0.069		0.99	0.34	7.29	1.33	0.130
	SE =	0.17	0.09	0.68	0.26	0.14	0.05	0.81	0.17	0.022		0.33	0.13	2.75	0.43	0.031
f	n =	10	10	10	11	10	10	10	10	10	0%	11	11	11	10	11
	mean =	11.23	3.16	28.2	8.6	12.73	3.17	25.0	6.9	7.282		8.23	3.04	37.4	4.8	7.389
	SD =	1.08	0.32	2.34	1.14	0.94	0.17	1.73	0.56	0.080		1.02	0.16	4.96	0.94	0.141
	SE =	0.36	0.10	0.78	0.36	0.30	0.05	0.58	0.17	0.027		0.32	0.04	1.56	0.30	0.044
											100%	9	7	7	10	10
												4.47	2.45	57.9	2.1	7.554
												0.83	0.33	4.66	0.85	0.214
												0.29	0.13	1.90	0.28	0.070
											Total Spawning Females	20	18	18	20	21
												6.54[b]	2.81	45.4[b]	3.5	7.468
												2.09	0.38	11.14	1.64	0.197
												0.48	0.09	2.70	0.37	0.031

t-test probability male vs. female

a. $p < 0.050$

b. $p < 0.010$

c. $p < 0.001$

Note: pH was not measured on seawater serum samples.

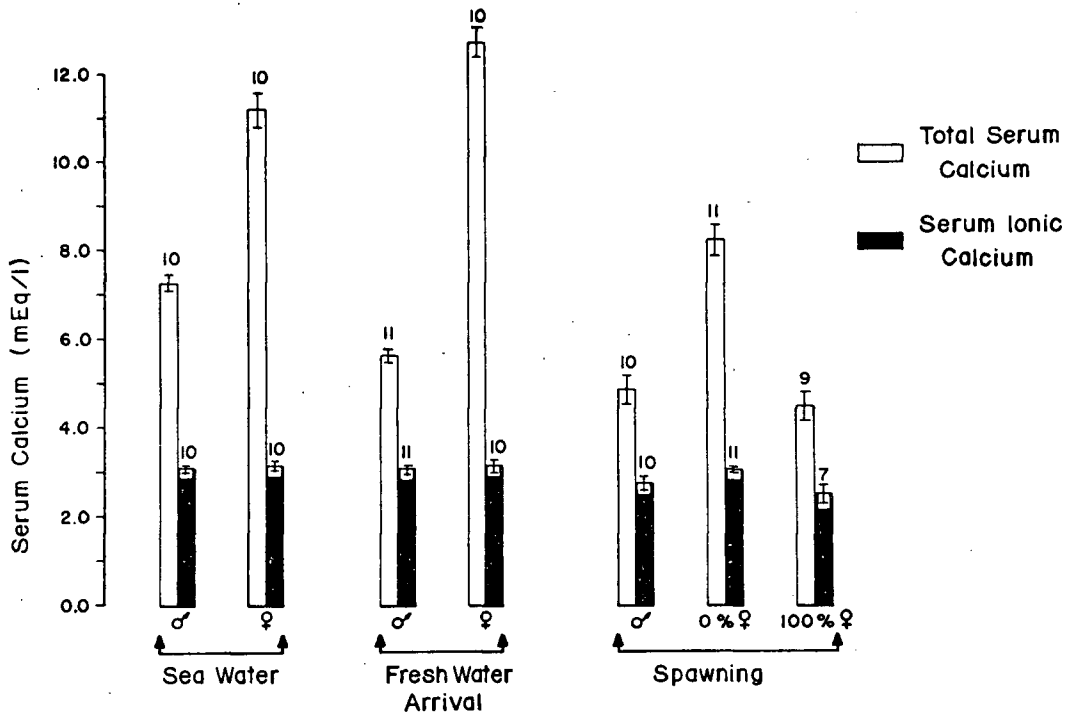

Figure 22. Serum ionic and total calcium changes in migrating Chilko sockeye (Chilko Migration 1972). Note constancy of ionic serum calcium levels.

Table XXV. Water Temperatures - Chilko Migration 1972

Collection Date	Location	Temperature of Water	
		On Location (surface temperature)	Ion electrode water jacket
July 21, 1972	Lummi Island (seawater)	14.5°C	12°C
August 28, 1972	Chilko Lake (freshwater)	14.4°C	13.5°C
September 24, 1972	Chilko River (freshwater)	10.6°C	12.5°C

It can be seen from the data that although serum total calcium levels markedly changed throughout the migration, the serum ionic calcium levels remained relatively constant. The spawning males showed a slight but significant ($p < 0.05$) decline in ionic calcium from the arrival level. Female serum ionic calcium remained stable until spawning when there was a significant decrease ($p < 0.001$) to the 100 percent spawned level. Since the total serum calcium fell dramatically and the ionic serum calcium remained fairly constant, there was a marked increase in percent ionic calcium from seawater to spawning in both sexes.

Although the female total calciums were significantly higher than those of the male (excepting the spawned out females), the

ionic calcium levels of both sexes were very similar. It is emphasized that the serum ionic calcium levels were the most stable of any of the plasma electrolytes measured and that these levels were maintained despite a marked increase in plasma percent water and decrease in haematocrit during the migration.

There was a marked decline in plasma protein throughout the migration and a rise in serum pH from arrival to spawning in both males and females.

Calcium and phosphate changes in the soft tissues (skin, muscle, gonads) and hard tissues (vertebrae, scales, premaxillae) of the migrating Chilko sockeye are presented in Table XXVI, pg. 148, and Table XXVII, pg. 149, respectively. These same results are illustrated in histogram form in Figures 23, 24, pages 150, 151, (soft tissues) and Figures 25, 26, pages 154, 155, (hard tissues). The soft tissue mineral contents are presented as mg calcium or phosphate per 100g fat-free dry weight (FFDW). The hard tissue mineral contents are presented as g calcium or phosphate per 100g ash weight. Only the premaxillae calcium and phosphate contents are expressed as gCa and PO_4 per 100g dry weight since ash weights for these bones were not available.

As illustrated in Figure 23, the skin had the greatest concentration of calcium (range 76-192 mgCa/100g FFDW) of the soft tissues. The female gonads also contained large amounts of calcium (150-175 mgCa/100g FFDW) and had 6 to 17 times more calcium than the male gonads. The latter possessed the lowest amounts of calcium (10-26 mgCa/100g FFDW) of the soft tissues. Measurements for the muscles ranged from 22 to 68 mgCa/100g FFDW. In comparing

Table XXVI. Soft Tissue Mineral Changes - Chilko Migration 1972

Tissue	Sex	(n)	SEAWATER						ARRIVAL CHILKO LAKE						SPAWNING					
			% Ash Wt. FF Dry Wt.			mg PO_4 100g FF Dry Wt.			mg Ca 100g FF Dry Wt			% Ash Wt FF Dry Wt.			mg PO_4 100 FF Dry Wt			mg Ca 100g FF Dry Wt		
			Mean	±	SE	Mean	±	SE	Mean	±	SE	Mean	±	SE	Mean	±	SE	Mean	±	SE
Gonads	m	9	14.49		0.71	2867.5		124.83	9.9		0.64	13.90		0.26	3551.0		73.9	13.1		0.86
	f	7	3.92[c]		0.15	805.1[c]		44.49	161.8[c]		6.03	4.02[c]		0.17	949.3[c]		28.3	175.0[c]		8.22
Muscles	m	7	5.56		0.33	857.7		79.24	48.1		5.61	4.64		0.45	1064.5		37.14	22.7		1.81
	f	9	5.07		0.26	908.2		40.98	68.3[a]		7.57	5.59		0.19	1107.7		21.67	31.9[b]		1.98
		7																		
Skin	m	8	1.67		0.14	422.4		24.71	141.7		27.30	2.82		0.08	598.5		17.17	166.7		24.21
	f	8	1.68		0.22	423.7		59.93	176.3		36.19	2.80		0.13	549.8		61.52	192.7		52.45
		9																		

		Sex	(n)	% Ash Wt FF Dry Wt. Mean	±	SE	mg PO_4 100g FF Dry Wt Mean	±	SE	mg Ca 100g FF Dry Wt Mean	±	SE
SPAWNING	Gonads	m	9	16.46		0.86	3861.8		140.2	26.4		3.68
		f	7	3.95[c]		0.20	856.0[c]		45.4	150.1[c]		6.82
	Muscles	m	7	5.05		0.23	1008.6		38.07	43.4		3.05
		f	9	5.83		0.27	1069.5		42.77	33.3		3.66
			7	5.36		0.49	1075.9		90.38	31.2		2.52
	Skin	m	8	-			547.0		30.05	78.0		10.97
		f	8	-			547.2		16.70	75.9		6.10
			9	-			528.6		14.65	110.0		12.80

t-test probability male vs. female

a. $p < 0.05$

b. $p < 0.005$

c. $p < 0.001$

Table XXVII. Hard Tissue Mineral Changes - Chilko Migration 1972

Tissue	Sex (n)	SEAWATER: % Ash Wt Dry Wt Mean ± SE	SEAWATER: g PO_4 100 g Ash Mean SE	SEAWATER: g Ca 100 g Ash Mean ± SE	ARRIVAL CHILKO LAKE: % Ash Wt Dry Wt Mean ± SE	ARRIVAL CHILKO LAKE: g PO_4 100g Ash Mean ± SE	ARRIVAL CHILKO LAKE: g Ca 100 g Ash Mean ± SE	SPAWNING: % Ash Wt Dry Wt Mean ± SE	SPAWNING: g PO_4 100g Ash Mean ± SE	SPAWNING: g Ca 100g Ash Mean ± SE
Scales	m 7	36.01 0.75	17.65 0.25	36.22 0.53	26.79 0.94	19.51 0.29	32.65 0.64	23.53 0.63	20.29 0.19	32.03 0.49
	f 6	34.01[a] 0.38	17.65 0.20	35.64 0.42	26.43 0.67	19.62 0.48	32.81 0.31	0% 24.77 0.68	20.36 0.40	32.94 0.39
	9							100% 24.87 0.86	19.59 0.47	32.14 0.79
Vertebrae	m 9	34.17 0.33	18.69 0.34	35.58 0.53	38.15 0.87	18.51 0.91	37.11 0.39	41.65 0.52	18.21 0.05	38.08 0.17
	f 9	34.07 0.40	18.81 0.29	36.19 0.23	39.75 0.52	18.18 0.06	36.21 0.48	0% 42.54 0.26	18.04 0.09	36.81 0.62
	9							100% 46.06 0.64	18.12 0.04	36.10 0.58

t-test probability male vs. female a. $p < 0.05$

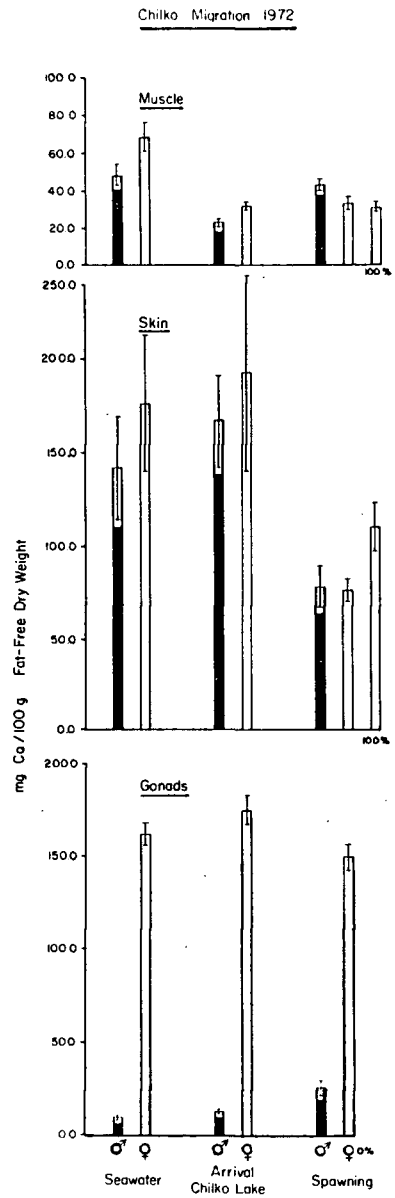

Figure 23. Soft tissue calcium changes in migrating Chilko sockeye.

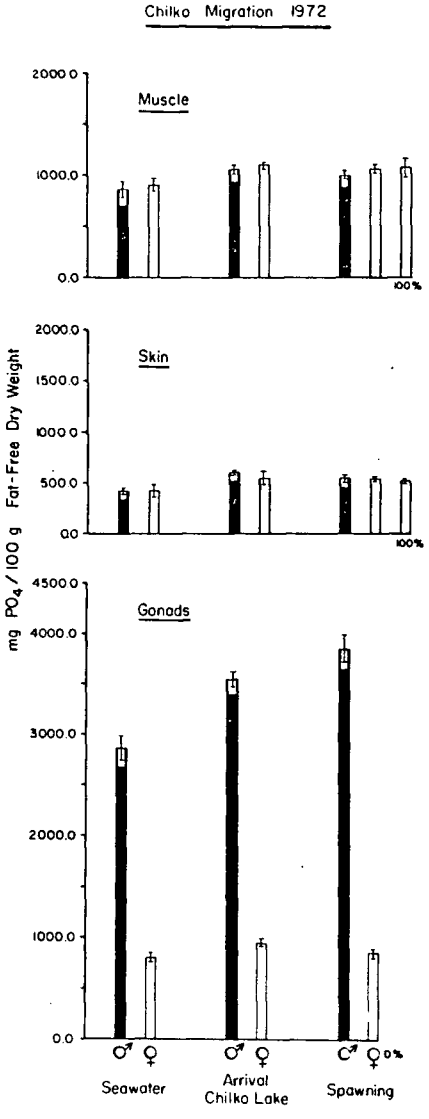

Figure 24. Soft tissue phosphate changes in migrating Chilko sockeye.

the 3 soft tissues, the skin showed the greatest variation in calcium content (as indicated by the SE bars) while the muscles showed the least.

In the females, the muscle calcium content decreased significantly ($p < 0.001$) from a high of 68 mgCa/100g FFDW in the sea to 32 mgCa/100g FFDW on arrival, at which level it remained stable. The male muscle calcium also fell significantly ($p < 0.005$) from 48 mgCa/100g FFDW in the sea to 23 mgCa/100g FFDW on arrival. Spawning males however, had a significantly ($p < 0.001$) higher level than the arrival males as the muscle calcium content again rose to the seawater level. In comparing the muscle calcium content for both sexes, the females had significantly higher levels in the seawater and arrival groups while the males had significantly higher measurements in the spawning group.

The calcium content of the skin for both male and female sockeye increased slightly on arrival to Chilko Lake. The female skin contained slightly more calcium but the difference was not statistically significant. In both sexes, the skin calcium content decreased (male $p < 0.005$, female $p < 0.025$) from arrival to spawning. Spawned out females had significantly ($p < 0.05$) higher skin calcium contents than the 0% spawning females.

As expected, the gonads of the female sockeye contained significantly ($p < 0.001$) greater amounts of calcium than the males at all stages of the migration. The female level rose slightly from sea to arrival then fell ($p < 0.05$) with spawning. In contrast, the male gonad calcium content increased significantly both from sea to arrival ($p < 0.01$) and from arrival to spawning ($p < 0.005$).

With regard to soft tissue phosphate (Figure 24), the male gonads contained the greatest concentration (2867 - 3862 mg PO_4/100g FFDW), the skin contained the least (422 - 547 mg PO_4/100g FFDW) and the muscles contained intermediate amounts (858 - 100 mg PO_4/100g FFDW). Only in the case of the gonads was there a sex difference in the soft tissue phosphate contents.

The muscle phosphate level in the male increased slightly ($p < 0.05$) from sea to arrival and remained constant from arrival to spawning. The female level also rose ($p < 0.001$) from sea to arrival, remaining stable throughout spawning.

The male skin phosphate content increased ($p < 0.001$) from sea to arrival. The spawning level was not significantly different from the latter. The female skin phosphate content did not change significantly from sea to spawning. Muscle and skin phosphate contents of both male and female sockeye followed the same basic pattern throughout the migration.

The male gonads exhibited a dramatic increase ($p < 0.001$) in phosphate content from the sea to arrival with a further slight increase on spawning. The testes contained 3-4 times more phosphate than the ovaries. Gonad phosphate content in the female increased ($p < 0.05$) from sea to arrival and then returned to the seawater level in the 0 percent spawned female.

