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Reproductive Endocrinology of Fishes

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SYNOPSIS. While evidence is currently lacking for the agnatha and elasmobranchs, the release of pituitary gonadotropin by the teleost pituitary appears to be under stimulatory control by the hypothalamus.

Gonadotropin has to date only been purified from teleost pituitary glands. Bioassay and biochemical data suggest that the teleost pituitary gland elaborates only one gonadotropin; however, there is some conflicting histological data on this point. Salmon gonadotropin has a molecular weight of approximately 29,000 at neutral *p*H and approximately 13,000 at low *p*H or after treatment with 8M urea or 1M propionic acid.

Radioimmunossays have recently been developed for carp and salmon gonadotropin. Immunological techniques have also been used to identify pituitary gonadotrops.

Pharmacological treatment of fish with methallibure has permitted inhibition of gonadal development while treatment with clomiphene citrate has stimulated ovulation. The role of corticosteroids and other steroid hormones in ovulation is still not fully elucidated. It is possible that the control of ovulation may differ between species.

Experiments are described which aim to enhance natural stocks of pink salmon by endocrine manipulation of sexual development in the male.

INTRODUCTION

Since the publication by Pickford and Atz in 1957 of the treatise on the physiology of the pituitary gland, there have been several excellent reviews on some or all aspects of the endocrine control of reproduction in fishes. Recent reviews include those of Van Oordt (1968), Dodd and Wiebe (1968), Barr (1968), Jorgensen (1968), Lofts (1968), Hoar (1969), Fontaine (1969), Ball and Baker (1969), Liley (1969), Yamamoto (1969), Yamazaki (1969), Dodd (1972), and Reinboth (1972).

The purpose of this review is not to attempt to cover the whole field of reproductive endocrinology in fish, but rather to review certain areas. Aspects not covered include the biosynthesis and metabolism of gonadal steroids and the endocrinology of reproductive behavior.

HYPOTHALAMUS AND RELEASING FACTORS

Evidence is lacking for hypothalamic control of gonadotropin release in the cyclostomes. In both *Polistotrema* (Gorbman et al., 1963; Nishioka and Bern, 1966) and *Myxine* (Olsson, 1959; Adam, 1963), the distal capillaries of the portal system terminate in the neurohypophysis and there is no evidence for a vascular connection between the neurohypophysis and the adenohypophysis. Furthermore, pituitary transplants into the eye or pharyngeal muscle were capable of stimulating sexual development in male and female lampreys (Larsen, 1969), indicating that the pituitary is capable of autonomous release of gonadotropin or is controlled by a blood-borne factor in the systemic circulation (Gorbman et al., 1963). In a recent study Fernholm (1972) has shown that 6.4% out of 264 *Myxine glutinosa* had modified adenohypophysial tissue between the adenohypophysis and the neurohypophysis or direct adenohypophysial-neurohypophysial contact. These fish were on an average larger than the population as a whole and included a higher percentage of male or sterile intersex specimens. Despite this contact there was no evidence of a vascular connection and culture of the adenohypophysis in the absence of the hypothalamus resulted in no appreciable change in adenohypophysial

I wish to thank Helen M. Dye, Michael Flynn, and James D. Funk for assistance in various aspects of the work described in this review and Mr. George Shaw, Ayerst Laboratories, Montreal, for the methallibure used in the pink salmon study.

structure.

Dodd (1972) in his recent review of ovarian control in cyclostomes and elasmobranchs, states that in the elasmobranchs as in the cyclostomes there is no evidence as yet for hypothalamic control of gonadotropic activity.

Since the work of Polenov (1950), the neurosecretory activity of the teleostean nucleus lateralis tuberis (NLT) has been associated with seasonal changes in reproductive activity. More recently, pituitary transplant studies confirmed the stimulatory hypothalamic influence on gonadotropic hormone release in the teleosts (Roy, 1964; Ball et al., 1965; Johansen, 1967).

Recent experiments have provided direct evidence for the hypothalamic control of gonadotropin release from the teleost pituitary gland. Stereotaxically placed electrolytic lesions in the goldfish NLT pars posterior and the posterior part of the NLT pars anterior caused a significant decrease in the gonadosomatic index of both males and females (Peter, 1970). Gonadotropin releasing factor (GRF) has been isolated from the mammalian hypothalamus and found to be a peptide containing nine amino acids. This factor, which will release both LH and FSH, lacks species specificity within the mammalia (Schally and Kastin, 1972*a*,*b*). Recently a hypothalamic extract from *Cyprinus carpio* was shown to have gonadotropin releasing activity when tested in vitro (Breton et al., 1972*a*). The carp GRF preparation was obtained by homogenization of mature, spermiating carp median eminence in 0.1 N HCl. The suspension was shaken for 1 hr at 4 C, centrifuged, and the supernatant fluid heated for 1 hr at 70 C prior to recentrifugation. Incubation of an extract from one hypothalamus with one-half pituitary gland resulted in a significant increase in the release of gonadotropin into the incubation medium as measured by the radioimmunoassay for carp gonadotropin (Breton et al., 1971). The addition of GRF from two hypothalami resulted in an even greater increase in gonadotropin release. The preparation was incapable of releasing gonadotropin from incubated whole pituitary glands suggesting that the pituitary surface is impermeable to GRF.

GONADOTROPIN

Agnatha and Chondrichthyes

While no gonadotropic hormone has been purified from the agnathan pituitary gland, Strahan (1959) has claimed its presence by means of bioassay. The ventral lobe of the pituitary of the elasmobranch *Scyliorhinus canicula* has recently been shown to contain a gonadotropin which augments P^{32} uptake by the testes of the day-old chick (Scanes et al., 1972). This gonadotropic activity varies quantitatively during the reproductive cycle (Scanes et al., as quoted by Dodd, 1972). Furthermore, a cross-reaction has been obtained between the ventral lobe or plasma of *S. canicula* and antibodies to avian LH (Dobson et al., as quoted by Dodd, 1972). In the holocephali an attempt to demonstrate the presence of gonadotropin, using the chick bioassay, in the rachendach-hypophysis, which is thought to be the homologue of the elasmobranch ventral lobe, failed (Scanes et al., as quoted by Dodd, 1972).

