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Department of 2006

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Date April 16, 1978
ABSTRACT

Thyroxine ($T^4$) administration by cholesterol pellet implantation, by immersion, or by feeding raised plasma $T^4$ levels of goldfish, *Carassius auratus*, above values of control fish (usually less than 1 µg/100 ml). Values for treated fish in the lower dosage groups generally fell between 1 and 4 µg/100 ml, while in the higher dosage groups plasma $T^4$ levels ranged from 5 to 16 µg/100 ml. Immersion was the most effective method of creating sustained physiological plasma $T^4$ elevations; both implantation and feeding are more suitable for flowing water systems where immersion is not feasible.

Thyroxine treatment of maturing fish resulted in a depression of gonadotropin cell activity, while immersion of immature fish in the goiterogen, propylthiouracil, stimulated gonadotropin cell activity slightly; this effect was reversed by $T^4$ replacement therapy. $T^4$ administration to intact goldfish accelerated ovarian development in immature individuals, but had no apparent effect in mature fish. In hypophysectomized individuals, $T^4$ alone failed to initiate vitellogenesis or maintain yolky oocytes; the hormone did, however, augment the ovarian response to salmon gonadotropin (SG-G100) and ovine luteinizing hormone (LH) following pituitary ablation.

Thyroxine treatment tended to increase relative liver weights in both intact and hypophysectomized fish, except when administered with LH. In the latter case, a combination of LH and $T^4$ prevented the usual liver hypertrophy after pituitary removal; either hormone alone, however, was ineffective, suggesting a synergistic action of the two hormones on liver function.

In intact and hypophysectomized goldfish, SG-G100 and LH stimulated thyroid function, as measured by histological criteria and plasma $T^4$ analysis.
The findings indicate that thyroid hormones act synergistically with gonadotropin to influence ovarian development in goldfish, and suggest that these effects are mediated, directly or indirectly, by a thyroxine-induced increase in ovarian sensitivity to gonadotropic stimulation. Thyroxine appears also to modulate gonadotropin production by a negative feedback on the hypothalamo-hypophysial axis; the physiological significance of this is, however, uncertain.
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INTRODUCTION

It is recognized that thyroid hormones influence the reproductive processes of vertebrates (see reviews by Pickford and Atz, 1957; Ball, 1960; Matty, 1960; Dodd and Matty, 1964; Reinboth, 1972; Leatham, 1973; Dodd, 1975). In teleosts, these hormones have been implicated in spawning (Ball, 1960) and the initiation of spawning migrations (M. Fontaine and Leloupe, 1960). Changes in thyroidal activity often coincide with sexual maturation (Hoar, 1955) and activity of the thyroid gland appears to increase during gonadal development (Ball, 1960). Experimentally induced hypothyroidism can slow gonadal development in both teleosts (Barrington and Matty, 1952; Scott, 1953; Pickford and Atz, 1957) and mammals (Leatham, 1973), while administration of thyroid hormones can stimulate sexual maturation in some fish (Grobstein and Bellamy, 1939; Hurlbut, 1975). There appears to be a complex interaction between thyroid hormones, sex steroids, and pituitary thyrotrophs and gonadotrophs in teleosts (Sage and Bromage, 1970b), with hypothalamic-pituitary control over the thyroid and reproductive systems closely linked (Sage, 1973). However, the actual mechanisms by which thyroid hormones affect reproductive processes have only recently been studied; while many facts have emerged, few generalizations have as yet been made.

Literature Review

Fish. Cycles of thyroid activity occur in many teleosts. In part, these cycles appear to be a response to seasonal environmental variations (Hoar, 1959). They may, however, have a functional relationship with the reproductive cycle as well (Swift, 1960; Matty, 1960; Sage, 1973). If associated
with reproduction, these cycles might serve as an ideal mechanism for co-
ordinating seasonal adaptation to environmental changes by altering sensitiv-
ity of the fish to its surroundings (Sage, 1973). Annual increases in
thyroid activity have been correlated with both ova maturation (Hickman, 1962;
Takashima et al., 1972; Ichikawa et al., 1974; Singh et al., 1974) and
spawning (Matty, 1960; Berg et al., 1959). It has been demonstrated that
this surge in thyroid activity may be at least partially independent of en-
vironmental influence. Peak thyroid activity occurs in summer in Platichthys
stellatus and in late fall in Coregonus clupeaformis; in neither case was it
possible to correlate activity with any one environmental factor (Hickman,
1962). A cyclic change in thyroid activity has also been observed in the
viviparous fish, Poecilia reticulata (Bromage and Sage, 1968); changes were
correlated with the gestation cycle, and occurred in the absence of changes
in temperature and day length. Thus, thyroid cycles may occur partially or
completely independently of changes in the environment, and may be associated
with the reproductive cycle.