In the hard tissues, it should be noted that while the vertebrae increased in mineral content (% ash/dry weight) throughout the migration, the scales demineralized. The mineral content of the male vertebrae increased ($p < 0.001$) from 34.17% in the sea to 41.65% at spawning. The female vertebrae increased ($p < 0.001$)

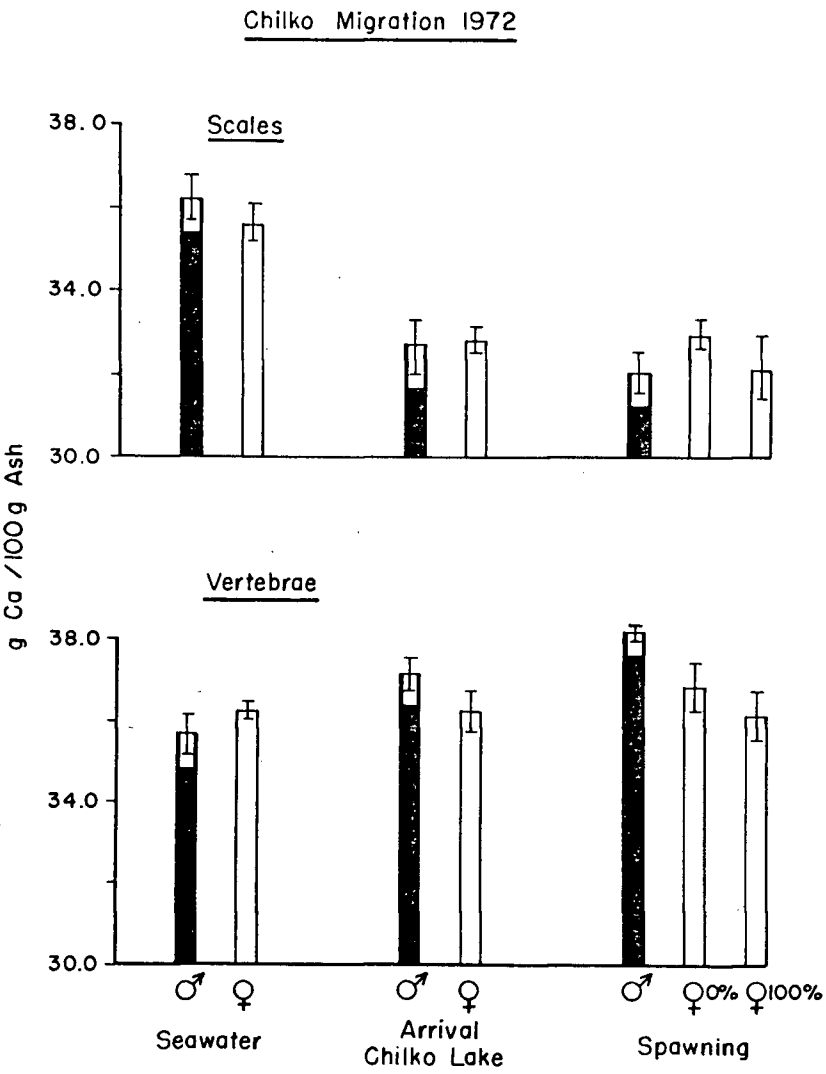

Figure 25. Hard tissue calcium changes in migrating Chilko sockeye.

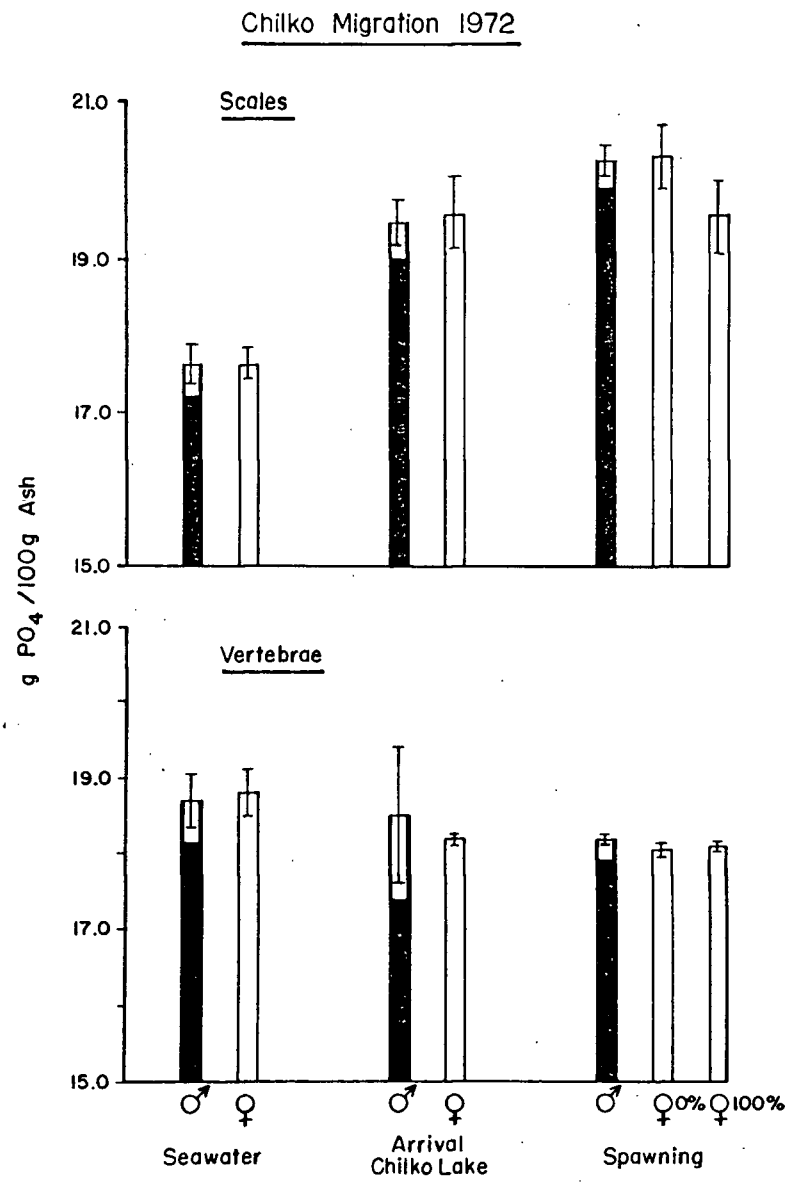

Figure 26. Hard tissue phosphate changes in migrating Chilko sockeye.

from the seawater value of 34.07% to 46.06% in the spawned out females. In the male, the scale mineral content decreased ($p < 0.001$) from 36.01% in the sea to 23.53% at spawning while the female level decreased ($p < 0.001$) from 34.01% in the sea to 24.87% in the spawned out females. Thus the scales of the male sockeye lost more mineral throughout the migration than did those of the female, whereas the female vertebrae mineralized to a greater extent than the males. This relationship is shown in Figures 27 and 28, pages 157 and 158 .

As seen in Figure 25, the calcium content of the male vertebrae increased from sea to arrival ($p < 0.05$) and further from arrival to spawning ($p < 0.05$). In the female, there was no significant change throughout the migration. A comparison of the vertebrae calcium content for both sexes reveals significantly ($p < 0.05$) higher calcium levels for the males than for the females in the spawning condition.

As well as losing mineral, the calcium content of the scales of both male and female sockeye decreased markedly during the freshwater migration. In the male, scale calcium decreased from sea to arrival ($p < 0.005$) with a further decrease at spawning. The female level also decreased significantly ($p < 0.001$) after the first stage of the migration but did not change with spawning.

As shown in Figure 26, the vertebrae phosphate content of both sexes decreased slightly with migration but the decrease was significant only between the female seawater and spawning vertebrae ($p < 0.05$).

In contrast, the male and female scale phosphate content

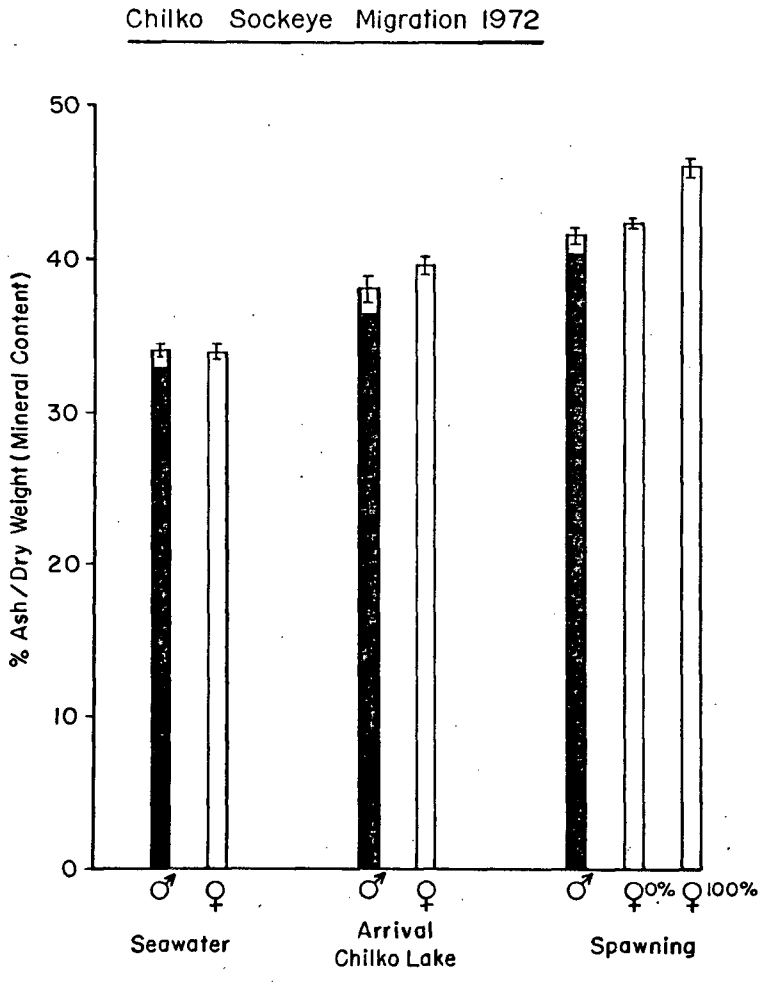

Figure 27. Vertebrae mineral content changes in migrating Chilko sockeye.

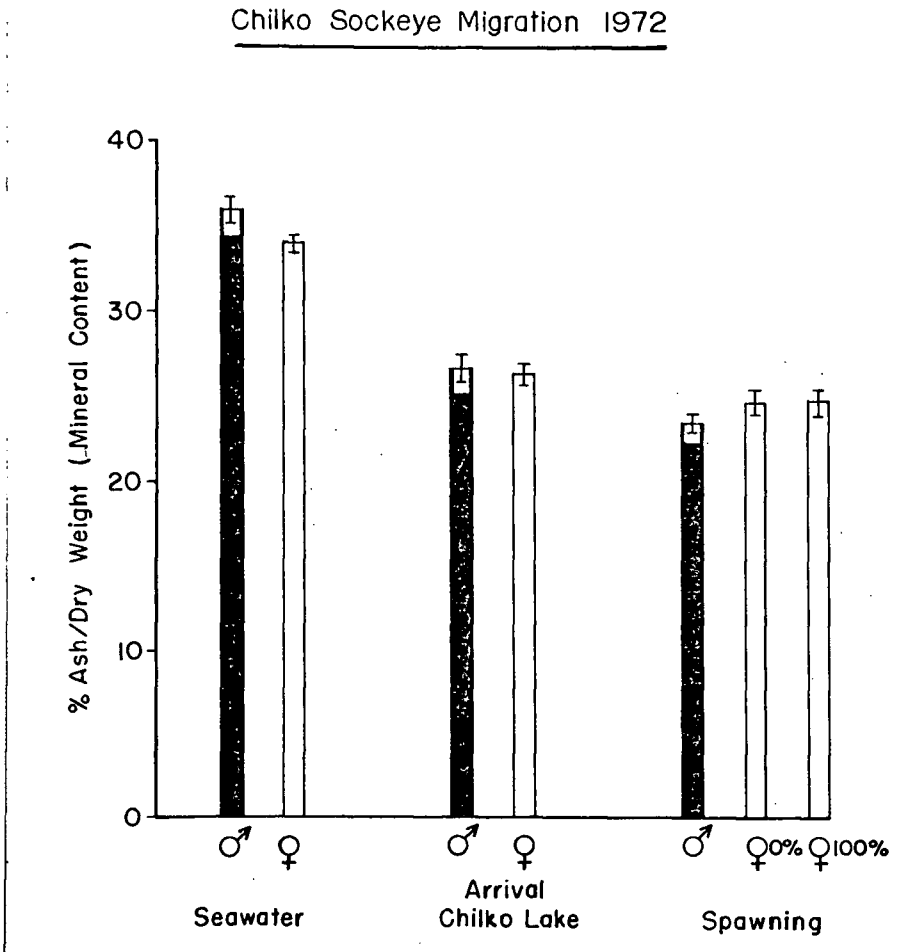

Figure 28. Scale mineral content changes in migrating Chilko sockeye.

showed a marked increase through the migratory stages. In the male, this increase was significant from sea to arrival ($p < 0.001$) and from arrival to spawning ($p < 0.05$). The female scale phosphate content also increased significantly ($p < 0.001$) from sea to arrival but there was little change with spawning.

Tchernavin (1937; 1938a; 1938b) has documented the phenomenal growth in the jaw bones of the spawning Atlantic salmon. This "breeding growth" as Tchernavin termed it, is particularly evident in the premaxillae (Vladykov, 1962). In males, the premaxilla practically doubles in length from the seawater to the breeding stage. The increase in size is due not only to the growth of the bone itself, but also to its fusion to an ossified plate which develops as a support base for the large breeding teeth (Tchernavin, 1938a).

Table XXVIII, pg. 160 , summarizes the dry weights, calcium and phosphate contents (g/100g dry weight) of single premaxillary bones taken from Chilko sockeye at each stage of the migration. Figure 29, pg. 161, illustrates the changes in dry weight of the premaxillae. The marked increase in weight for the spawning male reflects the extensive snout and jaw growth. Calcium and phosphate content of the premaxillae are illustrated in Figures 30 and 31, pages 162 and 163 . The spawning male had significantly ($p < 0.005$) more calcium and phosphate (g/100g dry wt.) than the spawning female.