Osteichthyes-Sarcopterygii

Gonadotropic activity has recently been detected in a saline extract of the pituitary gland of the lungfish *Protopterus* sp. (Burzawa-Gerard, 1969). In the frog spermiation assay (Fontaine and Chauvel, 1961) this preparation had an activity equivalent to 136 μg LH B5/mg. In the Steelman and Pohley (1953) assay for FSH in the rat, its activity was equivalent to 75.9 μg FSH S3/mg, while in the Parlow (1961) rat ovarian ascorbic acid depletion assay for LH no activity was detected, i.e., <1.4 μg LH B5/mg. The positive response in the Steelman and Pohley assay represents the first detection of pituitary gonadotropin of any fish using a mammalian recipient and correlates well with both the evolutionary position of the lungfish and the observation of Fontaine (1958) that an extract of the pituitary of *Protopterus annectens* possessed thyrotropic activity in a mammalian assay.

Osteichthyes-Teleostei

Bioassay. Studies on the isolation of gonadotropin from the pituitary gland of the teleosts have been under way for some time. The main stumbling block in such studies has been the development of a suitable bioassay. Otsuka (1956) working with *Oncorhynchus* (Pacific salmon) pituitary glands used immature mice as the recipient. A year later Robertson and Rinfret (1957) extracted gonadotropin from *Oncorhynchus tshawytscha* pituitary glands using a solution of acetone and acetic acid. The gonadotropin was precipitated by increasing the concentration of acetone and its biological activity was assayed by its effect on the growth rate of the immature trout testis. Other bioassays for piscine gonadotropin used at that time were the weaver finch assay (Witschi, 1955) and the frog spermiation assay (Fontaine and Chauvel, 1961). This latter assay was used in the purification of carp (*C. carpio*) gonadotropin (Fontaine and Gerard, 1963). Another group purifyng carp gonadotropin (Clemens et al., 1964) used the goldfish testicular hydration assay (Clemens and Grant, 1964). This gonadotropin was in fact referred to by Clemens and co-workers as the gonadal hydration factor. Purification of salmon gonadotropin was continued using the testicular growth response in immature trout (Schmidt et al., 1965). A disadvantage of this assay was the time required and therefore another assay was developed which was based on the spermiation response of hypophysectomized mature male goldfish (Yamazaki and Donaldson, 1968*a*). In this assay a response was obtained in only 24 hr, and human chorionic gonadotropin (HCG) could be used to standardize the assay. The goldfish spermiation assay was used for the purification of salmon (*O. tshawytscha*) gonadotropin (Donaldson and Yamazaki, 1968; Yamazaki and Donaldson, 1968*a*; Donaldson et al., 1972*a*). In 1970 we changed from the goldfish assay to the day-old chick testicular radiophosphate uptake assay (Florsheim et al., 1959; Breneman et al., 1962; Follet and Farner, 1966) which has the advantages of rapidity, sensitivity, precision, and the ability to respond to mammalian LH as well as salmon gonadotropin (Donaldson et al., 1972*a*), avian gonadotropin (Follet and Farner, 1966), reptilian gonadotropin (Channing et al., 1972), and amphibian gonadotropin (Donaldson et al., 1971). Fontaine and co-workers have continued to use the frog spermiation assay during the isolation of carp gonadotropin allowing for the seasonal variation in response to mammalian LH (Burzawa-Gerard and Fontaine, 1965). The activity of salmon gonadotropin has also been assayed against mammalian LH and FSH using testicular growth in the hypophysectomized lizard *Anolis carolinensis* (Licht and Donaldson, 1969).

Purification. To date gonadotropins of fairly high specific activity have been prepared and characterized from only two teleosts, the carp, *C. carpio* (Fontaine and Gerard, 1963; Burzawa-Gerard, 1971), and the chinook salmon, *O. tshawytscha* (Donaldson and Yamazaki, 1968; Donaldson et al., 1972*a*). Other purification studies not mentioned in the section on bioassay include those of Gronlund (1969) on *O. tshawytscha*, Breton (1968) on *Coregonus lavaretus* and *Gardonus* sp., and Sinha (1969 and 1971) on *Puntius gonionotus* (Puntius), *Aristichthys nobilis* (big head carp), *Hypophthalmichthys molitrix* (silver carp), and *Ctenopharyngodon idellus* (grass carp).

An outline of the purification procedure used for carp gonadotropin (Burzawa-Gerard, 1971, Burzawa-Gerard and Fontaine, 1972) and the procedure which we have developed for the purification of chinook salmon gonadotropin is presented in Figure 1. Despite the differences in the purification techniques and bioassays used to obtain the two gonadotropins, there is a reasonable degree of similarity in the specific activity of the two preparations relative to the starting materials (Table 1).

Physical characterization. Three techniques have been used to determine the molecular weight of piscine gonadotropin (Table 2). Molecular exclusion chromatography has been used by several investigators (Fontaine and Gerard, 1963; Clemens et al., 1964; Breton, 1968; Gronlund, 1969; Donaldson et al., 1972*a*). This technique tends