These increases in thyroidal activity, as well as altering sensitivity
to the environment in association with reproduction, may serve to stimulate
gonadal maturation. Normal gonadal activity in teleosts is dependent on the
presence of a functioning thyroid (Novales et al., 1973), and a seasonal
increase in hormonal iodine has been correlated with ova maturation in
Platichthys stellatus (Hickman, 1962). Thyroidectomy or treatment with
antithyroid drugs will inhibit gonadal maturation, whereas thyroid hormone
treatment tends to reverse this effect (Bern and Nandi, 1964). Grobstein
and Bellamy (1939) found that feeding thyroid powder to sexually immature
males of the genus Platypoecilius resulted in precocious maturation in the
two species tested. Conversely, destruction of the thyroid gland of Xiphophorous with $^{131}$I resulted in the retardation of gonadal maturation. Using the anti-thyroid drug thiourea, Barrington and Matty (1952) induced hypothyroidism in the minnow, Phoxinus phoxinus, and found that spermatogenesis was arrested. Thiourea treatment also retarded gonadal development in young zebra fish, Brachydanio rerio, although maturation of the testes in adults was unaffected (Scott, 1953). Administration of anti-thyroid drugs or thyroid hormones has sometimes affected ovarian maturation as well. Treatment with thiourea effects a profound drop in ovarian activity (Raizada, 1974) and blocked oocyte maturation (Mukherjee, 1976) in the catfish, Heteropneustes fossilis; further, it resulted in an inhibition of oocyte growth in P. phoxinus (Barrington and Matty, 1952) and Poecilia reticulata (Grosso, 1961). Thyroxine ($T_4$) treatment stimulated ovarian maturation in immature goldfish (Hurlburt, 1975), and triiodothyroxine ($T_3$) restored the ability of sturgeon oocytes to respond to hypophysial stimulation after prolonged captivity or cooling of the fish (Detlaf and Davydova, 1974). In addition, the increase in activity of the thyroid gland during gestation in P. reticulata (Bromage and Sage, 1968) may function in the maturation of the next batch of ova, since Ball (1962) has shown that the pituitary is not necessary for maintenance of gestation in Poecilia.

It appears that the thyroid is functionally involved in reproduction in elasmobranchs as well. The thyro-somatic index in the dogfish, Scyliorhinus caniculi, fluctuates annually and is highest in maturing females, indicating an increasing demand for thyroid hormones at this time. In addition, thyroidectomy prevented ovarian maturation in this species (Lewis and Dodd, 1974).
Other Vertebrates. An effect of thyroid hormones on gonadal function is also evident in other vertebrates. Complete maturation of oocytes in amphibia is dependent on a functioning thyroid (Pickford and Atz, 1957), and in *Rana cyanophyctis* both ovulation and spawning are reduced in hypothyroid individuals (Sarkar and Rao, 1971). In reptiles there is evidence of a relationship between thyroid function and many aspects of reproduction. Seasonal changes in thyroid function are correlated with spermatogenesis in *Naja naja* and *Elaphe taeniura* (Wong and Chiu, 1974), and in the lizards, *Sceloporous occidentalis* and *Agama agama*, vitellogenesis occurs at the time of greatest thyroid activity (Eyeson, 1970; Stebbins and Cohen, 1973); in addition, hyperthyroidism in *S. occidentalis* resulted in increased rates of ova maturation (Stebbins and Cohen, 1973). Ovarian weight and egg production in birds are stimulated by thyroid hormone administration (Maqsood, 1952; Rall et al., 1964; Hendrich and Turner, 1966), and in mammals thyroid hormones generally enhance both fertility and sexual behaviour (Rall et al., 1964) and can induce precocious sexual maturation (Pickford and Atz, 1957; Kanwar and Chaudry, 1976). Thyroidectomy of female rats leads to a reduction in ovarian weight, a retardation of uterine and vaginal development, and a decreased ovulatory capacity (Leatham, 1973; Peppler et al., 1975; Kovacs and Mess, 1976); neonatal hypothyroidism can result in permanent defects in the control of estrous cycles (Phelps and Leatham, 1976). In women, myxedema (a form of hypothyroidism) is frequently associated with menstrual irregularities and relative infertility, both of which respond positively to thyroid hormone treatment (Leatham, 1973). An interesting, though rare, example of thyroid-ovarian interactions in women is the occurrence of thyroid tumors within the ovary (Fredlund, 1976).
**Mechanism of Thyroid Action**