In order to determine the actual amounts of tissue calcium and phosphate per fish, the tissues of two freshwater arrival Chilko sockeye salmon (male and female) were dissected out, dried

Table XXVIII. Dry Weights, Phosphate and Calcium Contents of the Premaxilla Bone - Chilko Sockeye Migration (1972)

Sex	SEAWATER			ARRIVAL CHILKO LAKE			SPAWNING		
	Dry Wt Mean ± SE(n)	gPO_4 / 100g Dry Wt Mean ± SE(n)	gCa / 100g Dry Wt Mean ± SE(n)	Dry Wt Mean ± SE(n)	gPO_4 / 100g Dry Wt Mean ± SE(n)	gCa / 100g Dry Wt Mean ± SE(n)	Dry Wt Mean ± SE (n)	gPO_4 / 100g Dry Wt Mean ± SE (n)	gCa / 100g Dry Wt Mean ± SE(n)
m	54.10 ± 10.29(5)	4.75 ± 0.32(5)	7.87 ± 0.15(5)	75.49[b] ± 7.38(11)	6.63 ± 0.32(11)	12.19 ± 0.56(11)	213.51[b] ± 33.52(10)	8.82[a] ± 0.32(10)	16.07[a] ± 0.56(10)
f	37.92 ± 8.10(7)	4.30 ± 0.13(5)	8.41 ± 0.46(5)	40.34 ± 2.36(10)	6.01 ± 0.32(9)	11.40 ± 0.62(10)	0% 56.11 ± 6.90(10)	7.60 ± 0.17(10)	13.72 ± 0.35(10)
							100% 75.34 ± 7.10 (8)	7.50 ± 0.41 (8)	14.14 ± 0.76 (8)
							Total sp.f 64.66 ± 5.35(18)	7.55 ± 0.20(18)	13.91 ± 0.38(18)

t-test probability male vs. female

a. $p < 0.005$

b. $p < 0.001$

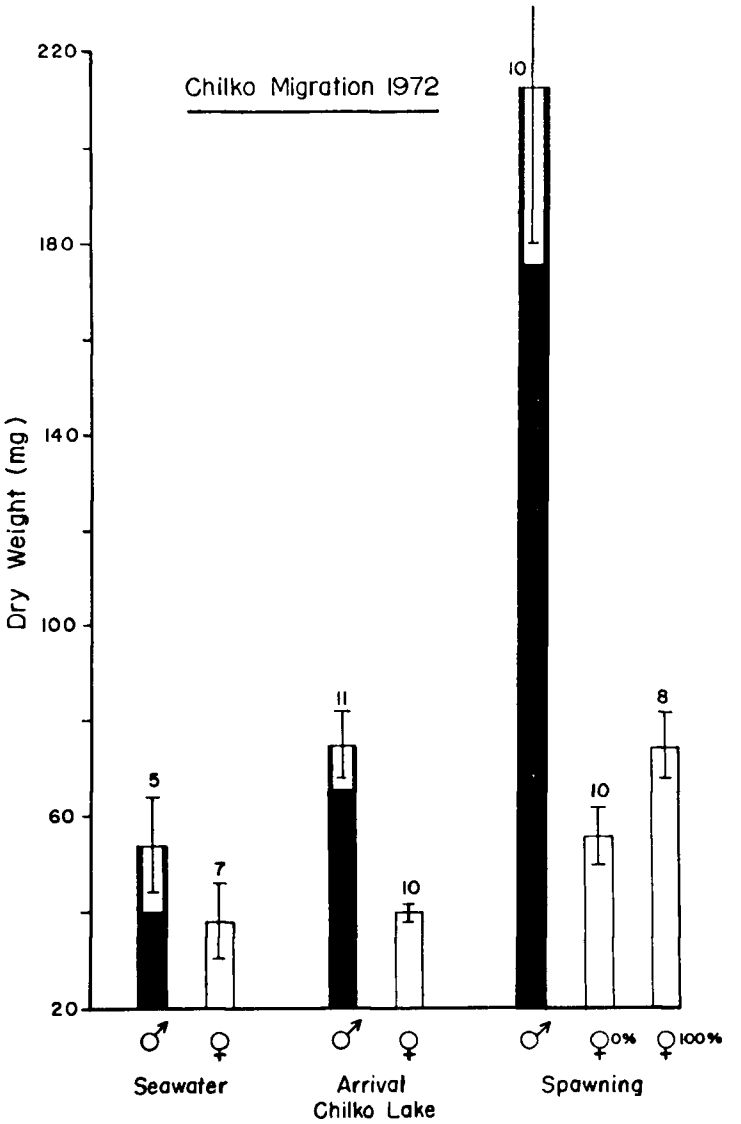

Figure 29. Premaxillae dry weight increases in migrating Chilko sockeye.

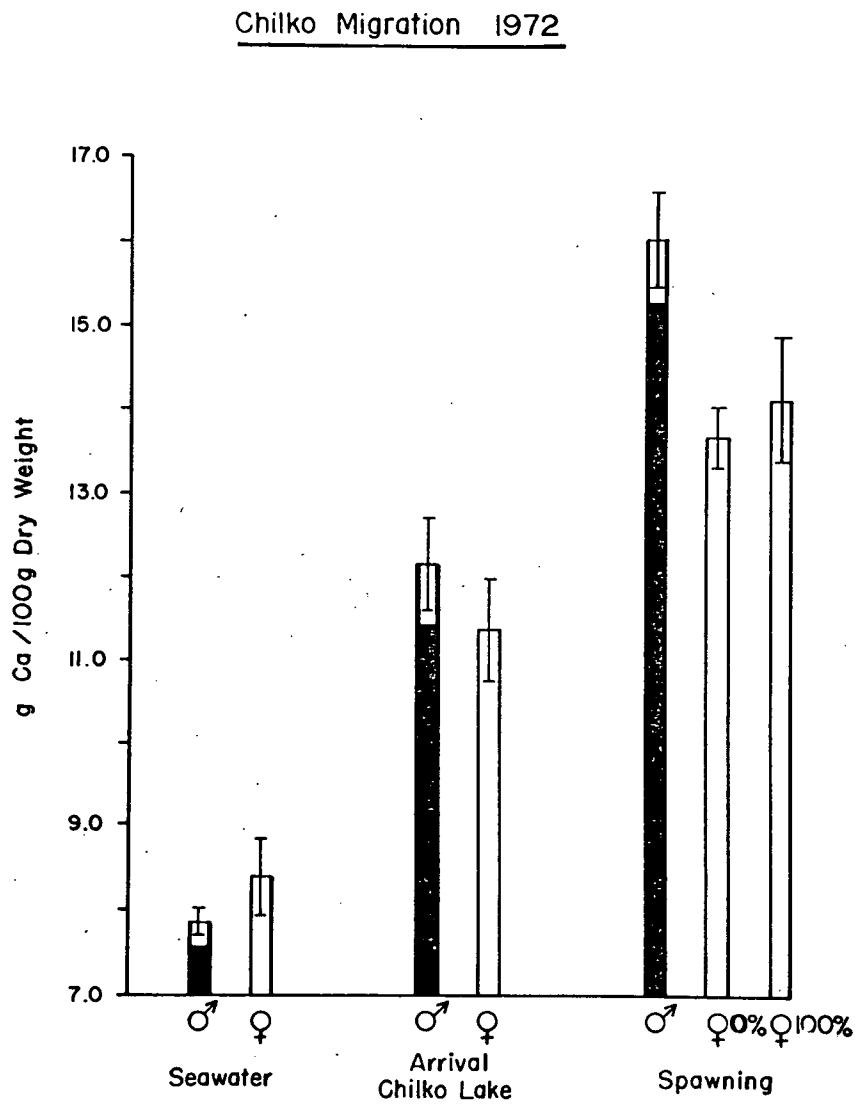

Figure 30. Premaxillae calcium content changes in migrating Chilko sockeye.

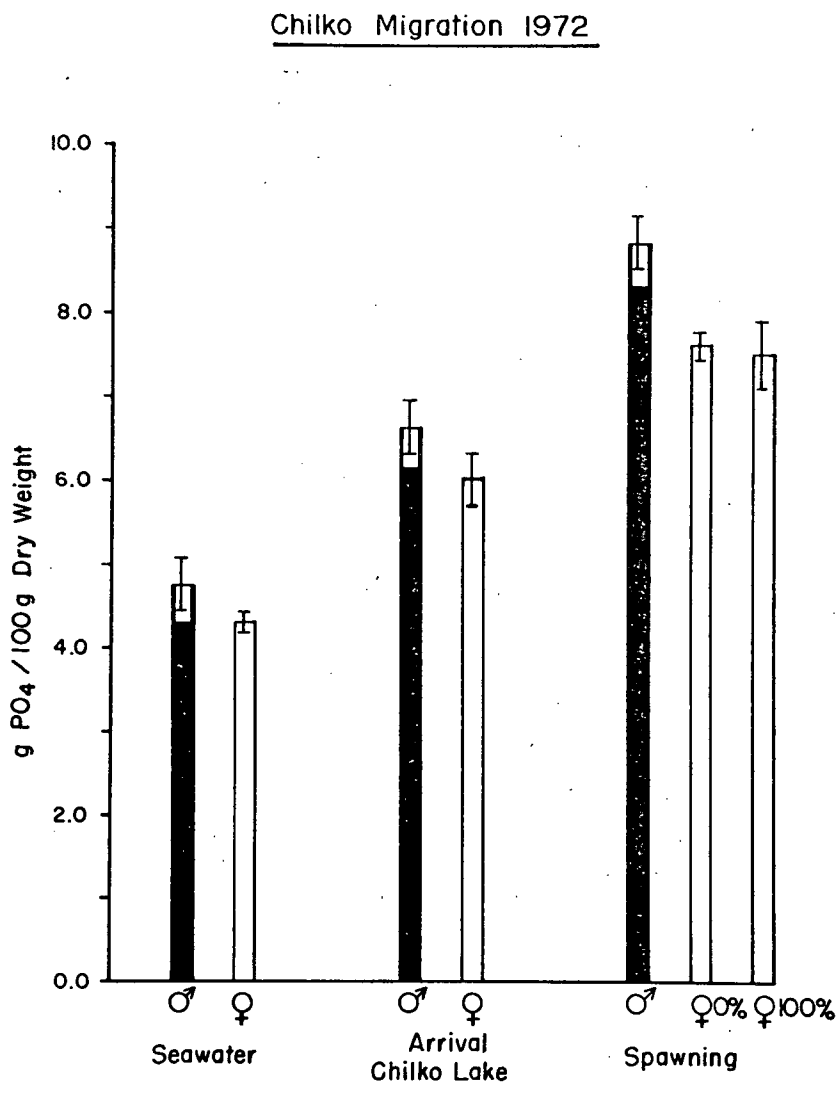

Figure 31. Premaxillae phosphate content changes in migrating Chilko sockeye.

and weighed. The percent dry weight organ (g)/total body wet weight fish (g) was calculated (Table XXIX, pg. 164). Data from a human cadaver was inserted in the table for comparison.

Table XXIX. Percentage Dry Weights of Tissues

	Total Body Wet Wt (Kg)	% dry wt /total body wet wt				
		Skin	Muscle	Gonads	Scales	Vertebrae	Total Skeleton**
male sockeye	2.540	1.693	24.399	0.581	0.079	0.736	2.669
female sockeye	2.526	1.742	21.770	3.486	0.071	0.732	2.514
human*	70.55	2.758	6.464	-	-	-	10.119

*Data taken from Mitchell et al, 1945.

**Total skeleton in sockeye includes vertebrae, ribs, tail, fin bones, gill apparatus and skull bones.

It is apparent from the table that the percent dry weight/total body wet weight for the skin is slightly higher in the human than in the fish. The sockeye have a much greater percent of muscle and a smaller percent of bone than the human. The higher percentage weight of the human skeleton compared to the salmon skeleton, probably reflects the supportative function of the human skeleton (Brown, 1957).

From the above dissection data and that given in Tables

XXVI and XXVII, pages 148, and 149 , it was possible to calculate the absolute amount of calcium and phosphate in each tissue of a male and female freshwater arrival Chilko sockeye (Table XXX, pg. 166). The absolute weight of the fish as well as the individual tissue weights changed during the migration. For example, it has been shown that salmon muscle and viscera weights decrease during migration while there are obvious increases in the absolute weights of the gonads and skeleton (Greene, 1926; Idler and Tsuyuki, 1958; Idler and Bitners, 1958). Therefore, the calculations would be different for seawater and spawning salmon. As expected, the major storage of calcium for the sockeye occurred in the hard tissues whereas the soft tissues contained slightly more phosphate. In the human, body calcium amounts to 1.5 - 1.6% body weight (Mitchell et al, 1945; Copp, 1970b) whereas in the sockeye, body calcium (total skeleton plus the 3 soft tissues measured) was approximately 0.42 - 0.44% body weight. The percent calcium and phosphate in the human skeleton is 1.58% and 0.72% respectively (Mitchell et al, 1945). Corresponding percentages in the sockeye skeleton were 0.40 - 0.43% for calcium and 0.21 - 0.23% for phosphate.

In summary, because of its great bulk, the muscle tissue contained the largest store of calcium and phosphate of the 3 soft tissues examined. The female gonads contained 60 times more calcium and slightly more phosphate than the male gonads. Due to their very low weight, the scales contributed little to the total storage of calcium and phosphate in the hard tissue. The scales did, however, contain more calcium than any of the soft tissues measured.

Table XXX. Calcium and Phosphate Content in Tissues of Average Chilko Freshwater Arrival Sockeye*

Tissue	Calcium (g) per tissue per fish		Phosphate (g) per tissue per fish	
	male	female	male	female
Soft Tissues				
Skin	0.075	0.068	0.270	0.193
Muscle	0.148	0.140	6.927	4.850
Gonads	0.002	0.122	0.550	0.664
Total Soft Tissue**	0.225	0.330	7.747	5.707
Hard Tissues				
Scales	0.173	0.158	0.103	0.095
Vertebrae	2.159	2.121	1.077	1.067
Premaxilla	0.019	0.009	0.010	0.005
Total Hard Tissue***	11.383	8.112	6.086	4.318

*Mean total wet weight: FWA male = 2667g, FWA female = 2011g

**Total soft tissue includes only skin, muscle and gonads.

***Total hard tissue includes all bones plus scales.

B. Plasma Calcitonin Levels in Coho Salmon: Effect of Sexual Maturation and Environmental Salinity

Physical parameters and plasma measurements of the 3 groups of coho salmon are presented in Table XXXI, pg. 168 , and their respective electrolyte and calcitonin levels are shown in Table XXXII, pg. 169 . The GSI, plasma calcitonin and plasma calcium levels for the 3 groups of coho are illustrated in Figure 32, pg. 170 .

As shown by the gonad-somatic index the adult coho were very sexually mature while both the coho grilse and freshwater coho were sexually immature. Plasma calcium levels were lowest in the immature freshwater coho and highest in the spawning adult coho. The coho grilse, even though they were living in seawater had lower plasma calcium levels (calcium level = 17.6 mEq/litre) than the freshwater adult spawning coho. Although plasma calciums in the females are slightly higher than in the males in each of the 3 groups, the differences are not statistically significant.

Plasma calcitonin levels for the adult spawning coho were markedly higher than the other two groups while the lowest levels were measured in the freshwater immature coho. A sex difference was noted only in the case of the adult spawning coho where the females had significantly higher ($p < 0.001$) plasma calcitonin levels than the males. The mean plasma calcitonin level of 4 coho "jacks" (2 year old, sexually ripe males, mean total wt. = 409 $\pm$ 107g) was 2,393 $\pm$ 754 pg/ml and was thus higher than the level found in the adult spawning coho (1,070 $\pm$ 294 pg/ml).

Table XXXI. Physical and Plasma Measurements - Coho Salmon Study

Parameter	Sex	Grilse Seawater Mean ± SE (n)	Immature Freshwater Mean ± SE (n)	Spawning Adults Freshwater Mean ± SE (n)
Total Weight	m	0.19 0.021 (5)	0.46 0.134 (4)	5.17 0.23 (15)
(Kg)	f	0.18 0.006 (5)	0.50 0.058 (10)	4.36[a] 0.22 (15)
Fork Length	m	25.3 1.0 (5)	35.1 2.7 (4)	77.8 0.9 (15)
(cm)	f	25.7 0.3 (5)	36.7 1.5 (10)	72.5[b] 1.1 (15)
Gonad/Somatic	m	0.03 0.00 (5)	0.5 0.21 (4)	4.7 0.00 (12)
Index	f	0.28[c] 0.04 (5)	0.9 0.14 (10)	>15.0
Haematocrit	m	44 3.8 (5)	19 2.5 (4)	47 1.4 (15)
(vols %)	f	45 3.8 (5)	12[a] 1.3 (10)	46 1.9 (15)
Plasma Protein	m	5.7 0.68 (5)	3.6 0.31 (4)	6.4 0.23 (15)
(g/100 ml)	f	6.4 1.29 (5)	3.6 0.10 (10)	6.5 0.38 (15)
Plasma	m	92.8 0.6 (5)	95.0 0.3 (4)	92.1 0.2 (15)
% H_2O	f	92.2 1.2 (5)	94.8 0.1 (10)	92.1 0.4 (15)
(g/100g)				

t-test probability male vs. female a. $p < 0.05$ b. $p < 0.010$ c. $p < 0.001$

Table XXXII. Plasma Electrolyte and Calcitonin Levels - Coho Salmon Study

Plasma Measurement	Sex	Grilse Seawater Mean ± SE (n)	Immature Freshwater Mean ± SE (n)	Spawning Adults Freshwater Mean ± SE (n)
Calcium	m	5.7 0.18 (5)	4.4 0.11 (4)	6.9 0.21 (15)
mEq/l	f	6.1 0.13 (5)	4.6 0.09 (10)	8.0 0.83 (15)
Phosphate	m	6.2 0.68 (4)	6.0 0.53 (4)	8.6 0.37 (15)
mEq/l	f	6.2 0.14 (5)	5.7 0.17 (10)	7.7 0.42 (15)
Sodium	m	–	145 1.6 (4)	167 1.1 (15)
mEq/l	f	–	146 1.4 (10)	163[a] 1.5 (15)
Potassium	m	–	3.8 0.18 (4)	3.2 0.27 (15)
mEq/l	f	–	4.3 0.17 (10)	3.0 0.30 (15)
Magnesium	m	–	–	2.02 0.06 (11)
mEq/l	f	–	–	1.79[b] 0.03 (15)
Calcitonin	m	814 169 (5)	292 239 (4)	1,070 294 (13)
pg/ml	f	1195 394 (5)	272 158 (10)	12,081[c] 1305 (15)