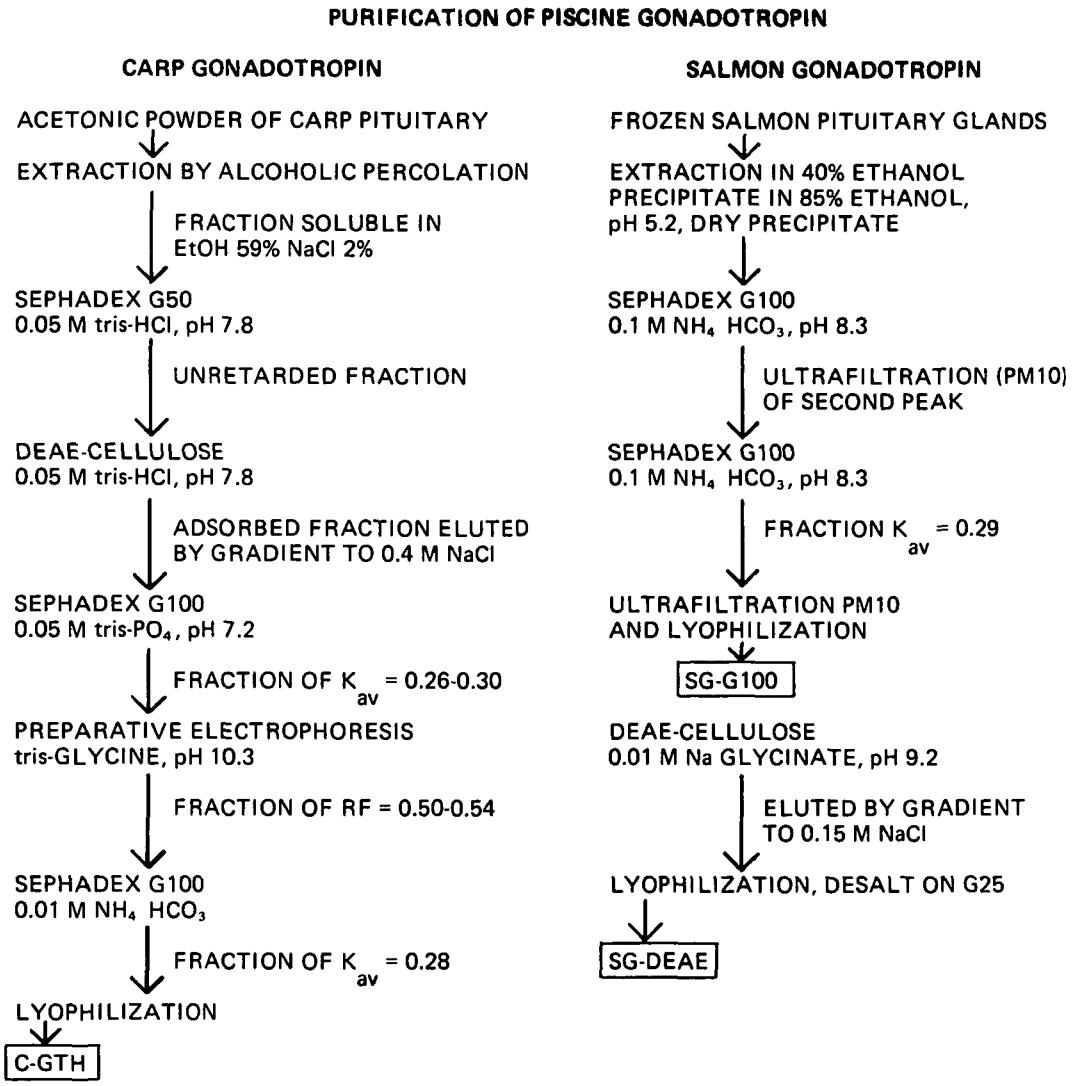

FIG. 1. Purification procedures for carp and salmon gonadotropin based on Burzawa-Gerard (1971), Burzawa-Gerard and Fontaine (1972), and Donaldson et al. (1972).

to overestimate the molecular weight if the proteins used to construct the standard curve are not glycoproteins (Donaldson et al., 1972*a*) or if the gel filtration medium selected is not of optimum pore size (Clemens et al., 1964; Gronlund, 1969). Another technique which has been used is centrifugation in a sucrose gradient in the preparative ultracentrifuge and comparison of sedimentation behavior with proteins of known molecular weight (Burzawa-Gerard and Fontaine, 1966; Burzawa-Gerard, 1971). Using this technique carp gonadotropin was observed to sediment as a major peak with a molecular weight of 27,000 together with a shoulder with a molecular weight of 15,000. This shoulder was found to have the same sedimentation velocity as a fast-running band having no gonadotropic activity which was observed when carp gonadotropin was subjected to preparative disc electrophoresis. Furthermore, when biologically active carp gonadotropin obtained by preparative disc electrophoresis was rerun using analytical disc electrophoresis, the fast-running low molecular weight band was obtained again,

TABLE 1. *Specific activity of carp* (Cyprinus carpio) *and salmon* (Oncorhynchus tshawytscha) *gonadotropin during the purification procedure.*

*Cyprinus carpio**			*Oncorhynchus tshawytscha***		
Stage of purification	Frog spermiation assay (mg LH NIH S1/mg)	S.A. relative to starting material	S.A. relative to starting material	Chick testes P^{32} uptake assay (mg LH NIH S16/mg)	Stage of purification
Acetone powder of pituitary glands	0.04	1	1	0.00135	Lyophilized powder of pituitary glands***
57% EtOH 2% NaCl extract	0.23	5.75	6.19	0.00836	40% EtOH extract precipitated at pH 5.2 in 85% EtOH
DEAE cellulose	0.34	8.5			Sephadex G100, first run
Sephadex G100	0.50	12.5	34.47	0.04653	Sephadex G100, second run
Preparative disc electrophoresis	1.50	37.5			

* Data obtained from Burzawa-Gerard, 1971.
** This bioassay data is presented in different form in Crim et al., 1972.
*** The actual starting material was frozen pituitary glands. These were lyophilized for the purpose of this bioassay.

TABLE 2. *Molecular weights of piscine gonadotropins.*

Species	Technique	pH	Sedimentation velocity	Molecular weight	Reference
Cyprinus carpio	Molecular exclusion chromatography	8.3		<30,000	Fontaine and Gerard (1963)
Cyprinus carpio	Ultracentrifugation sucrose gradient	—	2.84 $S_{obs.}$	31,000	Burzawa-Gerard and Fontaine (1966)
Cyprinus carpio	Ultracentrifugation sucrose gradient	—	1.3 $S_{obs.}$	15,000	Burzawa-Gerard (1971)
		—	2.2 $S_{obs.}$	27,000	
		2.2	1.5 $S_{obs.}$		
Cyprinus carpio	Molecular exclusion chromatography			50,000	Clemens et al. (1964)
Gardonus sp.	Molecular exclusion chromatography			30,000	Breton (1968)
				35,000	
Oncorhynchus tshawytscha	Molecular exclusion chromatography			42,000	Gronlund (1969)
Oncorhynchus tshawytscha	Molecular exclusion chromatography	7.5		40,000	Donaldson et al. (1972)
Preparation SG-G100	Ultracentrifugation	7.8	2.65 S_{20w}	29,400	
"	Ultracentrifugation	1.4	1.38 S_{20w}	12,800	
Preparation SG-DEAE 3	Ultracentrifugation	7.8	2.57 S_{20w}	28,500	
Preparation SG-G100	Molecular exclusion chromatography				Donaldson and Dye (unpublished)
	8M urea low molecular weight peak			13,600	
	1M propionic acid low molecular weight peak			13,800	

suggesting that carp gonadotropin, like the mammalian gonadotropins, consists of two subunits. This was confirmed when centrifugation of carp gonadotropin at low *p*H resulted in a sedimentation coefficient of 1.5 (Burzawa-Gerard, 1971) (Table 2).