The thyroid is involved in gonadal function in both poikilotherms and homeotherms, but the question remains as to exactly how the thyroid hormones affect the reproductive system. They may act directly on the gonads, or affect them indirectly via the hypothalamo-hypophysial axis. Furthermore, thyroid hormones have a profound effect on metabolic rates and may indirectly influence gonadal function in this way.

There is evidence that thyroxine stimulates the activity of steroid dehydrogenases in the rat ovary, thus increasing the rate of steroidogenesis, but whether this effect is direct or mediated via the pituitary is not known (Leatham, 1973). Eartly and Leblond (1954), working also with rats, observed that thyroxine stimulated testicular development when the pituitary was present, whereas no effect was evident in the absence of the pituitary. This indicates that thyroxine either acts on the hypothalamo-hypophysial axis, as the authors suggest, or that it affects the gonads via some other mechanism but is dependent on the presence of pituitary gonadotropin. No similar work on teleosts has been published.

A complex interplay between thyroid hormones, sex steroids, gonadotrophs, and thyrotrophs is evident in teleosts. Sage and Bromage (1970b) found thyroxine inhibited gonadotropin production in *Xiphophorous* spp. and *Poecilia reticulata* *in vitro*, while the thyrotropic cells of *P. reticulata* were stimulated by estrogens and inhibited by androgens *in vivo*. Sex steroids may also affect thyroid function directly, since it has been found that methyl testosterone can stimulate the thyroid gland in *Sparisoma* (Matty, 1960), *Poecilia reticulata* (Sage and Bromage, 1970b), and the hypophysectomized catfish, *Mystus vittatus* (Singh, 1969). In the catfish, estrogens and pro-
gesterone elicit a similar response, though not as strongly as that induced by methyl testosterone (Singh, 1969). Thus, there appears to be a reciprocal effect, the reproductive hormones influencing thyroid activity and the thyroid hormones in turn influencing the reproductive system.

The many interactions may be a result of evolutionary associations. Sage (1973) has proposed that the original role of thyroid hormones was in some manner associated with reproduction. The thyroid gland has evolved from the protochordate endostyle, a structure which secretes iodinated mucous used to trap food particles. There is, however, no evidence that thyroxine evolved functionally as a gastro-intestinal hormone in vertebrates. Its earliest role may have been an involvement in gonadal maturation; this perhaps led to other morphogenic effects on growth, development and metamorphosis.

The close relationship of thyroidal and gonadal control systems in teleosts supports this concept. Both gonadotropic and thyrotropic hormones (GTH and TSH) are glycoproteins, and TSH may have evolved from a gonadotropin-like molecule by gene duplication and subsequent divergence. There is no evidence for a pituitary control over thyroid function in cyclostomes. Thyrotropic activity is evident in elasmobranchs, and both this and gonadotropic activity are localized in the ventral lobe of the pituitary. There is a general parallelism between thyroid and reproductive function in this group, and it remains to be demonstrated whether or not two distinct thyrotropic and gonadotropic molecules are involved in their control (Dodd and Matty, 1964; Lewis and Dodd, 1974). In teleosts, TSH and GTH have evolved as separate hormones, but factors regulating their production remain closely linked. The many accounts of thyroid activity paralleling reproductive activity in teleosts are therefore not surprising. So close are these
parallels that Sage finds it difficult to imagine how the thyroid could have any effect that is not closely related to reproduction.