t-test probability male vs. female a. $p < 0.05$ b. $p < 0.010$ c. $p < 0.001$

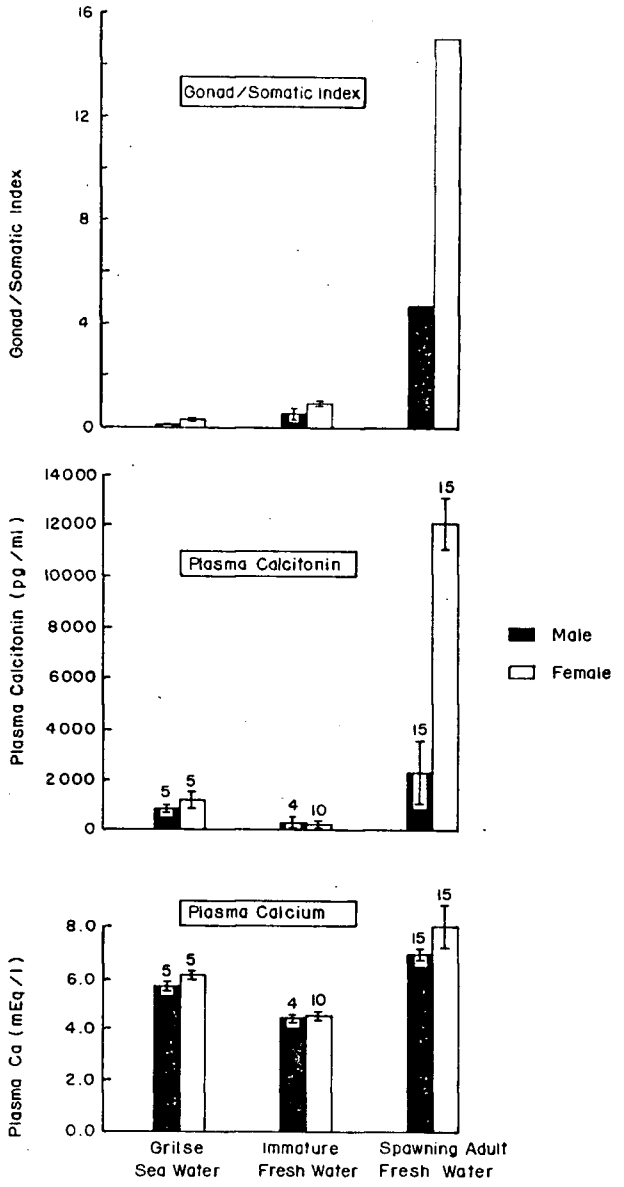

Figure 32. Plasma calcium, plasma calcitonin and gonad-somatic index measurements in 3 groups of coho salmon.

C. Plasma Calcitonin Levels in Spawning Adult Sockeye, Coho and Chinook Salmon

This section summarizes the plasma calcitonin levels for 3 species of Pacific salmon, sockeye, coho and chinook. It should be noted that the sockeye are the Chilko spawning sockeye salmon (0% spawning females, spawning males) from the 1971 migration study (Chapter IV, Section A). The coho are the freshwater spawning adults described in Section B of the present chapter. A description of the chinook salmon was given in Chapter I. Physical measurements, plasma calcitonin and electrolyte levels for the 3 groups are presented in Table XXXIII, pg. 172. The GSI indicates that all 3 species were very sexually mature.

Plasma calcitonin levels for the 3 salmon groups are illustrated in Figure 33, pg. 173. The coho and chinook levels were significantly higher (males $p < 0.005$ and females $p < 0.001$) than those measured for the sockeye.

Table XXXIV, pg. 174, summarizes the individual fish weights, ultimobranchial gland calcitonin content and plasma calcitonin level in coho and chinook salmon. Plasma calcitonin is presented in picograms per ml plasma and in mU per ml plasma (assuming a biological activity of 5000 U per mg for pure salmon calcitonin). It can be seen from the table that although the coho and chinook females had significantly higher plasma CT levels than the males, the UB gland calcitonin content did not reflect this sex difference. There was no significant difference between the UB gland calcitonin contents of the coho and chinook males or females.

Table XXXIII. Physical Measurements, Plasma Calcitonin and Electrolyte Levels in Adult Spawning CHINOOK, COHO AND SOCKEYE SALMON

Species	Sex	Weight Kg Mean ± SE(n)	Gonad/Somatic Index Mean ± SE(n)	Plasma % H_2O g/100g Mean ± SE(n)	Plasma Calcitonin pg/ml Mean ± SE(n)	Plasma Electrolytes mEq/l							
						Calcium Mean ± SE(n)		Phosphate Mean ± SE(n)		Sodium Mean ± SE(n)		Potassium Mean ± SE(n)	
Chinook	m	7.85	0.33(15)	4.6	0.44(15)	93.3	0.26(15)	2067	600(15)	5.3	0.17(15)	5.9	0.31(15)	161	1.3(15)	1.1	0.29(15)
	f	8.83[a]	0.33(14)	25.1[c]	0.87(13)	93.5	0.29(14)	13154[c]	2362(14)	5.8[a]	0.17(14)	6.9[a]	0.21(14)	166[a]	1.1(14)	1.2	0.29(14)
Coho	m	5.17	0.23(15)	4.7	0.00(12)	92.1	0.2(15)	1070	294(13)	6.9	0.21(15)	8.6	0.37(15)	167	1.1(15)	3.2	0.27(15)
	f	4.36[a]	0.22(15)	*15.0[c]	- (15)	92.1	0.4(15)	12081[c]	1305(15)	8.0	0.83(15)	7.7	0.42(15)	163[a]	1.5(15)	3.0	0.30(15)
Sockeye	m	2.78	0.13(14)	2.6	0.19(14)	95.5	0.34(15)	141	29(14)	4.1	0.24(15)	5.8	0.29(15)	140	2.9(15)	1.2	0.38(15)
	f	2.18[c]	0.08(12)	14.8[c]	0.31(12)	94.3	0.55(12)	1649[c]	240(12)	7.3[b]	0.84(12)	6.7	0.52(12)	133	6.1(12)	0.9	0.26(12)

t-test probability male vs. female a. $p < 0.05$ b. $p < 0.01$ c. $p < 0.001$

*estimate

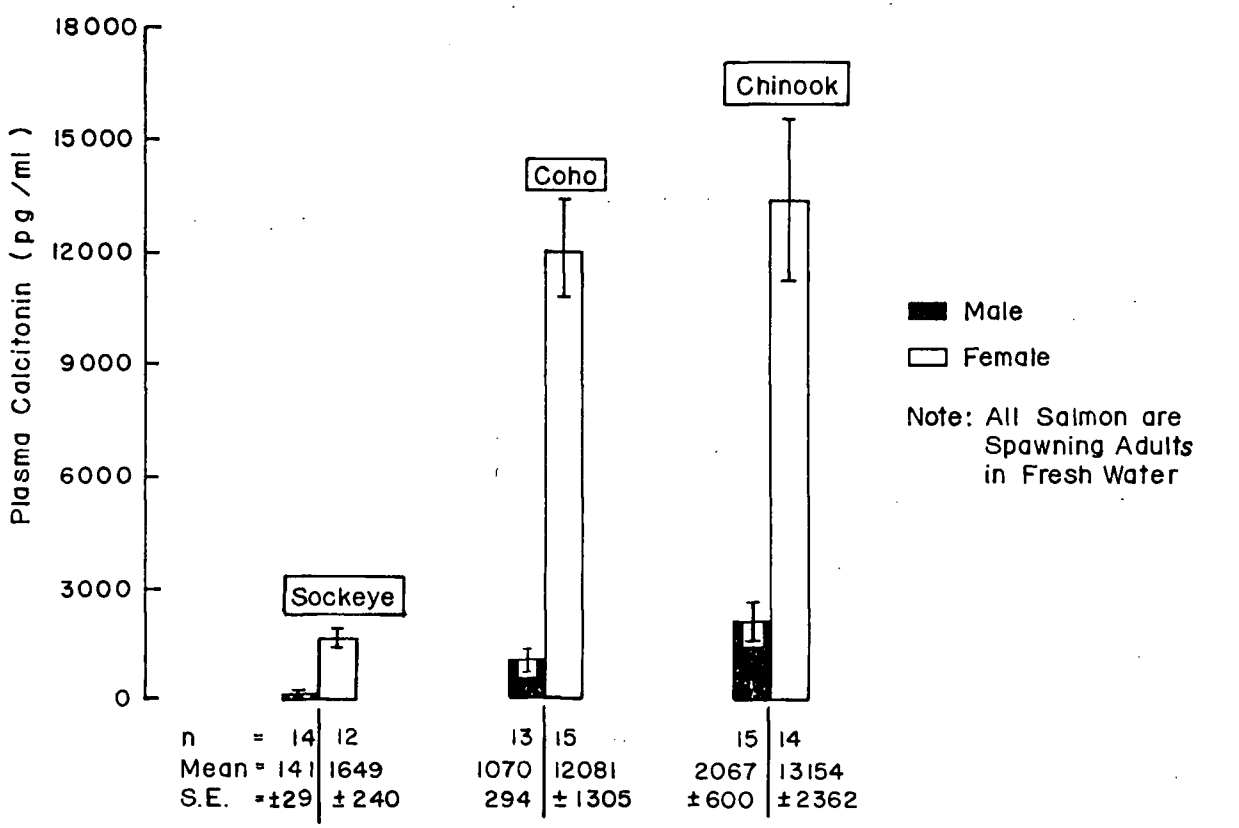

Figure 33. Plasma calcitonin levels in 3 species of salmon. Note higher female plasma CT level in each species.

Table XXXIV. Plasma and Ultimobranchial Gland Calcitonin Concentrations in Coho and Chinook Salmon

Species	Sex	Fish #	Total Wt Kg	UB Gland Calcitonin Content MRC U/gland	Plasma Calcitonin Level pg/ml	Plasma Calcitonin Level mU/ml*
Coho	m	19	4.32	112.9	2,575	12.88
		20	5.68	191.1	292	1.46
		21	5.46	557.1	180	0.90
		22	5.00	517.3	739	3.70
		24	5.46	313.2	0	0
		26	4.55	375.7	-	-
		28	4.55	153.2	880	4.40
	n =		7	7	6	6
	mean =		5.00	317.21	778[b]	3.89[b]
	SD =		0.50	162.82	860	4.30
	SE =		0.20	66.47	385	1.92
	f	1	4.55	594.0	16,800	84.00
		2	4.09	84.8	5,575	27.88
		5	4.09	66.7	8,725	43.63
		6	4.32	181.6	13,650	68.25
		8	5.46	494.7	11,150	55.75
		11	5.00	106.2	13,150	65.75
		12	3.86	173.8	7,375	36.88
	n =		7	7	7	7
	mean =		4.48	243.11	10,918	54.59
	SD =		0.53	196.37	3,645	18.23
	SE =		0.21	80.17	1,488	7.44
Chinook	m	2	7.73	233.7	1,166	5.83
		5	10.68	202.8	1,433	7.17
		6	8.18	166.5	2,816	14.08
		7	6.59	64.1	3,033	15.17
		8	6.91	192.6	1,166	5.83
		9	9.00	442.3	1,433	7.17
		10	9.77	417.6	1,475	7.38
		11	7.91	208.1	1,350	6.75
	n =		8	8	8	8
	Mean =		8.35	240.96	1,734[b]	8.67[a]
	SD =		1.31	119.10	698	3.49
	SE =		0.49	45.01	264	1.32
	f	18	8.55	277.6	4,333	21.67
		20	8.82	556.1	5,800	29.00
		21	9.09	258.9	36,333	181.67
		23	6.77	1209.9	18,166	90.83
		24	7.14	108.9	10,166	50.83
	n =		5	5	5	5
	mean =		8.07	482.28	14,960	74.80
	SD =		0.94	391.42	11,721	58.61
	SE =		0.47	195.71	5,860	29.30

t-test probability male vs. female a. $p < 0.025$ b. $p < 0.001$

*Plasma CT biological activity based on salmon CT specific biological activity of 5000 MRC U/mg.

Discussion

A. Chilko Sockeye Migration

Plasma

The decrease in plasma electrolytes which occurred throughout the Chilko migration may partially be explained by hemodilution (note the increase in plasma percent water).

Relatively high doses of cortisol, corticosterone, aldosterone, deoxycorticosterone and cortisone cause decreases in plasma sodium levels in freshwater fish (Henderson et al, 1970). Therefore, it is possible that the changes in plasma sodium throughout the migration might be related to the high adrenocorticosteroid levels found in both male and female salmon during and after spawning and death (Idler et al, 1959; Hane and Robertson, 1959; Robertson et al, 1961; Schmidt and Idler, 1962; Fagerlund, 1967; Henderson et al, 1970).

The fall in plasma sodium concentrations in the sockeye is consistent with a study done by Greene (1904), who found that the freezing point depression of chinook serum rose from -0.762°C in the sea to -0.612°C at spawning. Fontaine and Koch (1950) reported the freezing point depression of the serum of Atlantic salmon rose during the freshwater migration from -0.75°C to -0.66°C at spawning.

The very low plasma potassiums in the Chilko sockeye have also been found in chinook salmon (Urist and van de Putte, 1967)

and may be related to starvation. Further evidence of the importance of starvation in explaining the electrolyte changes in migrating salmon comes from Love et al (1968). They demonstrated a fall in plasma sodium, potassium and muscle potassium in starved immature cod, Gadus morhua, while muscle sodium rose. On feeding, this trend was reversed.

Plasma potassium, which rose in the freshwater arrival Chilko sockeye, may not reflect the increased cortisol and corticosterone levels at spawning since high doses of corticosteroids cause the plasma potassium level of freshwater fish to fall or remain constant (Henderson et al, 1970).

It is possible that the decline in plasma sodium levels is related to a decreased release of prolactin from the pituitary since some evidence has shown that this gland degenerates in spawning salmon (Robertson and Wexler, 1957, 1960). Subsequent work (van Overbeeke and McBride, 1967) has revealed that the degenerative changes in the sockeye pituitary, with sexual maturation and spawning, are only moderate. McKeown and van Overbeeke (1969), also working on the Chilko race of sockeye, detected no change in the granulation of the prolactin cells during the migration or subsequent spawning, whereas the granule density of the ACTH cells gradually increased throughout the latter part of the migration.

It has been reported that during their spawning migration in freshwater, flesh sodium concentration and water content of the sockeye salmon increases, while the flesh potassium concentration decreases (Vinogradov, 1953; MacLeod et al, 1958; Tomlinson et al, 1967). These results agree with our observations on plasma

sodium, potassium and percent water during the sockeye salmon migration. The higher flesh water content and plasma water in the males may be related to the higher level of 11-ketotestosterone found in spawning male sockeye (Idler et al, 1961b).

Another factor contributing to the decrease in plasma electrolytes in the migrating salmon has been reported by Miles (1971). Using migrating coho salmon, he measured an increase in the glomerular filtration rate from 1.48 ml/(kg x hr) in seawater to 9.06 ml/(kg x hr) in freshwater. Urine flow increased from 0.406 ml/(kg x hr) in the seawater to 4.65 ml/(kg x hr) in freshwater. Urinary excretion rates for sodium, potassium and calcium also increased from the sea to freshwater. The excretion of electrolytes, therefore, increases in freshwater despite the fact that there is no replacement of these ions from the diet.

There does not appear to be any consistent relationship between the female plasma calcitonin and electrolyte changes. Female plasma CT levels increased from sea to spawning, falling off after the eggs were shed, whereas the plasma calcium, sodium and phosphate levels declined steadily throughout the migration. Likewise, the male plasma calcitonin changes did not correlate with these electrolyte changes.

The decrease in haematocrit observed during the sockeye migration may reflect a decreased production of red blood cells due to the degeneration of the hemopoetic tissues (Robertson and Wexler, 1960). A fall in haemotocrit has also been noted in starving fish (Love, 1970). The decrease observed in the present study may be exaggerated since the gonads are developing rapidly

while the fish abstains from food.