In the determination of the molecular weight of salmon gonadotropin, molecular exclusion chromatography was used to obtain the Stokes radius of the molecule. This enabled us to calculate the diffusion coefficient (*D*) using the Stokes-Einstein equation. Sedimentation velocity (*s*) was obtained in the ultracentrifuge and molecular weight was obtained by substituting the experimentally obtained values for *s* and *D* in the Svedberg equation. The molecular weight of intact salmon gonadotropin at *p*H 7.8 ranged from 28,500 to 29,400 and compared closely with the values obtained for carp gonadotropin. Sedimentation of salmon gonadotropin at *p*H 1.4 resulted in an S_{20w} of 1.38 and an estimated molecular weight of 12,800 (Donaldson et al., 1972*a*). This latter value remains an estimate as we have not determined Stokes radius and thus *D* at low *p*H. Recently we have obtained further evidence for subunit formation in salmon gonadotropin (Donaldson and Dyé, unpublished). Gel filtration of salmon gonadotropin incubated overnight in either 8 M urea or 1 M propionic acid resulted in the formation of a peak of molecular weight similar to that of intact gonadotropin and a second peak of lower molecular weight (Fig. 2). Values for Stokes radius of standard proteins were obtained from Laurent and Killander (1964) and plotted arithmetically against $(-\log K_{av})^{1/2}$ (Ryan, 1969). The regression line was obtained by the method of least squares and the Stokes radii of the low molecular weight peaks were calculated for urea and propionic acid treatment as 22.65 Å and 22.97 Å respectively (Fig. 3) and diffusion coefficients were calculated to be 9.46×10^{-7} cm^2sec^{-1} and $9.33 \times 10^{-7} cm^2 sec^{-1}$. Using the sedimentation velocity of $S_{20w} = 1.38$ (Donaldson et al., 1972*a*), molecular weights for the subunits of 13,600 and 13,800 were obtained which compares with the value of M = 12,800 (Donaldson et al., 1972*a*) calculated using the value of *D* for bovine LH subunits (Reichert et al., 1969). To date it has not been possible to determine whether the low molecular weight protein is homogenous or whether it consists of two subunits having similar molecular weights but differing in amino acid composition as is the case with mammalian gonadotropins (Papkoff, 1972).

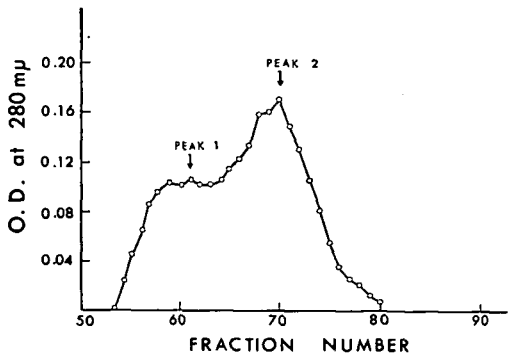

FIG. 2. Gel filtration on Sephadex G100 of salmon gonadotropin (SG-G100) incubated overnight in 8 M ammonium bicarbonate, fraction volume—5 ml.

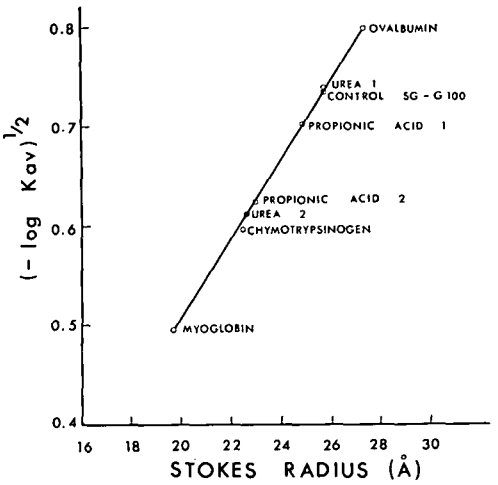

FIG. 3. Plot of the experimentally determined $(-\log K_{av})^{1/2}$ values for three reference proteins and their Stokes radii. $(-\log K_{av})^{1/2}$ values for control SG-G100, urea treated and propionic acid treated gonadotropin have been interpolated on the regression line.

Chemical characterization. The behavior on ion exchange columns of both carp and salmon gonadotropin suggests that they are acidic in nature and thus more similar to mammalian FSH than LH (Fontaine and

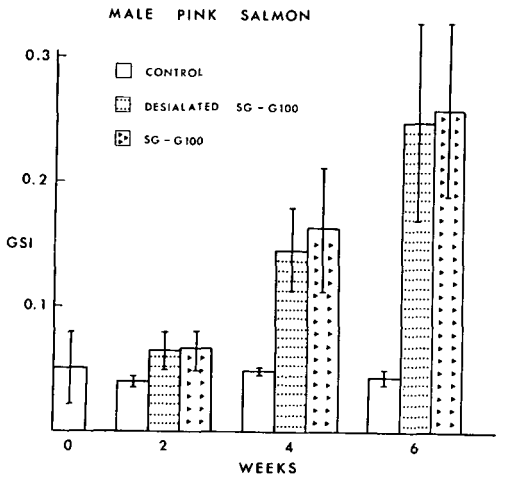

FIG. 4. Stimulation of gonadal growth in immature male pink salmon (*Oncorhynchus gorbuscha*) by intraperitoneal injection of intact and desialated salmon gonadotropin (SG-G100) three times per week for up to six weeks. The SG-G100 was desialated by Dr. H. Papkoff.

Gerard, 1963; Donaldson et al., 1972*a*). This is supported by the finding of a greater similarity between carp gonadotropin and FSH rather than LH in amino acid composition. Eight amino acids in FSH differ in numbers of residues by two or more when compared to carp gonadotropin, while in LH 13 amino acids occur with different frequency than the same amino acids in carp gonadotropin (Burzawa-Gerard, 1969). Carp gonadotropin is a glycoprotein and its carbohydrate content includes 8.6% hexoses, 4.9% hexosamines, and 0.35% sialic acid (Burzawa-Gerard, 1969). This sialic acid content is much lower than in ovine FSH (Papkoff, 1972) and a little higher than in ovine LH (de la Llosa et al., 1968). It has been known for some time that removal of sialic acid from FSH by neuraminidase destroys a large part of its biological activity (Gottschalk, 1957; Papkoff, 1965). The biological activity of LH is unaffected by neuraminidase treatment (Adams-Mayne and Ward, 1964; Papkoff, 1965). Recently Licht and Papkoff (1972) have shown that there is no clear phylogenetic pattern for the importance of sialic acid for gonadotropic activity in the lizard testis assay. In this assay salmon gonadotropin (SG-G100) lost much but not all biological activity after desialation, and thus more closely resembled ovine FSH which lost 90% than LH which lost 50% of its activity. This loss of activity by LH is attributed to FSH contamination (Licht and Papkoff, 1972). Desialated salmon gonadotropin had about 50% of the activity of the intact hormone when assayed by radiophosphate uptake in the chick testis (Donaldson and Dye, unpublished). In contrast, desialated and intact salmon gonadotropin were equally capable of stimulating testicular growth in immature pink salmon (*Oncorhynchus gorbuscha*) at a dosage of 1 μg/g body wt three times per week for 6 weeks (Fig. 4; Table 4). This dose may have been too high to detect a small to moderate degree of inactivation.