Thus, a functional and perhaps evolutionary relationship exists between the reproductive and thyroidal systems. The involvement of the thyroid in gonadal maturation is, however, an obviously complex problem, and one that is poorly understood. Previous studies have demonstrated that thyroxine administration may stimulate ovarian maturation in immature goldfish; this effect was dose-dependent, with low doses stimulating gonadal development, while at high doses both stimulatory and inhibitory influences were apparent (Hurlburt, 1975). However, it is still unknown whether thyroidal-gonadal interactions in teleosts are pituitary mediated, or whether they are mediated by effects of the thyroid on aspects of general or ovarian metabolism. Hence, the present study was designed to elucidate further the mechanisms by which thyroid hormones influence ovarian maturation in the goldfish, Carassius auratus. A physiological dose for thyroid hormone administration was first established, and the responses of the pituitary, ovary, and liver of intact fish to hyper- and hypothyroid states then investigated. In addition, the effects of thyroid hormones alone or in combination with piscine and mammalian gonadotropins on the ovary and liver of hypophysectomized fish were studied, in order to evaluate the nature and mechanisms of the thyroid's involvement in ovarian development in this species.
GENERAL TECHNIQUES

Maintenance of Goldfish

Goldfish (Carassius auratus L.) of the common comet variety were obtained from Hartz Mountain Pet Supply Company, Richmond, British Columbia and Grassyforks Fisheries Company, Martinsville, Indiana. Stock fish were maintained in flowing, dechlorinated fresh water under natural photoperiod, and fed regularly on brine shrimp. The fish were frequently diseased on arrival from suppliers; they were routinely treated in stock tanks. Skin parasites (Gyrodactylus spp. and Trichodaniis spp.) were eradicated by immersion in formalin (1:5000) for 30-60 min or methylene blue (2-4 ppm) for several days; the former treatment was found to be more successful. Secondary bacterial infection (principally Aeromonas hydrophilia) was common, and this was treated in the early stages of infection very successfully by immersion of the fish in 10% sea water containing chloramphenicol (10-15 mg/l) for three to five days.

The goldfish was used as an experimental animal since it is of a convenient size and easily obtained. Moreover, the structure of the ovary and the pituitary under varying environmental and hormonal conditions is well documented.

Histology and Histological Techniques

Ovary. The normal sequence of events in the growth of the goldfish oocyte includes the first, or pre-vitellogenic, growth stage and the second, or vitellogenic growth stage. The second growth stage is marked initially by the appearance of yolk vesicles (cortical alveoli) when the oocyte is about
150 μm in diameter, while the next phase of vitellogenesis, that of yolk granule formation, begins when the oocyte reaches a diameter of 350 to 450 μm (Yamazaki, 1965) (see Fig. 21).

Not all developing follicles mature to ovulation; many of them become atretic at some stage in development. Vitellogenic oocytes are regularly resorbed in the goldfish (Khoo, 1974), and Stacey (1977) has described pre-vitellogenic oocyte atresia in this species.

Histological changes in the ovary of the goldfish following hypophysectomy are documented by Yamazaki (1965) and Khoo (1974). All yolky oocytes undergo atresia following pituitary removal (Yamazaki, 1965). Those with more yolk become atretic faster than those with less yolk; thus, four weeks after the operation all yolk granule stage oocytes are in various stages of degeneration, while intact yolk vesicle oocytes may be found in the ovary up to 6 or 7 weeks post-hypophysectomy (Khoo, 1974). In fish hypophysectomized for a period of several months, a reduction in the number of pre-vitellogenic oocytes is apparent in the goldfish (Stacey, 1977), suggesting that these follicles may undergo atresia as well.

In the present study, ovaries were removed and weighed on termination of experiments. They were then fixed in Bouin's solution, embedded in paraffin, and sectioned at 7 μm. Yolk laden ovaries are brittle, and were often difficult to section routinely. This problem was overcome by soaking the trimmed blocks in 60% ethyl alcohol and glycerine (9:1) prior to sectioning. All ovaries were stained by the Masson's trichrome technique.

Ovarian development was analyzed in several ways. The gono-somatic index (GSI = gonad weight/body weight x 100) was calculated for each fish, and means for each experimental group determined. All values were trans-
formed by the arc sin square root method for analysis, and the GSI's for each group compared by analysis of variance (ANOVA) and Duncan's multiple range test for comparison among means.