The fall in plasma protein concentration during the sockeye migration has been reported by other workers (Jonas and MacLeod, 1960; Robertson et al, 1961; Qureshi et al, 1971). An explanation may derive from the fishes starvation, the uptake of protein into the developing gonads and/or a decreased plasma protein production due to the degeneration of the liver in the spawning salmon (Robertson and Wexler, 1960; McBride et al, 1965; Love, 1970). The plasma and muscle protein depletion in the migrating salmon may be enhanced by protein catabolism caused by the excess glucocorticoids (Robertson et al, 1961). The dramatic decline in plasma protein in both male and female sockeye appears greater than might be accounted for by blood dilution. Higher plasma protein levels in the female sockeye are likely produced by the liver under the action of estrogen in the sexually maturing female fish (Bailey, 1957; Urist and Schjiede, 1961; Ho and Vanstone, 1961; Phillips et al, 1964; Holmes and Donaldson, 1969; Love, 1970; McBride and van Overbeeke, 1971; Takashima et al, 1972).

The higher total plasma calcium found in the female salmon as compared to the male has been reported by other researchers (van Someren, 1937; Idler and Tsuyuki, 1958; Ho and Vanstone, 1961) and may be explained by the action of the female sex hormones. The decrease in total plasma calcium during the freshwater migration was also noticed in the sockeye salmon by Idler and Tsuyuki (1958) and in the Atlantic salmon by Fontaine et al (1969). These last authors noted that the fall in plasma calcium (25 - 35%) of the adult salmon (both sexes combined) during their freshwater

migration could not be accounted for solely by hemodilution. They speculated that the drop in plasma calcium could be caused by calcitonin since the UB gland appeared to be particularly active (histologically) in the spawning salmon.

Lopez (1969) has suggested that the corpuscles of Stannius may also be involved in lowering plasma calcium since these glands are very active in spawning Atlantic salmon, especially males.

The particularly steep decline in plasma calcium after spawning has also been observed by Woodhead and Woodhead (1965) in the female cod. A general drop in the electrolytes of the post-spawned salmon has been previously reported (Hoar, 1957b) Parry, 1961; Love, 1970). At least some of these electrolytes are used in the production of celomic fluid, which appears rather suddenly just at the time of spawning (Greene, 1904). The electrolyte composition (mEq/litre) of the celomic fluid in the Atlantic salmon has been measured as Na 151, K 3.2, Ca 7.1, Mg 2.6, Cl 116, HPO_4 4.0 and HCO_3 13.4 (Hayes et al, 1946).

It is interesting to note that whereas a significant decrease in total serum calcium occurred from seawater to the spawning condition, the ionic calcium remained quite constant. This undoubtedly reflects the physiological importance of the calcium ion in muscle contraction, nerve conduction, membrane permeability, etc. (Copp, 1972). The decline in serum ionic calcium in both spawning sexes may be partially explained by the rise in serum pH since the binding of calcium to serum proteins increases with increasing pH (Moore, 1969). It has been demonstrated that a rise in pH of 0.1 units is accompanied by a corresponding decrease in

ionized calcium of 0.1 mEq/l (Moore, 1969; Seamonds et al, 1972). In the present study, the decrease in ionic calcium level from freshwater arrival to spawning was slightly greater than could be accounted for by the accompanying increase in serum pH. The breakdown in control of ionic calcium in the spawned salmon is not surprising in view of the fact that the fish die within 10 days of spawning.

Moore (1969) measured the mean serum ionic calcium level of 18 normal human subjects. He obtained a measurement of 2.33 $\pm$ 0.006 mEq/l (temp. 25^{o}C; pH 7.42 $\pm$ 0.005). This is lower than most of the serum ionic calcium readings in the present study (range sockeye serum ionic calcium = 2.45 - 3.17 mEq/l) except for a few of the spawned out female sockeye. The total serum calciums of both male and female seawater and freshwater arrival sockeye (Table XXIV, pg. 144) are also higher (range 5.65 - 12.73 mEq/l) than the human level of 5.08 mEq/l reported by Moore.

The partition of calcium in the body fluids is influenced by many physical and chemical factors including body temperature, pH, concentration of serum protein, concentration of citrate and other organic complexes, ionic strength of the solution, plasma water and other conditions (Urist, 1963; Chan and Chester Jones, 1968; Moore, 1969). A comprehensive study of the factors involved would be necessary to clearly understand the serum ionic calcium changes in the migrating salmon.

Chan (1972) reported the normal plasma ionic calcium of freshwater Anguilla japonica to be 3.24 $\pm$ 0.24 mEq/l (using the specific calcium ion electrode). The normal plasma ionic calcium

level (Murexide method) of yellow and silver freshwater Anguilla anguilla was 2.76 ± 0.02 mEq/l (Chan and Chester Jones, 1968).

The fact that plasma calcitonin changes follow different patterns in the male and female throughout the sockeye migration, makes it unlikely that calcitonin is involved in the osmoregulatory adaptation changes from seawater to freshwater. In the female, variations in plasma CT are closely related to GSI changes and hence may be involved in the sexual maturation process. The increase in the female plasma CT level from seawater to 0% spawning is remarkable in view of the fact that it occurs concurrently with a significant hemodilution and decreasing haematocrit. The cause of the increase in plasma CT levels in the female may be related to a rise in production of calcitonin by the ultimobranchial gland (Table XXI, pg. 137) and/or to an increased secretion of calcitonin throughout the migration.

The increase in plasma CT levels observed in the female sockeye salmon is supported by the recent work of Deville and Lopez (1970). These authors reported an increased histological activity of the ultimobranchial gland of the Atlantic salmon, Salmo salar L., during migration and sexual maturation. At spawning, the ultimobranchial cell cytoplasm which was formerly packed with small, PAS positive granules, became clear and the UB gland hypertrophied. In the post-spawned salmon, the ultimobranchial gland underwent complete degeneration. Although these authors do not mention any sex difference in their observations, the UB gland histological alterations described could help to explain the plasma calcitonin changes in the female sockeye salmon in the present

study. Again, these authors speculate that the decrease in plasma calcium could be attributed to calcitonin secretion. However, this does not explain our results in the spawning female sockeye where the steep fall in plasma calcium is accompanied by a dramatic drop in the plasma CT level. Deville and Lopez (1970) also suggested that the increased calcitonin secretion in the maturing salmon could play a major role in inhibiting bone resorption in the breeding growth of the salmon skull.

Pang (1971b)has also reported that killifish UB glands were more active in freshwater than seawater fish. Results from Chapter I also indicated that the UB calcitonin content of seawater rainbow trout was slightly lower than that of freshwater trout.

The situation in the female sockeye appears to be somewhat analogous to Barlet's (1969) findings in milk cows. This author found that the hypocalcemia and hypophosphatemia occurring at calving and in milk fever, were associated with a significant rise of a "calcitonin-like" factor in the plasma.

The rise in the plasma CT level exhibited by the sexually maturing female salmon is similar to that shown by free plasma estrogens in the female channel catfish, Ictalurus punctatus (Eleftheriou et al, 1966). Cédard et al (1961) have reported a 6-fold increase in total estrogens in the blood of the spawning Atlantic salmon to a maximum of 7 micrograms/100 ml blood. The estrogen level returned to normal after spawning (the Atlantic salmon does not always die after spawning).

The fact that the female sockeye maintained significantly higher plasma calcitonin levels than the males at all stages of

the migration is striking. A sex difference in the plasma CT levels of fish has not been previously reported. Deftos et al (1972b)found plasma calcitonin levels in cows (165 pg/ml) to be significantly ($p < 0.05$) lower than those in bulls (303 pg/ml) even though total plasma calcium levels were not significantly different. Kenny et al (1972) have shown that male Japanese quail, 2 - 4 months of age, had significantly higher plasma CT levels than the females. In this case, the female total plasma calciums were significantly higher than those of the male. Thus, with regard to sex differences in plasma calcitonin levels, the salmon appears to be unique among the vertebrates.

The difference in plasma calcitonin levels between the sexes is clearly not related to serum ionic calcium levels since this parameter showed no sex difference throughout the migration. The only electrolyte that demonstrated a consistent sex difference was total plasma calcium, the females maintaining significantly higher levels than the males except for the spawned out females. This difference was not nearly so evident in the female and male spawning adult chinook and coho salmon (Chapter IV, Section C) although the females had slightly higher total plasma calciums than the males.

While there is a sex difference in plasma calcitonin levels in the maturing salmon, this does not hold for the sex steroids. Indeed, Cédard et al (1961) have shown that spawning Atlantic male salmon have slightly higher total estrogen levels than the female (not significantly different). Schmidt and Idler (1962) reported that the plasma of both male and female Chilko sockeye captured

immediately before spawning, contained high levels of testosterone and 11-ketotestosterone. Testosterone was predominant in the female plasma whereas 11-ketotestosterone was more abundant in the male. Both male and female plasma levels of these steroids decreased after spawning (Schmidt and Idler, 1962). In summary, female sockeye salmon plasma concentrations of testosterone, cortisol, corticosterone (in mature and post-spawned females) and calcitonin, are higher than in males at all stages of sexual maturation.

The plasma calcitonin, plasma electrolyte and tissue electrolyte (especially Na, K and Ca) changes in the migrating salmon may be most intimately related to the histological changes in the corpuscles of Stannius (Lopez, 1969; Heyl, 1970). In this regard, Heyl (1970) has observed changes in the general architecture and cell types of the corpuscles in the migrating and post-spawned Atlantic salmon which were more closely related to the time spent in freshwater than to gonadal development. It would be informative to examine the simultaneous histological changes of the corpuscles of Stannius and the ultimobranchial gland in the migrating sockeye salmon to reveal the relationship between these two glands. The greater histological activity observed in the spawning male Atlantic salmon corpuscles of Stannius by Lopez (1969), may give some insight into the observation of the sex difference in plasma calcitonin levels.

Tissues

It is difficult to assign a role to calcitonin in calcium homeostasis in the migrating, sexually maturing salmon when so many other hormonal changes are occurring at the same time. In fact, the pituitary, thyroid, interrenal, gonads, and corpuscles of Stannius (Hoar, 1953; Robertson *et al*, 1961; Hoar, 1963, 1965a, 1965b; Woodhead and Woodhead, 1965; Lopez, 1969; Love, 1970; Heyl, 1970) all appear to be involved with fish migration and/or sexual maturation in some way. Add to this the complications of osmoregulation, starvation and death, and the picture becomes exceedingly complex. Nevertheless, an attempt has been made to determine the tissue calcium and phosphate changes in the migrating salmon and the corresponding role of calcitonin.

This tissue study was prompted by the suggestion that calcitonin may play a part in the skeletal changes of the breeding salmon, since calcitonin inhibits bone resorption in mammals *in vivo* and *in vitro*. Tchernavin (1937) has shown that the alterations in the salmon skull are complicated and involve not only an absolute increase in size of certain bones but also changes in shape. A careful study by this worker (Tchernavin, 1938a, 1938b) revealed that all the tooth-bearing bones of the jaw (dentary, maxilla, premaxilla), the palatines and the vomer grow in size during the freshwater migration whereas the bones forming the gill covers, the branchiostegals and the postorbitals, resorb. The supra-ethmoid grows longer and broader at its anterior end, but is resorbed at its posterior end.

Besides these skeletal changes, the salmon lose the teeth they had in the sea ("feeding teeth") and develop an entirely new set of large "breeding teeth" (Rushton, 1926; Tchernavin, 1937, 1938a). These new teeth in the spawning male are several times larger than those of the female but the teeth of both sexes become firmly anchored to the jaw bones in the spawning condition. The growth of the bones and breeding teeth depends on the size of the fish and is invariably greater in the male. This bone and tooth development is quite remarkable considering that it occurs during the freshwater migration when the salmon have ceased to feed and in the very short time period of a few months! In the Atlantic salmon, which survive spawning and return to the sea, the skull and jaw bones slowly revert back to their original proportions and sizes.

Since the migrating Chilko sockeye in the present study were not feeding, it was important to determine the source of supply of calcium and phosphate for the growth of the bones and teeth. From Figure 23, pg. 150 , it can be seen that there was a slight decrease in muscle calcium content from seawater to freshwater levels but the total amount of muscle calcium per fish was not large (Table XXX, pg.166). The decrease in muscle calcium content during the migration may be related to the increased ACTH and corticosteroid levels (Chan et al, 1967; Chan et al, 1969; Henderson et al, 1970). The corpuscles of Stannius and ultimobranchial gland may also be involved in this mobilization of calcium from soft tissue (Chan, 1969; Chan 1972).

Greene (1926) has shown that the chinook salmon lose 51.6% of their total muscle mass by the time spawning is completed, so

the muscle supply of calcium and certainly phosphate might be larger than expected. Chan (1972) has reported that eel muscles contain five times more calcium than tetrapod muscles and that during starvation, the eel obtains food and calcium by digesting its own muscle. In the sexually maturing salmon, fat and protein from the degenerating muscle are transported to the developing gonads (Greene, 1926; Hoar, 1957b; Idler and Tsuyuki, 1958; McBride et al, 1960; Cowey, 1965).

The skin actually increased in calcium content from sea to freshwater arrival and then decreased at spawning (skin phosphate content remained fairly constant). The skin could contribute some calcium to the growing bones but the amount of skin calcium per fish is half that found in the muscle (Table XXX, pg. 166).

The sockeye skin contained considerably more calcium (75 - 192 mg Ca/100g FFDW) than rabbit skin (50 - 85 mg Ca/100g dry wt) or the skin of dog and man (31 - 59 mg Ca/100g dry wt)(Irving, 1957).

A further consideration is that the skin of sexually maturing salmon has been shown to increase in thickness at spawning (Greene, 1926; Robertson and Wexler, 1960). It has since been demonstrated that the increased skin thickness, red coloration and increased size and number of epidermal cells can be produced in salmon by androgen injections, i.e. 11-ketotestosterone and methyltestosterone. (Idler et al, 1961b; Fagerlund and Donaldson, 1969; McBride and van Overbeeke, 1971; Yamazaki, 1972). These skin changes are important to the salmon since the skin of teleosts is generally considered permeable to water while it is only slightly or not at

all permeable to organic substances and ions (van Oosten, 1957).

The female gonad calcium and phosphate contents (mg/100g FFDW) remained fairly stable during the migration (Figures 23 and 24, pages 150 and 151). However, the average fat-free dry weight of the female gonads increased from 31.99 $\pm$ 2.96g in the sea to 102.58 $\pm$ 4.08g in the 0% spawning females. This growth would require approximately 114 mg of calcium and 614 mg of phosphate. It is possible that calcitonin, in conjunction with the female sex steroids plays a role in this development. The male gonads increased from 11.26 $\pm$ 0.88g in the sea to 15.25 $\pm$ 1.14g in fresh-water and then decreased to 7.97 $\pm$ 1.36g at spawning. Hence they would not require such large amounts of calcium and phosphate as the females.

These increases in gonad calcium and phosphate concentrations may contribute to the fall of plasma calcium and phosphate during the maturation process in the female sockeye. However, from the data it can be seen that both male and female plasma calcium and phosphate decreased approximately the same amount from sea to 0% spawning yet more of these electrolytes were being stored in the female gonad than in the male.

The changes in the phosphate content of the gonads and muscle of both sexes may reflect to some extent changes in the synthesis and storage of nucleic acids. Creelman and Tomlinson (1959) found that migrating sockeye salmon experienced major losses of RNA phosphorus from the flesh, alimentary tract and male gonad, while the gonads of both sexes gained large amounts of DNA phosphorus.

The female sockeye gonad calcium content and % ash/FFDW measured in the present study (150 - 175 mg Ca/100g FFDW; 3.92 - 4.02% ash/FFDW) are consistent with the results of Ogino and Yasuda (1962). These workers studied the unfertilized eggs of the rainbow trout, Salmo gairdneri, and found the calcium content to be 182 mg Ca/100g dry wt and the % ash/dry weight 3.66%.

The teleost scale consists of two parts: an outer calcified or bony layer with growth-ridges and an inner fibrous or lamellar layer which is partially calcified (Crichton, 1935; van Oosten, 1957; Harden-Jones, 1968; Brown and Wellings, 1969).

The phenomenon of scale resorption in migrating salmon has been reported by many workers (Hutton, 1924; Crichton, 1935; van Someren, 1937; Tchernavin, 1938b; Robertson and Wexler, 1960). Van Someren (1937) found that the resorption process particularly affects the posterior portion of the scale and may be mediated by "osteoclast-like" cells. Moss (1961), however, has reported that most teleost scales are acellular. It is the outer, calcified layer which is resorbed to a greater extent than the fibrous layer.

It was observed that resorption of the scales in the male salmon was frequently more severe than in the female. The scales of spawning salmon are also extremely difficult to remove (descaling in seawater salmon occurs frequently and easily). According to Yamazaki (1972) this phenomenon may be due to testosterone.