IMMUNOLOGICAL STUDIES OF PISCINE GONADOTROPIN

Antiserum to carp gonadotropin obtained from rabbits, when tested against carp gonadotropin in immunoelectrophoresis, gives two precipitation arcs which are believed to correspond to the monomer and dimer seen in preparative disc electrophoresis. No precipitation arc was observed between this anti-serum and ovine LH or FSH (Burzawa-Gerard and Fontaine, 1972). Recently a radioimmunological assay for carp gonadotropin has been developed (Breton et al., 1971). This assay has been used to investigate changes in the plasma concentration of gonadotropin in the goldfish. A circadian rhythm of plasma gonadotropin concentration was observed in non-ovulating female goldfish in July on a 15-16 hr photoperiod and maintained in an aquarium whose temperature ranged from about 18 C at 8 AM to about 32 C at 4 PM. The gonadotropin concentration ranged from 5.75 ng/ml at 8 AM to a maximum of 9.55 ng at 11 AM and a minimum of 3.34 ng at midnight. Male goldfish did not exhibit a marked circadian rhythm (Breton et al., 1972*b*). There was a surge of gonadotropin released on the day of ovulation which was significantly higher (49.3 ng/ml) than the concentration on the day before or the day after ovulation (Breton et al., 1972*b*). This sudden release of gonadotropin at the time of ovulation corres-

ponds with the degranulation of pituitary gonadotrops observed using the light microscope (Pickford and Atz, 1957; Ramaswami, 1962; Ball, 1965; Olivereau, 1967; Hoar, 1969) and recently by electron micrography (Leatherland, 1970). Bioassay studies of pituitary gonadotropin content also indicate an increase in pituitary gonadotropin content during maturation followed by a drop after spawning (Gerbil'skii, 1940; Barr and Hobson, 1964; Swift and Pickford, 1965; Clemens and Johnston, 1965; Singh, 1970). Although ovulation was preceded by several warm days in the study of Breton et al. (1972*b*), it is not possible to distinguish from their data the relative importance of temperature and photoperiod in inducing the ovulatory surge of gonadotropin release.

Recently, Crim et al. (1972) have developed a procedure for the radioimmunoassay of salmon gonadotropin. Antibodies were obtained by injection of SG-G100 into rabbits and these antibodies were used in conjunction with SG-G100 which had been further purified on DEAE cellulose and then labeled with I^{131}. The technique is currently capable of measuring down to 6 ng/ml of gonadotropin in plasma. Using this technique, we have assayed the potency of salmon gonadotropin at three stages of purification and compared the results to those obtained in the chick testis radiophosphate uptake assay (Table 3). The data are fairly close, suggesting that the gonadotropin being measured in the bioassay and the protein being measured by the radioimmunoassay are the same substance.

Wild female pink salmon (*O. gorbuscha*) captured on the spawning beds after ovulation, but prior to the completion of spawning, had higher concentrations of gonadotropin in the plasma than mature spermiating male salmon. The plasma concentration in females was 74.9 ng/ml compared to 13.2 ng/ml in the males.

TABLE 3. *Relative potency* for extracts of salmon pituitary glands determined by bioassay and radioimmunoassay.*

Pituitary preparation	Bioassay†	Radioimmunoassay
Lyophilized fresh pituitaries	0.16	0.27
Crude extract	1.0	1.00
SG-G100	5.56	6.00

* Relative potencies computed in terms of the crude extract which is assigned the value of 1.0.
† Day old chick testis P^{32} uptake test.
Data from Crim et al. (1972).

All *Oncorhynchus* species die after the first spawning. Some time ago Robertson and Wexler (1962) and McBride et al. (1963) showed that death could be prevented by gonadectomy prior to spawning. Sexual development in the salmon is associated with an increase in cortisol secretion rate which can be reversed by gonadectomy (Donaldson and Fagerlund, 1968, 1970) and reinduced by injection of androgen or estrogen into gonadectomized fish (Fagerlund and Donaldson, 1969; Donaldson and Fagerlund, 1969). Wild pink salmon reach sexual maturity, spawn, and die exactly two years after fertilization of the ova. Male pink salmon fingerlings which were stimulated to precocious sexual maturity at one year of age by injection of salmon gonadotropin did not die when they reached maturity (Funk and Donaldson, 1972). However, when a second group of male pink salmon were kept in heated sea water to stimulate growth before and during gonadotropin injection, the majority died after reaching sexual maturity at one year of age (Donaldson, unpublished). Thus, gonadotropin and the steroid hormones appear to play a key role in the postspawning death of Pacific salmon.

CYTOLOGY OF THE PITUITARY GONADOTROPS

Agnatha and Chondrichthyes

There is as yet little agreement regarding the identity of the gonadotrops in the lamprey. The situation has recently been reviewed by Sterba (1969) and Ball and Baker (1969). In the myxinoids a basophil in the anterior region of the adenohypophysis which stains with aldehyde fuchsin and forms signet ring cells after gonadectomy has been identified as a possible gonadotrop (Olsson et al., 1965).

In the elasmobranchs, the gonadotrops appear to be located in the ventral lobe; however, there is no clear-cut agreement re-

garding the specific identity of the gonadotrop (Ball and Baker, 1969).

Osteichthyes-Sarcopterygii

In the lungfish, *Protopterus* sp., three basophils have been identified in the pars distalis (Godet, 1964; Kerr and van Oordt, 1966). The first type to appear is regarded as the thyrotrop; a second type which are very abundant and formed only in adult fish are considered to be FSH cells; and a third type resemble amphibian LH cells (Kerr and van Oordt, 1966).

Osteichthyes-Teleostei

Two excellent reviews have recently appeared which include sections on the teleost gonadotrops (Ball and Baker, 1969; Sage and Bern, 1971). In some species, two gonadotrops have been identified, e.g., the eel (Olivereau and Herlant, 1960), The Pacific salmon (Olivereau and Ridgeway, 1962*a*), the goldfish (Olivereau, 1962; Leatherland, 1972), and the mullet (Leray, 1966; Abraham et al., 1967; Olivereau, 1968), while in other species or investigations only one gonadotrop has been identified, e.g., *Poecilia latipinna* (Ball and Baker, 1969), the catfish (Sundararaj, 1959), the blind Mexican cavefish (Mattheij, 1970), the Pacific salmon (McBride and van Overbeeke, 1969), and the goldfish (Yamazaki, 1969).

Recently the gonadotrops and thyrotrops of the carp *C. carpio* have been identified by immunocytology using antibodies developed against carp gonadotropin and carp thyrotropin. The gonadotrops were localized in the proximal pars distalis, and there was no evidence for the presence of more than one type. The gonadotrops were also detected using antiovine LH (Billard et al., 1971*a*).