Next, the diameter of the largest oocyte in a randomly chosen histological section was measured. As oocyte size is directly related to the stage of maturity and yolk deposition in goldfish (Khoo, 1974), maximum oocyte diameter may be used as a sensitive criterion of the stage of ovarian maturity. As with GSI values, these data were analyzed by ANOVA and Duncan's multiple range test.

In experiments with hypophysectomized fish, in which hormone administration often not only affected size but also the number of oocytes, the stage of ten follicles (both developing and atretic) in a section was then measured; these follicles were sampled randomly using the microscope stage coordinates. Data were pooled for each group, and the number of atretic and developing oocytes calculated. Data were analyzed by the Kolmogorov-Smirnov test for cumulative distributions. This technique allows determination of the proportion of ovaries occupied by developing and vitellogenic oocytes and by degenerated follicles, while avoiding tedious cell counts.

**Thyroid.** Lower jaws containing thyroid tissue were fixed in Bouin's solution and transferred to decalcificant (5% formalin:5% formic acid) for one week. They were then embedded in paraffin, sectioned serially at 5 μm, and stained by the Masson's trichrome technique.

Epithelial cell height was used as a histological index of thyroid activity. The highest and lowest cell heights in ten follicles were measured, and mean values for each follicle determined. The mean epithelial cell
height for individual fish was then calculated. Results were analyzed by ANOVA and Duncan's multiple range test.

Numbers of thyroid follicles were not determined, since Chavin (1956) found this parameter extremely variable in the goldfish, with little or no correlation between follicle number and thyroid activity.

Pituitary. The bony capsule containing the pituitary was removed and fixed in Bouin Holland-saturated mercuric chloride (9:1). Following decalcification (1 week in 5% formalin:5% formic acid) tissues were embedded in paraffin, sectioned serially at 5 μm, and stained with the alcian blue (pH 2.5)-periodic acid Schiff (PAS)-orange G method of Herlant (1960). This method not only allows for differentiation between basophilic and acidophilic cells, but also for distinguishing between the basophilic cell types. Gonadotropic and thyrotropic cells are basophils, the former staining with both alcian blue and PAS while the latter stain mainly with alcian blue by this technique. Staining with PAS alone produces a positive pink coloration in both cell types in goldfish (Nagahama, 1973). GTH and TSH cells may also be distinguished by their shape and location in the pituitary; in the goldfish, GTH cells are generally round or elliptical and are situated in the mesoadeno-hypophysis, whereas TSH cells are polygonal and are located in the proadeno-hypophysis (Nagahama, 1973).

The activity of GTH cells was studied according to the criteria established by Sage and Bromage (1970a), by which reduced cell and nuclear size reflect relative inactivity. Cell and nuclear diameters of 15 to 20 GTH cells were measured in a mid-sagittal section of each pituitary, and mean values of each fish calculated. Results were analyzed by ANOVA and Duncan's multiple range test. In addition, the amount of PAS positive granulation
and the frequency of intracellular spaces were examined; a decrease of one or both of these factors may indicate that a cell is relatively inactive (Nagahama, personal communication).

**Hypophysectomy**

Goldfish were hypophysectomized using a modification of Yamazaki's (1965) technique as demonstrated by N. E. Stacey (personal communication). The fish were first anaesthetized in ice water containing 0.01% tricane methane sulphonate (MS 222) for about 30 minutes. Fish acclimated previously at 12°C needed a slightly longer period for anaesthesia than those acclimated at 20°C, but survival rate of the former was much higher, presumably because temperature shock was less severe. Fish were next placed on the "operating table" (a grooved sponge) with the left side uppermost and covered with crushed ice.

The gular membrane anterior to the left operculum was slit with a small scalpel, and the left operculum together with the first two gill arches retracted. An oblique incision of about 1.0 mm was made in the underlying dorsal mucosa, exposing the parasphenoid bone. A hole was then drilled with a round dental burr immediately posterior to the pterygoid bone and laterally to the path of a conspicuous pair of nerves (probably the facial [VII cranial] nerves). The pituitary was thus exposed, and was then removed with a curved pipette aspirator. Bleeding was often profuse during the operation, but ceased soon afterwards.

After the operation the fish were placed in buckets containing cold (4°C), well oxygenated 25% sea water (about 7°/oo salinity). Upon recovery, the fish were transferred to holding tanks (8°C; 25% sea water); the tanks were then allowed to warm slowly to room temperature. With the exception of
Experiment IVb, 10 mg chloramphenicol/litre was added to the holding tanks for the first week to prevent infection; after this time the wound was healed, and the exposed area of the skull was covered with connective tissue. Treatment with chloramphenicol increased survival rates of 70-80% to greater than 90%.