Van Someren (1937), in studying the Atlantic salmon, found no correlation between blood calcium level and scale re-

sorption and suggested that the latter process was not necessarily related to breeding but to starvation. He noted that the degree of resorption was proportional to the length of time spent in freshwater, i.e. time of cessation of feeding. Resorption continued after spawning and ceased only when the fish resumed normal feeding in the sea.

Results obtained from the sockeye scales are in substantial agreement with previous work on fish scales (van Someren, 1937; van Oosten, 1957; Moss and Freilich, 1963; Brown and Wellings, 1969). The seawater sockeye scales of the present study had mineral contents of 36.01% (male) and 34.01% (female). Brown and Wellings (1969) found that the mineral content of teleost scales varies from 16 to 59%. The calcium (36.22 - 32.14g Ca/100g ash) and phosphate (17.65 - 20.36g PO_4/100g ash) contents of the sockeye scales were similar to those of sockeye bone.

The present study supports the observation that the male salmon scales resorb to a greater degree than the female scales since the decrease in mineral content was slightly more marked in the males (Figure 28, pg. 158). Besides losing mineral, the calcium content (gCa/100g ash) of the scales of both sexes declined quite sharply (Figure 25, pg. 154). Associated with the decline was a significant rise in phosphate content (g PO_4/100g ash). This may reflect the fact that there are 2 pools of phosphate in the scales, organic and inorganic.

Foerster and Reeve (in Van Someren, 1937), suggested that the calcium resorbed from the scales in maturing salmon is utilized by the growing bones and teeth. These authors believe

that the greater development of secondary sex characteristics in the male (jaw bones, teeth, hump) cause the more extensive scale resorption in the male since an increased supply of Ca and PO_4 would be necessary for these developing tissues. This theory, although attractive, can provide only a partial explanation. As previously calculated (Table XXX, pg.166), the scales do not contain large amounts of calcium and phosphate since their absolute weight per fish is very small. It is quite likely that the Ca and PO_4 lost from the scales remain in the salmon, since the scales are covered by a thin layer of epidermis even during resorption. The calcium could possibly be transported to the skin calcium reservoir and from there contribute to bone and gonad growth or calcium homeostasis. The calcium might also remain in the skin and function to decrease water permeability. This process would account for the fact that the skin Ca and PO_4 content increased from sea to freshwater arrival, the time at which a major decline in scale mineral content occurred. In this connection it has been shown in growing speckled trout, Salvelinus fontinalis, that the calcium content of the skin increases as the scales become larger (Phillips et al, 1953).

The vertebrae, in contrast to the scales, showed a significant increase in mineral content throughout the migration (Figure 27, pg. 157). The vertebral calcium content remained stable in the females and increased in the males (Figure 25, pg.154), while the vertebrae phosphate content decreased in both sexes (Figure 26, pg.155). Thus, it is clear that the vertebrae bones are not supplying Ca and PO_4 to the growing tissues since they,

themselves, are mineralizing.

The data in Table XXXV, pg. 192, were based on actual weights of the growing tissues plus results from the migration study, in order to determine the approximate calcium utilization in the growing tissues.

Table XXXV. Calcium Utilized by Growing Tissues in Maturing Sockeye Salmon

	Calcium (mg)	
Tissue	male	female
Teeth	183	114
Premaxillae	66	18
Vertebrae	132	170
Jaw bones	600	200
Gonads	-	114
Total	981	616

Some of the calcium required by the developing tissues could be supplied by the scales, muscle and skin. As mentioned previously, Tchernavin has shown that while there is growth of the teeth, jaw bones, palatines and vomer, resorption occurs in the gill-covers, branchiostegals, and postorbitals. Tchernavin (1938b) also suggested that the resorbing bones could supply the calcium and phosphate for those bones which were growing. This statement has some basis in fact, since in the freshwater arrival male and female sockeye, the total dry weight of the bones which resorb

weighed almost twice as much as the bones which grow. In any case the skeleton, not including the growing bones, could probably supply much of the needed calcium and phosphate. The chicken can mobilize 10 percent of its bone in one day (Simkiss, 1961; Taylor, 1970). If the sockeye salmon could mobilize a similar portion of its skeletal calcium store (Table XXX, pg. 166), it would be able to supply most of the mineral required by the developing tissues.

Other workers have shown in fish that the muscle (Chan et al, 1967 ; Chan, 1972) and skin (van Oosten, 1957; Podoliak and Holden, 1965; Fleming, 1967) constitute important storage compartments for exchangeable calcium. However, Simmons (1971) has pointed out that the skin does not appear to be a major reservoir for calcium in marine fish. Starvation in goldfish and carp has been reported to be associated with scale resorption (Ichikawa, 1953; Yamada, 1956, 1961). Some of the calcium needed for the growing sockeye tissues could possibly be supplied from the environmental water by absorption through the gills, fins and oral epithelia (see Simmons, 1971 for references). Using Ca^{45}, it has been established that calcium ion transport across the gills is more efficient in freshwater than in seawater fish and the major repositories for this absorbed calcium are the bone and skin. Tracer studies, using Ca^{45} and P^{32}, would reveal the extent to which these ions are absorbed from the water by the sexually maturing salmon.

Since calcitonin inhibits bone resorption in mammals,it is curious that the mature male salmon, which showed the more extensive scale

resorption and bone growth, exhibited lower plasma calcitonin levels than the females. Other endocrine glands, such as the pituitary, thyroid, gonads, corpuscles of Stannius and possibly the adrenal cortex are also likely involved in the skeletal changes in the maturing salmon (Davidson, 1935; Gardner and Pfeiffer, 1943; Hoar, 1957a;Robertson and Wexler, 1962; Love, 1970; Lopez, 1970a, 1970b; Simmons, 1971; Chan, 1972).

B. Plasma Calcitonin Levels in Coho Salmon: Effect of Sexual Maturation and Environmental Salinity.

As was found with the Chilko sockeye, the highest plasma calcitonin levels were exhibited by the spawning coho females. A sex difference in plasma calcitonin levels (female plasma CT was higher than the male) was clearly evident only in the spawning adults, again indicating a relationship between calcitonin and sexual maturation. The finding of high plasma calcitonin levels in sexually ripe coho "jacks" would appear to indicate that plasma CT is also slightly elevated during male sexual maturation.

In contrast to the sockeye, the high coho plasma calcitonin levels were associated with high plasma calcium levels. The very low plasma CT levels found in the freshwater immature coho may partially reflect the high plasma percent water and low haematocrits found in this group. High plasma calcitonin levels in the spawning adult coho do not appear to be correlated with any of the plasma electrolytes measured.

C. Plasma Calcitonin Levels in Spawning Adult Sockeye, Coho, and Chinook Salmon.

Table XXXIII, pg.172 , shows that in all 3 species of salmon, the female plasma calcitonin levels were significantly ($p < 0.001$) higher than those of the male. Since the UB gland calcitonin contents of the coho and chinook showed no sex difference, the high circulating plasma CT level in the females may be explained by differences in secretory and/or clearance rates of calcitonin in the male and female salmon. The females also had significantly higher total plasma calciums in the chinook ($p < 0.05$) and sockeye ($p < 0.01$).

The higher plasma calcitonin levels found in the chinook and coho compared to the sockeye, could be due to many factors. Since these fish represent 3 separate species under the same genus, Oncorhynchus, there are many morphological, physiological and biochemical variations among them. The different plasma calcitonin levels may reflect different ages, growth rates, size or distances travelled during the freshwater migration. The sockeye travelled approximately 500 miles in freshwater compared with less than 10 miles in the other species. The low plasma electrolyte and calcitonin levels found in the sockeye may partially be explained by the high plasma percent water found in this group. The high plasma water was probably related to the fact that the sockeye had spent more time in freshwater than the other 2 groups.

The female sockeye (Table XXI, pg.137) had much lower UB gland calcitonin concentrations than the female chinook or coho salmon (Table XXXIV, pg.174), and this would help to account for

the lower plasma CT levels found in the sockeye.

Thus, the fact that high female plasma calcitonin levels have been measured in 3 species of spawning salmon suggests that calcitonin is related to sexual maturation, at least in the female. The plasma CT level in the spawning female salmon is much higher than that reported for any mammal, except for some patients with medullary thyroid carcinoma (Deftos and Potts, 1970; Deftos et al, 1972a) and comparable or higher than concentrations found in birds and fish (Copp et al, 1972b; Kenny et al, 1972). Tashjian et al (1972) also using the radioimmunoassay, confirmed these observations that fish have higher circulating plasma calcitonin levels than most mammalian species. He found that coho salmon (age 1 - 3 years) adapted to freshwater, and fed a commercial diet, had higher plasma CT levels than unfed coho adapted to saltwater for 2 - 4 months. The data also suggested that freshwater 2 year old female coho (GSI not measured) had higher plasma CT levels than males of the same age.

In summary, the role of calcitonin in calcium homeostasis in fish can be expected to be unique among the vertebrates, since they lack parathyroid glands and can obtain calcium from their environment. Results from the Chilko sockeye migration suggest that fish are different from mammals with respect to calcitonin secretion. In the sheep and pig, hypercalcaemia causes the release of calcitonin (Copp, 1970b; Cooper et al, 1971), whereas hypocalcaemia is associated with high plasma CT levels in the 0% spawning female sockeye.

V. EFFECT OF ESTROGEN ON SERUM IONIC CALCIUM IN TROUT AND GONADECTOMY AND ESTROGEN ON PLASMA CALCITONIN AND CALCIUM IN SALMON

Introduction

Hypercalcaemia has been observed in many female teleosts during the breeding season (Hess et al, 1928; Pora, 1935, 1936; van Someren, 1937; Fontaine, 1956; Garrod and Newall, 1958; Phillips et al, 1964; Booke, 1964; Fleming et al, 1964; Urist and Van de Putte, 1967; Oguri and Takada, 1967; Woodhead, 1968; Woodhead and Plack, 1968; Fontaine et al, 1969; Urist et al, 1972). The increase in plasma calcium is associated with a rise in vitellin, a calcium-binding phosphoprotein which is produced in the liver of those lower vertebrates possessing yolky eggs (Urist and Schjeide, 1961; see Simkiss, 1961, 1967). Estrogen injection into both male and female teleosts elevates total plasma calcium (Bailey, 1957; Fleming and Meier, 1961; Urist and Schjeide, 1961; Ho and Vanstone, 1961; Clarke and Fleming, 1963; Fleming et al, 1964; Oguri and Takada, 1966, 1967; Chan and Chester Jones, 1968; Woodhead, 1969a; Urist et al, 1972). Several of these workers have noted concomitant increases in serum proteins, phosphorus, lipids and vitamins. These constituents are thought to be mobilized for the developing gonads.

The mechanism of action of estrogen in fish and the source of the mobilized calcium has not been clarified. Estrogen appears

to affect only the protein-bound fraction of calcium (Bailey, 1957; Urist and Schjeide, 1961; Chan and Chester Jones, 1968; Urist et al, 1972).

The results of experiments outlined in Chapter IV indicated a sex difference in plasma calcitonin levels in the salmon and that the plasma CT level in the female increased with sexual maturation. These plasma CT changes did not appear to be correlated with total plasma calcium or other electrolyte changes. This chapter will report the effect of gonadectomy and estrogen replacement on plasma calcitonin levels in sockeye salmon. The effect of estrogen on serum ionic and total calcium changes in immature trout was also investigated. The purpose of these experiments was to provide further insight into the inter-relationships of calcium metabolism, calcitonin and sexual maturation.

Materials and Methods

The chapter is divided into two sections. In Section A, the effects of estrogen injection on serum ionic and total calcium in sexually immature rainbow trout, Salmo gairdneri, are outlined. As was noted in Chapter IV, the female salmon exhibited higher plasma CT levels and total calcium levels than the male. It was important, therefore, to determine whether estrogen elevated ionic calcium since in mammals increased ionic calcium levels cause the release of calcitonin. Sexually immature trout were used in these experiments to minimize the influence of endogenous estrogen secretion and its effects on calcium metabolism.

Section B outlines the effects of gonadectomy and estrogen

replacement on plasma calcitonin and total calcium levels of adult sockeye.

A. Effect of Estrogen on Serum Ionic and Total Calcium in Immature Rainbow Trout

Two groups of 10 fish each were put into separate 50 gallon fibreglass tanks of running water. The fish were then acclimated to laboratory conditions for one week. They were fed trout chow pellets daily, except on the day of injection.

The control group received 0.1 ml cottonseed oil per fish. The experimental group received estradiol cypionate (estradiol-17β in the form of the cyclopentyl propionate ester; Upjohn Pharmaceutical Co.) at a dose of 0.1 mg estradiol/ 0.1 ml cottonseed oil per fish. Injections were performed intraperitoneally once per week for 6 weeks, on fish which were under light anesthetic (2-Phenoxyethanol, 1.0 ml/gal; Eastman Kodak Co.). This experiment was conducted from May 17 to June 28, 1972, during which time the water temperature in the two tanks ranged from 7.5 - 8.5^{o}C.

Blood samples were collected from the caudal vein of un-anesthetized fish within 2 minutes of capture. To ensure that the blood was well oxygenated, the gills were perfused with aerated water during the sampling procedure. Other methods of collection and analysis of samples were outlined in General Materials and Methods.

Six rib bones were dissected from each of the 20 trout for calcium and phosphate content analysis. Serum ionic calciums were measured at a water temperature of 8.5 $\pm$ 1.0^{o}C, the water temperature at the time of sacrifice.

B. Effect of Gonadectomy and Estrogen Replacement on Plasma Calcitonin and Electrolyte Levels

A group of sexually immature sockeye of the Great Central Lake race were captured during their spawning migration in June 1971. These fish were then transported to the Fisheries Research Board Technical Station, Vancouver, B.C. The methods of capture and transportation were those used by McBride et al (1963). In the laboratory, the fish were maintained on a natural photoperiod and seasonal water temperatures.

In the period from July 15 - 29th, a group of these fish were gonadectomized by Jack McBride, Fisheries Research Board of Canada, using the technique he developed (McBride et al, 1963). A second group was left intact and held under similar conditions in the laboratory.

The salmon were divided into 3 experimental groups:

(1) Intact controls (normal sockeye) - 5 males, 6 females
This group had intact gonads and were maturing normally. The fish were not fed during the experiment. In nature, these sockeye spawn from late September until the end of November (McBride et al, 1963). It should be pointed out that 4 of the normal males (fish #N8 - N11) were sockeye "jacks" but as shown by their gonad-somatic indices, they were quite sexually mature. These jacks mature in their 3rd year whereas the sockeye normally mature in their 4th or 5th year of life (Clemens and Wilby, 1961).

(2) Gonadectomized control sockeye - 1 male, 9 females

Fish in this group received 1.0 ml intramuscular injections of cottonseed oil once per week for 7 weeks. They were fed daily throughout the experimental period. Five females were sacrificed on February 9, 1972, six months following the gonadectomy operation.

(3) Gonadectomized estrogen-injected sockeye - 3 males, 3 females

These fish received intramuscular injections of estradiol cypionate (1.0 mg estradiol/ml cottonseed oil; Upjohn Pharmaceutical Co.) once a week for a period of 7 weeks. They were fed on each experimental day.

The injection experiment began October 13, 1971, 2 - 3 months following gonadectomy and the fish were sacrificed on December 1, 1971, one week following the final injection. All injection and blood sampling operations were performed on salmon lightly anesthetized in 2-Phenoxyethanol. Water temperatures throughout the injection experiment ranged from 6 - 12^{o}C.

Procedures for the collection and analysis of samples were outlined in General Materials and Methods. Plasma samples were collected for calcitonin measurement. Ribs were dissected from each salmon in the December 1, 1971 group for analysis of calcium and phosphate content.

It should be noted that approximately the same dosage of

estradiol cypionate was given to the rainbow trout in Section A and the sockeye salmon.

Results

A. Effect of Estrogen on Serum Ionic and Total Calcium in Immature Rainbow Trout

Physical parameters and plasma electrolyte measurements for the 2 groups of trout are presented in Table XXXVI, pg. 203. The total weights and fork lengths of the estrogen-treated group were slightly higher than for the controls. The 2 groups of female trout had significantly higher GSI values than the males but both sexes were very immature.

Figure 34, pg. 204, illustrates the serum ionic and total calcium and plasma inorganic phosphorus levels for the immature trout. The male and female electrolyte measurements were combined since no sex differences were observed. As can be seen from the data, estrogen injection significantly elevated plasma inorganic phosphorus ($p < 0.001$) and serum total calcium ($p < 0.001$). Despite a 9-fold increase in serum total calcium, the serum ionic calcium level did not change.