In the sockeye salmon (*Oncorhynchus nerka*), fluorescein labelled antiovine LH produced fluorescence in cells in the rostroventral region of the proximal pars distalis. No reaction was obtained with antiovine FSH or antibovine TSH (McKeown and van Overbeeke, 1971). The gonadotrops of the sockeye salmon have also been examined using the electron microscope and were found to contain large globular inclusions and small secretory granules. This gonadotrop corresponds to the beta-cells of Olivereau and Ridgeway (1962). Another cell which had a large amount of cytoplasm consisting largely of endoplasmic reticulum and having a vesicular nature was also identified as a possible gonadotrop matching the gamma-cell of Olivereau. This latter cell type is suggested as being involved in the early phase of gonad maturation during the spring and summer, while the former cell type is thought to be involved in the final stage of gonad development (Cook and van Overbeeke, 1972). While chinook (spring) salmon gonadotropin (SG-G100) has only been prepared from the pituitary glands of sexually mature fish (Donaldson et al., 1972), the fact that it is capable of stimulating all stages of sexual development including spermatogenesis, spermiation, vitellogenesis, and ovulation (Table 4) suggests that there may be only one gonadotropin produced in the pituitary gland of the Pacific salmon.

PHARMACOLOGICAL MANIPULATION OF THE PITUITARY-GONADAL AXIS

Two synthetic drugs have been used to alter the rate of release of pituitary gonadotropin in fish. While the initial reason for their use was primarily the investigation of the pituitary-gonadal axis, there is a possibility that these or other drugs may be used to manipulate the reproductive development of fish in aquaculture. Methallibure (I.C.I. 33,828) (Paget et al., 1961) was originally used in fish by Hoar et al. (1967) to inhibit pituitary gonadotropic function and thus achieve a form of physiological hypophysectomy in *Carassius auratus, Gasterosteus acculeatus,* and *Cymatogaster aggregata*. In the latter species which has to date resisted attempts at hypophysectomy, methallibure inhibited spermatogenesis, vitellogenesis, and gonadal steroidogenesis (Wiebe, 1967, 1968). In the adult guppy (*Poecilia reticulata*) methallibure is capable of suppressing, but not stopping, the transformation of spermatogonia into spermatocytes, while in the juvenile spermatogenesis was

TABLE 4. *Biological activities of salmon* (Oncorhynchus tshawytscha) *gonadotropin SG-G100.*

Species	Test	Dose range over which effect was observed		Reference
		Salmon gonadotropin SG-G100	Other hormones	
Carassius auratus	Spermiation after hypophysectomy	0.1–3.0 μg/g	0.1–10.0 IU HCG	Yamazaki and Donaldson (1968*a*)
Carassius auratus	Spermatogenesis after hypophysectomy	100 μg/fish 3X/week for 3 weeks		Yamazaki and Donaldson (1968*b*)
	Vitellogenesis after hypophysectomy	100–500 μg/fish 3X/week for 3 weeks		
	Ovulation after hypophysectomy	0.1–30 μg/g		
Carassius auratus	Restoration of spermiation and testicular 3β-ol steroid dehydrogenase activity after long term hypophysectomy	200 μg/fish 3x/week for 3 weeks	50–1000 μ/g androgen restored spermiation	Yamazaki and Donaldson (1969)
Poecilia reticulata	Restoration of vitellogenesis and sexual behavior in fish hypophysectomized 10–13 days	10–20 μg/fish every 2 days for 20 days		Liley and Donaldson (1969)
Heteropneustes fossils	Restoration of spermatogenesis and seminal vesicles in long term hypophysectomized fish	0.1–100 μg/fish/day for 30 days	1–50 μg LH-S14 or 100 μg testosterone	Sundararaj et al. (1971)
	Restoration of spermiation	100 μg/fish/day for 30 days	LH-S14 no effect at 50 μg; testosterone no effect at 100 μg	
Heteropneustes fossils	Ovarian maintenance after hypophysectomy	1–5 μg/fish/day for 20 days		Sundararaj et al. (1972)
	Ovulation and spawning; intact and hypophysectomized 6 hr	50–100 μg/fish		
	Restoration of vitellogenesis in fish in early preparatory or mid-postspawning period hypophysectomized 5 days	100–250 μg/fish/day for 23 days; body weight 43 g		
Oncorhynchus gorbuscha	Spermatogenesis and spermiation in immature fish; spermatozoa used to fertilize normal eggs	1 μg/g 3X/week for 3 months		Donaldson et al. (1972)
Oncorhynchus gorbuscha	Spermatogenesis and spermiation in immature fish	1–10 μg/g 3X/week for 3 and 2 months respectively		Funk and Donaldson (1972*a*)
Oncorhynchus gorbuscha	Vitellogenesis in immature fish	1 μg/g 3X/week with or without 1.5 μg/g estradiol for up to 8 months		Funk and Donaldson (1972*b*)

TABLE 4. *Biological activities of salmon* (Oncorhynchus tshawytscha) *gonadotropin SG-G100.*

Species	Test	Dose range over which effect was observed: Salmon gonadotropin SG-G100	Dose range over which effect was observed: Other hormones	Reference
Mugil cephalus	Accelerated spermatogenesis and spermiation in maturing fish	1.25–12.5 μg/g 3X/week for 4 weeks		Donaldson and Shehadeh (1972)
	Accelerated vitellogenesis in maturing fish	11 μg/g 3X/week for 4 weeks		
Mugil cephalus	Ovulation in mature fish (oocyte dia. 750 μ)	0.1 μg/g/day for 4 days		Shehadeh et al. (1972)
	Induction of spermiation in nonspermiating or slightly spermiating fish		17 Methyl testosterone 1–5 μg/g/2 days for 30–42 days or 0.2 IU HCG /g/2 days for 42 days	
Mugil cephalus	Ovulation and spawning in mature females (oocyte dia. 690 μ)	11.9–15.3 μg/g body wt; one third of the dose initially, followed by the remainder 24 hours later		Shehadeh and Kuo (1972)
Oryzias latipes	Induction of in vitro ovulation	0.04–400 μg/ml incubation medium	4 μg/ml LH	Hirose and Donaldson (1972)
Anolis carolinensis (lizard)	Testicular maintenance after hypophysectomy of mature lizard	20–40 μg/g/day for 14 days	10–100 μg LH-S11/day; 0.1–1.0 μg FSH-S4/day	Licht and Donaldson (1969); Licht and Pearson (1969)
	Testicular recrudescence outside breeding season	10–20 μg/g/day		
Gallus domesticus	P^{32} uptake in testis of day old chick	8–32 μg/chick	1–4 μg LH/chick; 7–14 μg FSH/chick	Donaldson et al. (1972) Donaldson et al. (unpublished)
Carassius auratus	Adenyl cyclase activity in ovarian homogenate	7–117 μg/ml	1.2–117 μg/ml carp gonadotropin	Fontaine et al. (1972)
Oncorhynchus tshawytscha	Cyclic AMP in testis slices	0.05–1.0 μg/incubation salmon gonadotropin		Menon and Smith (1971)
Plecoglossus altivelis	Ovulation in intact fish	1–2 injections 0.02–0.8 μg/g	1,250 IU HCG ≅ 10 μg SG-G100	Ishida et al. (1972)
Salmo gairdneri	Oocyte maturation in vitro	0.5 μg/ml	2.5 μg/ml carp pituitary extract	Jalabert et al. (1972)