Following hypophysectomy, there is a gradual loss of skin pigmentation which takes several days or weeks to complete; this whitening of the body may be used as an index of operational success. In addition, completeness of hypophysectomy was determined upon termination of experiments in the following manner. The pituitary region was first examined under a binocular microscope to check for pituitary remnants. If remnants seemed to be present, this region was fixed in Bouin's solution, decalcified, and sectioned at 10 μm. Upon staining, serial sections were examined histologically for adenohypophysial tissue.

The only disease attacking hypophysectomized fish resembled "red fin disease" (Van Duin, 1973). This was characterized initially by a lowering of the dorsal fin and a general listlessness. In later stages there was a rupturing of blood vessels close to the surface. If detected before the disease was well advanced, the fish could be cured effectively by immersion in potassium dichromate (1:25,000) for one week. During this period the fish would not feed.
SECTION I. THE EFFECTS OF THYROXINE ON PLASMA THYROXINE LEVELS
INTRODUCTION

Hormone dosage is an important factor when considering the effects of $T_4$ on ovarian maturation, since mild hyperthyroidism may stimulate while extreme hyperthyroidism may inhibit ovarian development in both goldfish (Hurlburt, 1975) and mammals (Leatham, 1973). Thus, before commencing further studies on the interactions between the gonadal and thyroidal systems in goldfish, a detailed study of the effects of hormone dosage and of various vehicles for $T_4$ administration on plasma $T_4$ levels was undertaken. Several methods have been employed to increase plasma $T_4$ levels in teleosts, including injection, immersion, feeding, and pellet implantation. Their effectiveness in creating chronic physiological elevations has, however, only recently been studied. Eales (1974) investigated the effects of immersion and intraperitoneal injections on plasma $T_4$ levels in several species of teleosts. Intraperitoneal injection was found to be impractical as major surges of plasma $T_4$ are created after injection of large dosages, while small dosages of $T_4$ were cleared rapidly from the blood, so that more frequent injections, involving considerable stress to the fish, would be needed. Immersion was found to be highly effective in creating sustained and predictable elevations in plasma $T_4$ in the species studied (Eales, 1974).

In the present study, the effectiveness of immersion and two additional vehicles of $T_4$ administration (feeding and $T_4$-cholesterol pellet implantation) were investigated.
MATERIALS AND METHODS

The following experiments were conducted at 20°C, and with the exception of the third experiment, fish were fed regularly on brine shrimp throughout the experimental period.

a. Pellet Implantation

Fish were divided into four groups and implanted with T₄-cholesterol pellets. Cholesterol and L-thyroxine (both from Sigma Chemical Company, St. Louis, Missouri) were mixed thoroughly with a metal spatula, and pelleted in 10 mg lots by means of a hand press (Parr Instruments Company, Moline, Illinois). The final amount of T₄ in the pellets of the four groups was nil (control), 60 µg, 150 µg, and 750 µg respectively. Fish were lightly anaesthetized in MS 222 (1:5000); a small incision was made in the skin between the lateral line and dorsal fin and a 2 mm stainless steel spatula used to tunnel into the musculature. The pellet was inserted by means of fine forceps, and a drop of terramycin (50 mg/ml) applied to the wound which was then sealed with Eastman 910 adhesive. Fish were sampled before the start of the experiment, and again on days 5, 12, 21, and 28.

b. Immersion

Fish were divided into three groups and held in 50-litre glass aquaria. The aquaria were cleaned and filled with fresh water on the day before the start of the experiment. At noon on day 0, half the water was removed from each tank and replaced with fresh water to which was added T₄ dissolved in 5 ml of 0.1 N NaOH. The amount of hormone dissolved was such that the final T₄ concentrations in the three tanks were nil (control), 2 µg/100 ml, and
10 µg/100 ml respectively. Half the water in each aquarium was again exchanged at noon on days 2, 4, and 6, and the amount of $T_4$ added at these times was half that added originally. Fish were sampled just prior to the initial immersion in $T_4$-treated water, and again on day 0 at 1600 hours and at 1200 hours on days 1, 3, 5, and 7.