Table XXXVII, pg. 205, presents the percent ash/dry weight, and calcium and phosphate contents of the rib bones of the control and estrogen-injected trout. There was no significant difference between the 2 groups in any of the 3 parameters.

Table XXXVI. Physical Measurements, Plasma and Serum Electrolytes in Control and Estrogen-Treated Trout

Group	Sex		Total Weight g	Fork Length cm	GSI	Hct	Plasma Inorganic Phosphorus mEq/l	Serum Ionic Calcium mEq/l	Serum Total Calcium mEq/l	% Ionic Calcium
Control Cotton-seed	m	n=	6	6	6	6	5	6	6	6
		mean=	136	24.1	0.08	22	5.52	2.74	4.88	56.23
		SD=	10.57	0.38	0.06	5.93	0.82	0.17	0.43	1.97
		SE=	4.73	0.17	0.03	2.65	0.41	0.07	0.19	0.88
	f	n=	4	4	4	4	2	4	4	4
		mean=	123	24.3	0.22	24	5.90	2.65	4.73	56.26
		SD=	8.46	0.69	0.02	5.12	0.55	0.15	0.30	3.95
		SE=	4.88	0.40	0.01	2.95	0.55	0.08	0.17	2.28
Total Controls	m&f	n=	10	10	10	10	7	10	10	10
		mean=	131	24.2	0.14	23	5.63	2.70	4.82	56.24
		SD=	11.67	0.52	0.08	5.67	0.77	0.17	0.39	2.93
		SE=	3.89	0.17	0.03	1.88	0.31	0.05	0.13	0.98
Experi-mental Estrogen	m	n=	6	6	6	6	5	6	6	6
		mean=	149	24.0	0.12	24	11.68	2.85	36.63	7.90
		SD=	13.95	0.94	0.02	7.27	0.71	0.17	4.01	1.14
		SE=	6.24	0.42	0.01	3.26	0.36	0.07	1.79	0.51
	f	n=	4	4	4	4	4	4	4	4
		mean=	150	24.6	0.22	25	11.83	2.82	35.38	7.97
		SD=	2.74	0.54	0.02	2.12	0.39	0.13	1.35	0.29
		SE=	1.58	0.31	0.01	1.22	0.23	0.07	0.78	0.17
Total Estrogen	m&f	n=	10	10	10	10	9	10	10	10
		mean=	149[a]	24.2	0.16	24	11.74[b]	2.84	36.13[b]	7.92[b]
		SD	10.96	0.85	0.06	5.83	0.60	0.15	3.28	0.90
		SE	3.65	0.28	0.02	1.94	0.21	0.04	1.09	0.30

t-test probability: total controls vs. total estrogen a. $p < 0.005$ b. $p < 0.001$

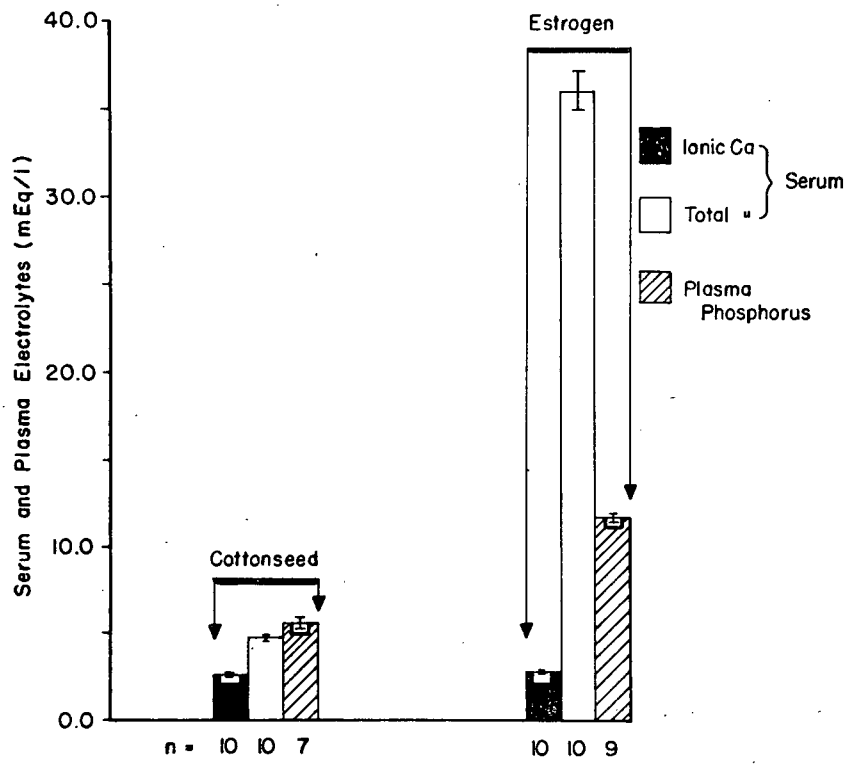

Figure 34. Serum ionic and total calcium and plasma inorganic phosphorus levels in immature trout - effect of estrogen.

Table XXXVII. Bone Measurements in Control and Estrogen-Treated Trout.

Group	n	% Ash / Dry Wt mean ± SE		g PO_4 / 100g Ash Mean ± SE		gCa / 100g Ash mean ± SE	
Control Cotton-seed	8	61.53	0.88	17.91	0.15	34.29	0.52
Experi-mental Estrogen	6	63.38	0.36	17.97	0.28	33.91	0.18

B. Effect of Gonadectomy and Estrogen Replacement on Plasma Calcitonin and Electrolyte Levels

Physical measurements, plasma calcitonin and plasma electrolyte levels for the 3 groups of salmon are summarized in Table XXXVIII, pg. 207. Plasma calcium levels are shown in Figure 35, pg. 208, and the corresponding individual plasma CT levels are illustrated in Figure 36, pg. 209.

In the intact control group, the females had significantly higher plasma calcium ($p < 0.05$) and magnesium levels ($p < 0.05$) than the males. These electrolyte levels were slightly lower in the gonadectomized controls than in the intact controls. However, the plasma calcium ($p < 0.001$), inorganic phosphorus ($p < 0.001$) and magnesium ($p < 0.001$) in the gonadectomized estrogen group were significantly elevated over corresponding levels for the gonadectomized controls (sexes combined). Plasma sodium and potassium showed little variation among the 3 groups.

The intact control sockeye exhibited the same sex difference in plasma calcitonin levels noted in the migrating Chilko sockeye salmon in Chapter IV. In the normal intact males, plasma CT levels were undetectable. Plasma CT values for the gonadectomized control and gonadectomized estrogen groups were both less than 400 pg/ml.

The percent ash/dry weight, and calcium and phosphate contents of the rib bones for the 3 groups of sockeye are presented in Table XXXIX, pg. 210. The intact control male sockeye had significantly higher percent ash/dry weight ($p<0.01$) and lower calcium ($p<0.05$) and phosphate contents ($p<0.05$) than the intact control females. The other groups displayed no large differences in any of the parameters measured.

Table XXXVIII. Physical Parameters, Plasma Calcitonin and Plasma Electrolytes of Intact Control, GX Control and GX Estrogen Sockeye

Group	Sex		Total Weight g	GSI	Hct	Plasma Calcitonin pg/ml	Plasma Electrolytes mEq/l Ca	PO_4	Mg	Na	K
Intact	m	n=	5	5	5	undetect-	5	5	5	5	5
Control		mean=	757	4.02	33	able	4.55	5.36	1.43	147	2.48
		SD	197.90	0.70	5.52		0.33	0.29	0.12	5.49	0.47
December 1,1971		SE	98.95	0.35	2.76		0.16	0.14	0.05	2.75	0.24
	f	n=	6	6	6	6	6	6	6	6	6
		mean=	1793[c]	18.46[c]	36	6603	5.78[a]	5.23	1.70[a]	150	2.02
		SD=	418.50	2.63	3.47	2075.65	1.07	0.78	0.23	2.87	0.75
		SE=	187.16	1.18	1.55	928.26	0.48	0.35	0.10	1.28	0.33
Gonadectomized	m	n=1	1475	-	27	400	4.70	4.82	1.56	153	1.90
Control											
	f	n=	4	-	4		4	4	3	3	3
December 1,1971		mean=	1594		26	400	4.77	4.46	1.57	152	2.27
		SD=	444.19		2.24		0.22	0.10	0.08	2.05	0.17
		SE=	256.45		1.29		0.13	0.05	0.05	1.45	0.12
February 9, 1971	f	n=	5	-	5		5	5	5	5	5
		mean=	1036		20	400	4.60	3.68	1.26	146	2.64
		SD=	130.13		3.72		0.40	0.19	0.30	8.17	0.15
		SE=	65.07		1.86		0.20	0.09	0.15	4.09	0.07
Gonadectomized	m	n=	3	-	3		3	3	3	3	3
Estrogen		mean=	1367		17	400	23.99	10.05	3.78	144	2.60
		SD=	455.22		3.09		2.16	0.43	0.21	1.41	0.14
December 1, 1971		SE	321.89		2.19		1.53	0.30	0.14	1.00	0.10
	f	n=	3	-	3		3	3	3	3	3
		mean=	1425		19	400	30.21	12.32	4.91[b]	141	2.37
		SD=	73.60		2.45		6.14	1.81	0.27	1.25	0.31
		SE=	52.04		1.73		4.34	1.28	0.19	0.88	0.22

t-test probability male vs. female

a. $p < 0.05$
b. $p < 0.01$
c. $p < 0.001$

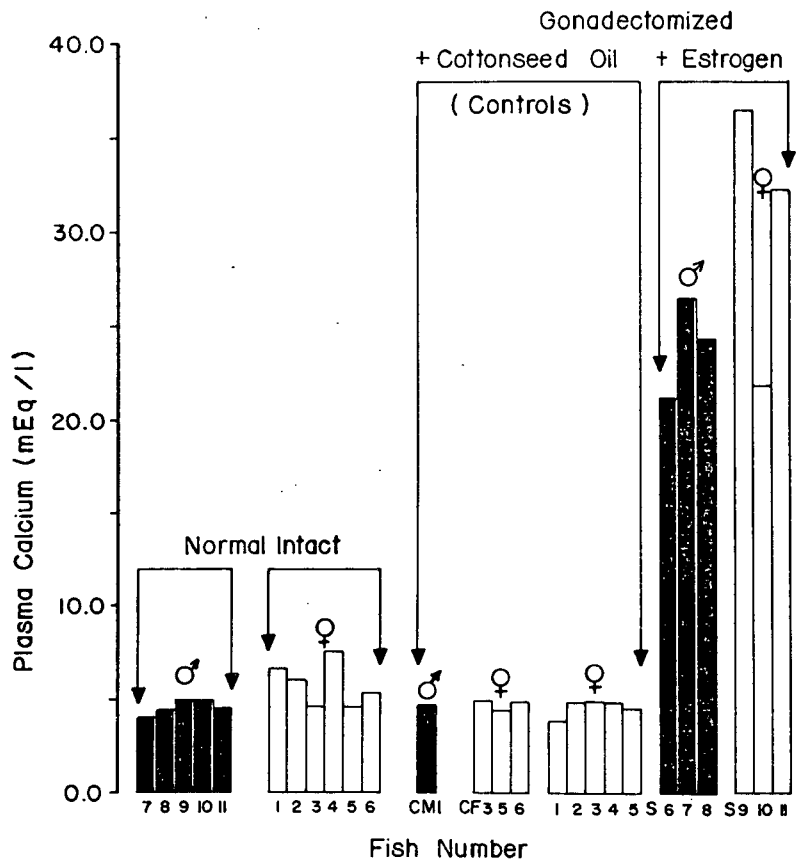

Figure 35. Total plasma calcium levels in sockeye - effect of gonadectomy and estrogen replacement.

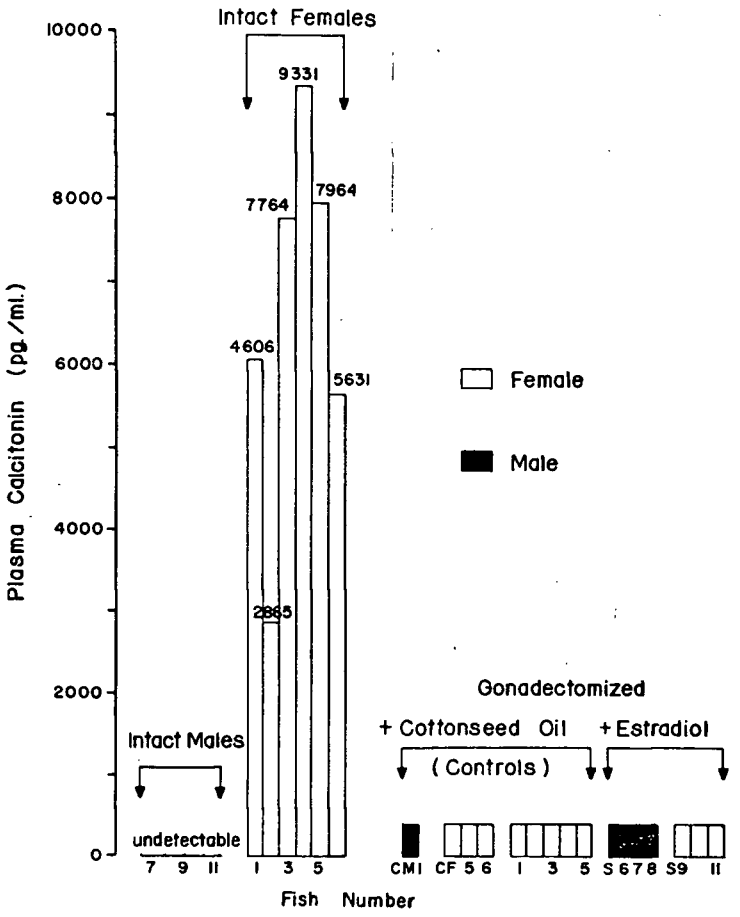

Figure 36. Plasma calcitonin levels in sockeye - effect of gonadectomy and estrogen replacement.

Table XXXIX. Bone Measurements in Intact Control, GX Control and GX Estrogen Sockeye

Group	Sex		% Ash / Dry Wt	gPO_4 / 100g Ash	gCa / 100g Ash
Intact Control	m	n =	5	5	5
		mean =	68.00	16.64	35.10
		SD =	4.57	0.87	1.70
		SE =	2.28	0.43	0.85
	f	n =	5	5	5
		mean =	59.83[b]	18.29[a]	37.97[a]
		SD =	1.09	1.02	1.39
		SE =	0.54	0.51	0.69
Gonadectomized Control	m	n =	1	1	1
			60.25	17.54	37.70
December 1, 1971	f	n =	4	4	4
		mean =	60.51	18.97	37.19
		SD =	1.09	1.61	1.04
		SE =	0.63	0.93	0.60
Gonadectomized Estrogen	m	n =	3	3	3
		mean =	60.85	17.89	37.01
		SD =	0.90	0.04	0.39
		SE =	0.64	0.03	0.27
	f	n =	3	3	3
		mean =	59.92	17.44	36.42
		SD =	1.39	0.68	1.03
		SE =	0.98	0.48	0.73

t-test probability male vs. female a. $p < 0.05$

b. $p < 0.01$

Discussion

Total calcium concentration in the serum of vertebrates is present in three distinct fractions:

1. protein-bound calcium
2. complexed calcium bound to anions such as bicarbonate, phosphate and citrate
3. ionic calcium

The complexed and ionic calcium fractions constitute the ultrafiltrable or diffusible fraction and it is the ionic calcium that is the physiologically active form of calcium in the body (see Moore, 1969, 1970; Simkiss, 1967; Chan and Chester Jones, 1968). In mammals, the ionic calcium level is precisely regulated and under the control of calcitonin and parathyroid hormone (Copp 1969c, 1970a).