completely halted at the spermatogonial stage (Pandey, 1970; Pandey and Leatherland, 1970). The effects of methallibure in the guppy have also been reported by Martin and Bromage (1970), and Billard et al. (1970). In an ultrastructural study Leatherland (1969) showed that methallibure appears to block the synthesis of gonadotropin in the pituitary of *C. aggregata* and has no effect on the neurosecretory pathways of the hypothalamus. Further evidence for an effect on the pituitary rather than at the gonadal level was provided when LH was observed to override the effect of methallibure in *C. aggregata* (Wiebe, 1969). In the goldfish, the inhibitory effects of methallibure on spermatogenesis were nullified by simultaneous injection of 10 μg/g body wt carp pituitary acetone powder three times per week. In fact the fish were spermiating after 10 days of treatment. HCG 10 IU/g body wt maintained the gonadosomatic index but did not promote the conversion of spermatogonia to spermatocytes in the presence of methallibure. In fish receiving no hormonal treatment, spermiation occurred at 17 C 20 days after cessation of methallibure treatment (Billard et al., 1971*b*).

Hyder (1972) has investigated the effects of methallibure on gonadal development in the tropical, continuous-breeding teleost *Tilapia*. Methallibure prevented the transformation of spermatogonia into spermatocytes and sperm release, but did not prevent the development of spermatocytes into spermatozoa. The interstitial cells were reduced in number and size and the testicular testosterone concentration was lower in the treated fish. Simultaneous treatment with *Tilapia* pituitary extract or HCG 50 IU/fish counteracted the effects of the methallibure, while testosterone propionate 250 μg/fish induced spermiation but not spermatogenesis. The ovaries of methallibure-treated *Tilapia* contained oocytes up to the late perinucleolus stage, vitellogenesis was inhibited, and ova containing yolk were reabsorbed. Simultaneous treatment with *Tilapia* pituitary extract reinitiated vitellogenesis and stimulated the thecal cells. HCG 500 IU/fish had no effect on the ovaries of *Tilapia* receiving methallibure, while FSH had a marginal stimulatory effect. On the basis of these findings, Hyder (1972) has proposed that *Tilapia* may produce two gonadotropins or one gonadotropin with two different subunits, one HCG-like responsible for stimulating the testis and one non-HCG-like which is responsible for ovarian development. The verification of this hypothesis will have to await the isolation and bioassay of *Tilapia* gonadotropin(s). Hyder's finding that spermiation in *Tilapia* is stimulated by *Tilapia* pituitary extract, HCG, or androgen confirms our observation in the goldfish (Yamazaki and Donaldson, 1969), that the effect of gonadotropin on spermiation is probably mediated via a stimulation of androgen biosynthesis.

While the above findings suggest that methallibure acts at the level of the hypothalamic hypophysial axis in teleosts, recent data from an amphibian *Rana esculenta* indicate that it may also have an effect at the target organ level. In long term pars distalis-ectomized frogs, methallibure completely blocked the stimulatory effect of pars distalis extract on ovarian and oviduct weight and largely blocked the stimulation of testicular and thumb pad development Rastogi and Chieffi, 1972).

The effect of methallibure in *Tilapia aurea* and *T. mossambica* has been investigated from an aquacultural point of view by Dadzie (1970). Addition of the drug to the food resulted in a more rapid effect than addition to the water. Secondary sexual characteristics and spawning behavior were abolished. After 25 days the testes contained only spermatogonia and spermatozoa and vitellogenous oocytes were atretic. In the meso-adenohypophysis there was a gradual decrease in the staining intensity of the basophils. *Tilapia* receiving methallibure grew slightly faster than control fish. In a 2-week period following cessation of treatment, female *Tilapia* grew faster than controls. The growth rate obtained using methallibure to inhibit sexual development compared favorably with the growth rate obtained by monosex culture of male hybrids (Dadzie, 1970).

The second drug whose effects on gonadotropin release in fish have been studied is

clomiphene citrate (MRL-41). In mammals clomiphene is a competitive estrogen inhibitor and interferes with feedback inhibition of gonadotropin release by estrogen. It has no estrogenic, androgenic, antiandrogenic, or progestational activities. Since 1961 it has been used extensively in clinical medicine to induce ovulation in anovulatory females (Jones, 1968). Recently clomiphene has been used to induce ovulation in mature goldfish. The drug was injected intraperitoneally at a dosage of 1 or 10 μg/g body wt per day for 4 days. Ovulation was induced in 90% of the fish by the fourth day in both groups. The ova were successfully fertilized and hatched into normal fry (Pandey and Hoar, 1972). This important finding will open up a whole new field of possibilities in the difficult area of induced ovulation in acquacultured fish, e.g., mullet, milkfish, and certain Chinese and Indian carp.

POSSIBLE ROLE OF THE INTERRENAL IN OVULATION

Sundararaj and Goswami (1966*a*) showed that the mammalian pituitary gonadotropin LH and the placental gonadotropins HCG or PMS were capable of inducing ovulation in vivo in hypophysectomized mature female catfish. The corticosteroid hormones cortisol, cortisone, and especially DOCA were also effective while the gonadal hormones estradiol, testosterone, and progesterone and several pituitary hormones including ACTH were ineffective. More recently we have shown that salmon gonadotropin is also effective in inducing ovulation in vivo in the hypophysectomized catfish (Sundararaj et al., 1972*a*).

In vitro experiments carried out by Kirshenblat (1959) showed cortisone to be capable of inducing ovulation of *Misgurnus fossilis* oocytes while LH was ineffective. In *Heteropneustes fossilis* in vitro ovulation was induced by cortisol and DOC (Goswami and Sundararaj, 1971) but not by salmon gonadotropin (Sundararaj et al., 1972*b*). In another study Sundararaj and Goswami (1966*b*) showed that the steroid 11β-hydroxylase inhibitor SU 4885 (Metopirone) interfered with the induction of ovulation in vivo in the catfish by LH but did not interfere with the induction of ovulation by DOCA.