c. Feeding

Fish were divided into three groups, and starved for one week before the experiment. On day 0, they were fed a meal-gelatin diet prepared according to Peterson et al. (1967) at a level of 3% body weight. The $T_4$ was first ground in a mortar and pestle, and added in suspension to the aqueous meal mixture. This was blended for several minutes before addition of the gelatin (cooled to 35°C). In later experiments, when $T_4$ was administered for a period of weeks, the meal-gelatin diet was frozen and stored in this manner. The control group received the unmodified diet, while $T_4$ was added to the diet of the two treatment groups in proportions of 10 ppm or 100 ppm. The fish were observed to consume all food within one-half hour after addition of the diet to the tank. Samples were taken prior to treatment, and again at 2, 4, 8, 12, 24, and 48 hours after feeding.

Sample times in each experiment were chosen according to the nature of the treatment. Hence, $T_4$ levels in pellet implanted fish were studied over several weeks to determine the duration as well as the pattern of $T_4$ uptake by the plasma. In the immersion experiment, fish were sampled for 1 week only, as the method is presumably effective as long as fresh $T_4$ is added to the water. Oral administration differs from the other two methods in that the fish is exposed to exogenous $T_4$ for a relatively short time after feeding;
thus the pattern of $T_4$ uptake and subsequent excretion after a single feeding was studied.

**Blood Sampling and Plasma $T_4$ Analysis**

Sampled fish were anaesthetized in MS 222 and blood drawn from the caudal artery by heparinized Natelson blood collecting tubes (Fisher Scientific Company). The blood was then centrifuged, and the plasma collected and stored at $-10^\circ C$ for up to two and one-half weeks. Plasma stable $T_4$ levels were determined by the competitive protein-binding technique, using Tetralute kits obtained from Ames Company Division, Miles Laboratories, Rexdale, Ontario. The standard method was modified according to Ames Technical Services Bulletin no. 1-75-D, increasing sensitivity of the test to encompass the relatively low plasma $T_4$ levels previously encountered in teleosts (Higgs and Eales, 1973).

Competitive protein-binding analysis of plasma $T_4$ is known to be both accurate, and highly reproducible (Braverman et al., 1971). In addition, Higgs and Eales (1973) found the Ames Company Tetralute kit to be the most useful for studies of fish plasma levels, as it is rapid, precise, and lacks the exactly timed stages of other kits tested in their laboratory. In the present study, duplicate analyses were usually not possible due to the low amounts of plasma obtainable from experimental animals. However, in the few instances that they were performed, results were found to be reproducible within 0-0.1 µg $T_4$/100 ml plasma.
RESULTS

Fig. 1 shows the mean plasma $T_4$ levels in control and $T_4$-implanted fish. Control values remained below 1 µg/100 ml and usually below 0.5 µg/100 ml plasma for the duration of the experiment, while $T_4$ levels in treated fish rose rapidly after pellet implantation. In the highest and medium dosage groups, these rose to 15-30 times the control values for the first three weeks. Those of the lowest dosage group peaked at a mean of about 4 µg $T_4$/100 ml plasma after 5 days, and fell to within the control range after 3 weeks. Variability within each sample group was greatest at the medium dosage, while at the highest and lowest dosages, values generally fell within 2 µg and 1 µg of the mean respectively.

Mean plasma $T_4$ levels for fish immersed in $T_4$-treated water are given in Fig. 2. Except for the sample 4 hours after the initial immersion, all values represent means of fish sampled 24 hours after addition of fresh $T_4$ solution. $T_4$ levels in control fish remained below 1.0 µg/100 ml plasma. In the high dosage group, plasma $T_4$ levels rose steadily, reaching a mean of 5.5 µg/100 ml after 5 days. Plasma levels in the low dosage group increased sharply in the first few hours, but fell to about 1 µg/100 ml after 24 hours. After 7 days, plasma $T_4$ levels in this group had risen to a mean of 2.5 µg/100 ml. Values for both dosage groups are similar to those observed in other teleosts after immersion (Eales, 1974). Variability in several of the samples of the higher dosage group was considerable, while in the lower dosage group, values generally fell within 1 µg of the mean.