The serum ionic calcium level of the estrogen injected trout in the present study remained quite constant despite a marked elevation in total calcium. These results are in close agreement with previous work on amphibians, reptiles and birds (see Simkiss, 1961, 1967). The effect of estrogen on ultrafiltrable or ionic calcium in teleosts has been investigated in only a few cases but the general pattern appears to be similar to the above oviviparous vertebrates. Bailey (1957) reported that a single intraperitoneal injection of 0.5 mg estradiol benzoate into goldfish, produced a 10-fold increase in total calcium level on the 20th day post-injection whereas the ultrafiltrable (mostly ionic) calcium level remained stable. Chan and Chester Jones (1968) showed

that the ionic calcium level of the freshwater European eel, Anquilla anquilla L., remained constant after injection of estrogen (Premarin 100 microg/100 g per day for 6 days) despite a significant ($p < 0.01$) rise in total calcium. Recently, Urist et al (1972) reported that estradiol valerate injection into male and female lungfish, L. paradoxa, resulted in a dramatic elevation of total protein and total calcium with essentially no change in inorganic phosphorus or ultrafiltrable calcium.

It is interesting to note that although estrogen injection also causes hypercalcaemia in birds (Riddle and Dotti, 1936; Urist et al, 1958; Taylor, 1970; Simkiss, 1967) amphibians (Urist and Schjeide, 1961; Simkiss, 1961, 1967) and reptiles (Dessauer and Fox, 1959; Urist and Schjeide, 1961; Clarke, 1967; Prosser III and Suzuki, 1968; Simkiss, 1961, 1967), the effect of estrogen on plasma calcium in mammals is more variable and much less conspicuous (Day and Follis, 1941; Gardner and Pfeiffer, 1943; Manunta et al, 1957; Young et al, 1968; Sorensen and Hindberg, 1971).

The serum ionic calcium levels of the trout in this thesis (range 2.39 - 3.03 mEq/l) are within the range measured in the migrating Chilko sockeye (males, 2.12 - 3.49 mEq/l; females, 1.84 - 3.83 mEq/l). These results are also similar to the values measured by Chan and Chester Jones (1968) in the European eel under various experimental conditions (plasma ionic calcium range 2.70 - 2.84 mEq/l, Murexide method). The above observations indicate that teleosts are capable of precisely regulating their ionic

calcium levels and provide further evidence to support the contention that the ionic calcium concentration or activity is one of nature's "physiological constants" (McLean and Hastings, 1935).

Estrogen injection significantly elevated the plasma inorganic phosphorus levels in the trout ($p < 0.001$) and gonadectomized sockeye ($p < 0.001$, sexes combined). Bailey (1957) also reported an increase in plasma inorganic phosphorus in goldfish on treatment with estrogen. Ho and Vanstone (1961) showed that intramuscular injections of estradiol monobenzoate (0.2 mg per day for 4 days) into sexually maturing male and female sockeye salmon, caused significant ($p < 0.01$) increases in both protein and lipid phosphorus as well as total calcium ($p < 0.01$). In contrast, Chan and Chester Jones (1968) did not observe an increase in plasma inorganic phosphorus on injection of estrogen into Anguilla anguilla L. while Urist et al (1972) observed a large increase in total phosphorus with little change in inorganic phosphorus.

The effect of estrogen on plasma magnesium levels has rarely been investigated. A significant increase in plasma magnesium was observed in the gonadectomized sockeye on treatment with estrogen ($p < 0.001$, sexes combined). Day and Follis (1941) reported a slight (not significant) rise in serum magnesium of young rats treated with estradiol benzoate. The only report on the effect of estrogen on serum magnesium in fish appears to be the work of Oguri and Takada (1967). These authors observed an increase in the serum magnesium levels of goldfish from control levels of 1.63 mEq/l (males) and 1.50 mEq/l (females) to 3.5 - 5.6 mEq/l, 8 and 9 days following a single injection of 4.7 mg and

7.5 mg of estradiol.

In the intact control group of sockeye, there was no sex difference in the plasma inorganic phosphorus, sodium or potassium levels although the female plasma calciums and magnesiums were both significantly higher ($p < 0.05$) than the males.

In comparing the plasma electrolytes of the intact control females and the gonadectomized control females, it is interesting to note that removal of the gonads, and thus removal of the source of estrogen, resulted in significant declines in plasma calcium ($p < 0.05$), inorganic phosphorus ($p < 0.01$) and magnesium ($p < 0.05$). This decline was associated with a decrease in plasma CT levels from a mean of 6603 $\pm$ 928 pg/ml to less than 400 pg/ml. It is not known whether the decrease in circulating level of plasma CT in the gonadectomized sockeye is related to a reduction in secretory rate or an increased metabolic destruction. The fact that estrogen replacement dramatically increased total plasma calciums but did not restore the plasma CT levels, indicates that the factors governing the circulating level of calcitonin may be quite complex. Results in the male sockeye are even more complicated since they indicate that the plasma calcitonin level may rise on gonadectomy. Plasma cortisol has also been shown to decrease following gonadectomy of male and female sockeye (Donaldson and Fagerlund, 1970).

Gonadectomy appears to have little effect on plasma calcium levels in rats (Rice et al, 1968; Sorensen and Hindberg, 1971). Castration effectively lowers plasma calcium levels in the toad Xenopus but the same operation in dogs produces a marked rise in serum calcium (Gardner and Pfeiffer, 1943). The only report of

the effect of gonadectomy on plasma electrolytes in fish, is that of Pickering and Dockray (1972). These authors showed that gonadectomy of freshwater female lampreys resulted in a significant increase in plasma calcium levels. No change occurred in the freshwater male lampreys.

The effect of estrogen on plasma calcium, phosphorus and magnesium levels was more marked in the female gonadectomized sockeye than in the male. This observation has been reported by other workers (Ho and Vanstone, 1961; Oguri and Takada, 1967; Woodhead, 1969a; Urist et al, 1972). Data from the present study indicate that the more marked elevation of plasma calcium due to estrogen in the females occurred even after removal of the gonads.

The hypercalcaemic effect of estrogen in fish does not appear to depend on a source of dietary calcium since most of the experiments reported in the literature were conducted on fasting fish (Bailey, 1957; Ho and Vanstone, 1961; Oguri and Takada, 1966; Chan and Chester Jones, 1968; Woodhead, 1969a). Both the trout and gonadectomized sockeye were fed but examination of the stomach contents of the gonadectomized sockeye at the time of sacrifice revealed that these fish were eating very irregularly.

A great deal of literature has been published on the effects of gonadal hormones on vertebrate bone metabolism. The majority of evidence indicates that in many species, estrogen inhibits bone resorption (Day and Follis, 1941; Gardner and Pfeiffer, 1943; Urist et al, 1948; Budy et al, 1952; Linquist et al, 1960; Lafferty et al, 1964; Young et al, 1968; Skosey, 1970; Sorensen and Hindberg, 1971). In birds (see Simkiss, 1961, 1967) and

mice (Urist et al, 1950) estrogen stimulates new bone formation. The actions of androgens on skeletal metabolism are not so well documented although in some cases, estrogens and androgens act synergistically. The effects of the sex steroids on bone metabolism depends on the species, age of the animal, the hormone(s) used, the dosage and time course and many other factors.

This investigation indicated that estrogen had no effect on the percent ash/dry weight, or the calcium and phosphate contents of the rib bones of either the immature trout or the gonadectomized sockeye. Thus, although estrogen produced a dramatic increase in total serum calcium and a rise in plasma inorganic phosphorus, the bone mineral content did not change. Fleming et al, (1964) pointed out that only a small amount of calcium need be mobilized to obtain a significant hypercalcaemia. It is possible that the methods used in this study were not sensitive enough to detect these changes in bone mineral. Calcium could also be mobilized from the soft tissues or absorbed via the gills from the environment (Fleming et al, 1964; Simmons, 1971).

Reports on the effects of estrogen on fish bone have been few and variable. Bailey (1957) observed elevated serum calcium levels in estrogen-treated or preovulatory goldfish but found no histological or X-ray evidence of bone deposition. Clark and Fleming (1963) reported that estrogen injection into mature female killifish, Fundulus kansae, elevated total serum calcium but had no detectable effect on bone histology or the bone calcium content as measured by the Von Kossa technique. Furthermore, Woodhead

1969b) has reported that intramuscular injection of estradiol 17-β, 3-benzoate (1.0 mg/kg, 4 injections on alternate days) into female lesser spotted dogfish, Scyliorhinus canicula, resulted in a significant ($p < 0.001$) increase in total plasma calcium from a control level of 10.3 ± 0.5(SD) to 11.4 ± 0.6(SD) mEq/l. This observation is relevant to the discussion since elasmobranchs possess a cartilaginous skeleton. These findings support the hypothesis that the effects of estrogen on serum and on bone are distinctly separate.

In contrast to the above results, some workers have shown that sexual maturation of the female eel, induced by hormones, was accompanied by bone deformation (Boetius et al, 1962; Fontaine et al, 1964). Moreover, Lopez and Martelly-Bagot (1971) showed that injection of carp pituitary extract into female Anguilla anguilla L. produced sexual maturation accompanied by hypercalcaemia and hyperphosphatemia. These authors also noticed a marked proliferation of osteoclasts concomitant with significantly enlarged resorption surfaces, increased osteolysis and demineralization of the intercellular substance, without histological modification of the organic matrix. Analysis of the bone by X-ray diffraction revealed that the mineral was lost from the amorphorous phase of the bone mineral rather than the crystalline apatite. These skeletal changes were thought to be caused, in part, by the gonadotrophic production of estrogens. Thus, there appears to be species differences in the response of fish bone to estrogens and this may depend on whether the bone structure is cellular or acellular.

Only two reports on the effects of androgens on calcium metabolism in fish appear in the literature. A single injection of 1.0 mg of testosterone proprionate into goldfish, had no effect on total serum calcium (Bailey, 1957). Recently, Peterson and Shehadeh (1971) observed a dramatic increase in total plasma calcium of the male and female mullet, Mugil cephalus L., following intraperitoneal injection of 25.0 mg crystalline methyltestosterone (hormone injected on alternate days for one month). Injection of 5.0 mg of partially purified salmon gonadotrophin also elevated the total plasma calcium level in the female mullet.

Indirect evidence on the effects of the sex steroids on bone development in salmon has been shown by McBride and co-workers (McBride et al, 1963; van Overbeeke and McBride, 1971; McBride and van Overbeeke, 1971). These authors have demonstrated that gonadectomy of mature salmon not only prolongs their life span beyond the time at which they normally would have spawned and died, but also leads to the arrest of the external secondary sexual characteristics such as the snout and teeth development and red skin colour. Plate 11, pg. 219, shows the 5 gonadectomized control female sockeye sacrificed February 9, 1972. Note the sea-green backs and silver sides which give these fish the appearance of sexually immature seawater salmon (Plate 7, pg. 130).

Van Overbeeke and McBride (1971) injected 2.50 mg of 11-ketotestosterone and 17α-methyltestosterone into gonadectomized sockeye twice weekly for a period of 7 weeks. These sockeye, after

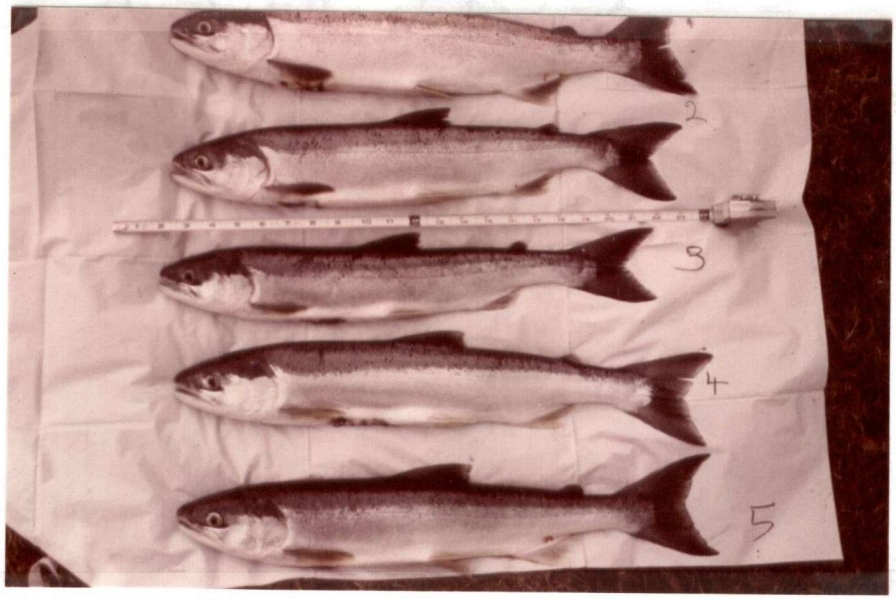

Plate 11. Gonadectomized female sockeye (Great Central race).

Note similar colouration and body shape to seawater Chilko sockeye (Plate 7).

the 7 weeks of androgen treatment, all showed the red spawning colouration as well as hooked snouts and premaxillary teeth. Thus the androgens definitely play a role in skeletal metabolism in the salmon.

In summary, in trout and salmon, estrogen appears to influence total calcium without influencing ionic calcium or hard tissue calcium content. Androgens appear to affect skeletal development but have minimal effects on plasma calcium. The role of calcitonin in the above processes remains to be elucidated. The factor(s) governing the secretory rate of calcitonin in trout and salmon are under investigation.

SUMMARY

In the General Introduction, it was stated that the objective of this thesis was to investigate calcium metabolism in fish and the possible physiological role of calcitonin in this process. A brief summary of the thesis findings appears below.

Measurement of the ultimobranchial gland calcitonin content of trout and salmon under a variety of conditions displayed great variation. The UB gland CT contents found in the present study are among the highest reported for lower vertebrates and confirm the original observation of Copp in 1967 that the fish ultimobranchial gland is a rich source of calcitonin. No consistent correlation was found between the UB gland CT contents and plasma calcium or phosphate, sex, sexual maturation, smolting, changes in environmental calcium levels or species differences. The lower concentrations of calcitonin in fingerling trout may indicate a relationship between calcitonin and growth.

The biological half-life for salmon calcitonin (SCT) was measured in cannulated trout and salmon. The half-life of SCT was 27.6 minutes in trout and 48.0 minutes in salmon. This is a rather slow disappearance compared to the half-life of SCT in mammals.

Salmon calcitonin injection had no effect on plasma calcium levels in fingerling or adult rainbow trout. SCT infusion was also ineffective in lowering plasma calcium and other electrolytes in cannulated adult female sockeye salmon. Renal excretion of calcium,

sodium and magnesium, as well as urine flow, were not significantly altered by SCT infusion into these salmon.

Results from cannulated trout and salmon indicated that these fish can regulate plasma calcium and phosphate very efficiently. Data from estrogen-injected trout and migrating salmon, showed that while total plasma calcium changed dramatically, the ionic calcium level remained remarkably constant and well-controlled. Estrogen injection, in addition to causing hypercalcaemia and hyperphosphatemia, significantly elevated plasma magnesium levels in the salmon.

Evidence of the hormonal status of calcitonin in fish was obtained when calcitonin was detected in the plasma of salmon using the salmon calcitonin radioimmunoassay. As is the case in mammals, calcitonin is continuously secreted under basal conditions. The circulating level of plasma CT in salmon was higher than that found in mammals and comparable to measurements in birds and other fish.

A sex difference in the circulating plasma calcitonin levels (females higher than males) was found in three species of adult salmon. This is one of the first reports indicating a sex difference in the circulating level of plasma calcitonin. The higher circulating level of plasma CT in the female may be related to an increased secretory rate since the ultimobranchial glands of male and female salmon contained approximately the same amounts of calcitonin. The cause of the increased secretory rate is not known but it is clearly not related to ionic calcium levels.

Plasma calcitonin levels of female salmon increased significantly during migration from sea to freshwater. These plasma CT levels reached maximum values just prior to spawning, after which they fell off precipitously. Plasma CT levels in the male decreased from sea to freshwater and returned almost to their original levels at spawning. The plasma calcitonin changes during the migration do not appear to be related to any of the plasma or tissue calcium and phosphate alterations. The increase in plasma CT during sexual maturation in the migrating female sockeye and the decrease after spawning, parallels the changes seen in the gonad-somatic index. The high levels of plasma CT in the female decreased following removal of the gonads. Estrogen replacement did not restore the plasma calcitonin levels in the gonadectomized females. These observations have led to the suggestion that calcitonin may be involved in sexual maturation in some way although it is not caused by high estrogen levels. This suggestion is not new since Lewis et al (1971) have recently proposed that calcitonin may play a physiological role in pregnancy and lactation in rats by protecting the skeleton against the osteolytic action of parathyroid hormone.

Many of the questions posed in the General Introduction have been answered through the experiments outlined in this thesis. However, new directions of research in the investigation of calcium metabolism and calcitonin in fish have been revealed. These processes appear to be quite different in fish when compared to mammals, indicating that the function of calcitonin during evolution has changed.

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