In contrast with the above findings, piscine gonadotropin is capable of inducing ovulation in vitro in the medaka (*Oryzias latipes*) (Hirose and Donaldson, 1972). As in the catfish, in vitro ovulation in *Oryzias* is also induced by the corticosteroids. Thus, in some species such as the catfish, gonadotropin may stimulate ovulation by induction of corticosteroidogenesis in extra ovarian tissue, possibly the interrenal, while in other species such as the medaka, ovulation may be caused by gonadotropin-induced ovarian corticosteroidogenesis. A recent in vitro study in *Leptocottus armatus, Gillichthys mirabilis,* and *Microgadus proximus* has shown that the ovarian tissue of these fish is capable of corticosteroid biosynthesis in vitro (Colombo et al., 1972); on the other hand, gonadotropin-induced corticosteroid biosynthesis has yet to be conclusively demonstrated in the piscine interrenal.

While oocyte maturation and ovulation cannot be disassociated in *Oryzias* and *Heteropneustes,* these events have been separated in a salmonid. Jalabert et al. (1972) have recently shown that normal oocyte maturation in fragments of trout (*Salmo gairdnerii*) ovary can be induced in vitro by progestogens or by piscine gonadotropin but not by corticosteroids, estrogen, or androgen. No hormonal treatment resulted in in vitro ovulation although ovulation unaccompanied by normal oocyte maturation was achieved in some cases by incubation in coelomic fluid. Jalabert et al. (1972) propose the existence of two mediators in the trout, one a progestogen which is produced by the ovary after gonadotropin stimulation and causes oocyte maturation, and a second one whose nature and site of production is unknown, which is induced by gonadotropin or the first mediator and causes ovulation.

MANIPULATION OF REPRODUCTIVE DEVELOPMENT IN PINK SALMON

The pink salmon *O. gorbuscha* has a rigid two-year life cycle and in the Fraser River of British Columbia only one-year class is

present which returns to spawn in the river in odd numbered years. Attempts to develop a self-perpetuating even year stock of fish by transplanting fertilized eggs from other rivers in northern British Columbia which have even year runs of fish have failed, possibly because the stock in each river is adapted to a particular set of ecological conditions and migration route. To circumvent this difficulty, an attempt is being made to experimentally accelerate or decelerate sexual development in Fraser River pink salmon in the laboratory so that they mature after either one year or three years and provide progeny for stocking the Fraser River in the even years with indigenous fish. To date, male pink salmon have been accelerated to sexual maturity one year earlier than normal using thrice-weekly injections of salmon gonadotropin over a period of 2-3 months (Funk and Donaldson, 1972). Spermatozoa from these accelerated males have been used to fertilize ova obtained from wild females in northern British Columbia and the eggs hatched to produce fry having 50% of their genetic complement from the Fraser River stock (Donaldson et al., 1972*b*). In 1972 larger amounts of milt were obtained from one year old pink salmon by hatching the eggs and raising the fish in water at 12 C. This higher water temperature led to rapid growth and also hastened the testicular response to the exogenous gonadotropin (Donaldson, unpublished). Ova fertilized using the spermatozoa from these accelerated males were raised to the eyed stage in the laboratory and then planted in the gravel beds of an artificial spawning channel which flows into a tributary of the Fraser River. The fry are expected to emerge from the gravel and migrate to the sea in April 1973 and return as full-grown adults to spawn naturally in September 1974, a year when no other pink salmon are present in the river. Owing to the small number of eggs planted, only a small number of adults are expected to return in this pilot experiment.

Attempts to accelerate the sexual development of female pink salmon by one year have to date been unsuccessful. Injection of gonadotropin or gonadotropin plus 17β-estradiol over a period of 210 days, resulted in the formation of ova in the post-primary yolk stage which had a diameter of 1.3-4.0 mm. Owing to the small size of these juvenile salmon, only a small number of ova developed, the remainder becoming atretic. The mechanism which determines which ova develop and which become atretic is not known. Ova developed most rapidly in fish which received 17β-estradiol in addition to salmon gonadotropin (Funk et al., 1972). This was probably a result of the estrogen stimulating the biosynthesis of vitellin in the liver which is then transported to the ovary via the systemic circulation and made available to the ova undergoing vitellogenesis (Ho and Vanstone, 1961; Holmes and Donaldson, 1969). In the fish which received gonadotropin alone, the stimulation of vitellin biosynthesis would depend entirely on the estrogen biosynthetic capacity of the developing ovary.

As it does not appear to be feasible to obtain sexually mature female pink salmon at one year of age, the second approach has been to delay sexual maturation and extend the life of the fish over a three-year period rather than the unvarying two-year life cycle seen in nature. One approach which is proving successful has been the extension of the two-year photoperiod cycle over a period of three years (Vanstone, 1972). A second approach involves the use of methallibure. In a first experiment injection of methallibure commenced in November of the first year and continued until September of the following year at which time the pink salmon would normally be mature. Three groups were carried through to September, control fish on normal photoperiod, control fish on 12 hr light-12 hr dark photoperiod, and methallibure-injected fish on 12 hr light-12 hr dark photoperiod. The response in the male salmon was very clear-cut. The control fish on normal photoperiod had the largest testes at the beginning of September, the fish on 12:12 photoperiod had testes 36% as large as the controls, and the fish on 12:12 photoperiod plus methallibure had testes 0.66% of the size of the control testes. In the female pink salmon the GSI's were in the same order as in the males, but the ab-

solute differences were not as marked as in the males. In females sampled at the end of August and beginning of September, the GSI in the 12:12 photoperiod fish was 68% of controls and the GSI of the 12:12 photoperiod plus methallibure fish was 29% of controls. The mean oocyte diameter in the latter group was 30% of the control value (Flynn and Donaldson, 1972). The greater inhibition noted in the testis relative to the ovary may be explained by the fact that vitellogenesis had commenced just prior to the initiation of methallibure treatment while spermatogenesis had not yet begun. In the juvenile guppy, methallibure treatment completely inhibited gonadal development, while in the adult only partial suppression was achieved (Pandey, 1970). Currently we have under way an experiment to determine whether complete inhibition of vitellogenesis can be achieved in the pink salmon if methallibure treatment commences prior to the initiation of vitellogenesis.

In this review I have attempted to show that while there are still many mechanisms to be fully elucidated our knowledge of the comparative endocrinology of reproduction in fish has reached a point where we can apply our findings towards the enhancement of natural fish stocks and also to the controlled reproduction of aquacultured species.

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