Fig. 3 gives the plasma $T_4$ levels of fish fed diets containing $T_4$. Values for control fish were less than 1 µg/100 ml plasma, with the exception
Figure 1. Plasma $T_4$ concentrations of fish implanted with pellets containing 0, 60, 150, and 750 $\mu g$ $T_4$.

Means and standard errors of values from 3 to 5 fish are represented. Mean body weights (g) and ranges were: 22.7, 18.1-35.5 (controls); 16.4, 11.1-20.4 (60 $\mu g$ $T_4$); 24.9, 16.1-30.8 (150 $\mu g$ $T_4$); 24.2, 15.8-38.9 (750 $\mu g$ $T_4$).
Figure 2. Plasma $T_4$ concentrations of fish immersed in $T_4$ solutions of 0, 2, and 10 μg/100 ml for up to 7 days.

Means and standard errors of values from 3 to 6 fish are represented. Mean body weights (g) and ranges were: 10.7, 7.9-14.9 (controls); 10.4, 7.3-14.4 (2 μg/100 ml); 11.4, 8.0-17.8 (10 μg/100 ml).
Figure 3. Plasma $T_4$ concentrations of fish fed diets containing $T_4$ in proportions of 0, 10, and 100 ppm for up to 48 hours after feeding.

Means and standard errors of values from 3 to 6 fish are represented. Mean body weights (g) and ranges were: 10.9, 9.6-12.5 (controls); 11.1, 8.3-17.2 (10 ppm); 11.0, 7.8-14.9 (100 ppm).
The diagram shows the concentration of T4 in micrograms per 100 ml over time in hours. The concentration peaks at 100 at 8 hours, then drops significantly. There are additional lines indicating lower concentrations at 10 and 0 micrograms per 100 ml, which remain relatively stable throughout the time period.
of two fish in which plasma $T_4$ levels of 1.1 and 1.8 $\mu$g/100 ml were recorded. $T_4$ levels in the higher dosage group rose dramatically in the first few hours, reaching a peak after 4 hours. Variability in this and the 8- and 12-hour samples was high, but was reduced and $T_4$ levels stabilized after 24 and 48 hours. Plasma $T_4$ levels in the fish fed 10 ppm $T_4$ were considerably lower and less variable than those of the fish fed 100 ppm $T_4$; values were again highest after 4 hours, with plasma $T_4$ levels dropping to a mean of 1.3 $\mu$g/ml after 48 hours.

**DISCUSSION**

Eales (1974) defines a physiological dose of $T_4$ as one which raises plasma $T_4$ to a level which the fish itself could maintain by endogenous production. In the goldfish, plasma $T_4$ concentrations of untreated fish were similar to those found in other teleost species by Higgs and Eales (1973) and Leloup and Hardy (1976) (usually less than 1 $\mu$g/100 ml), but were considerably lower than those reported by Refetoff et al. (1970) in *Salmo irideus* and *Catastomus commersoni* (4.5 and 4.3 $\mu$g/100 ml respectively). Bovine TSH administration may raise endogenous plasma $T_4$ levels to over 3 $\mu$g/100 ml and occasionally up to 5 $\mu$g/100 ml in the brook trout (Chan and Eales, 1976), and perhaps also in the goldfish. Hence, a level of 4 to 5 $\mu$g $T_4$/100 ml plasma was considered the upper physiological limit in the following studies on the goldfish.

All methods of $T_4$ administration elevated plasma $T_4$ levels of treated fish. At the highest dosages, plasma $T_4$ levels were several times the physiological values, while the lowest dosages generally raised plasma $T_4$ levels to the upper physiological range.
The $T_4$-cholesterol pellet implantation method was found to be a convenient means of elevating plasma $T_4$ levels especially in flowing water systems where immersion is not feasible. A minimum of handling time and stress is incurred, since many fish may be implanted in a day and then left undisturbed for a period of three to four weeks. Few problems were encountered with infection, and all fish began to feed well within a day. If, however, injection with another hormone in addition to $T_4$ treatment is considered, this method is impractical as frequently the wound may be aggravated during handling and serious infections may occur.

Immersion of fish in $T_4$-treated water is also a convenient and effective method of $T_4$ administration. The finding that exchange of only half the water every second day, rather than a complete exchange every day, created chronic plasma $T_4$ elevations is significant for two reasons. Firstly, stress to the fish is reduced as they need not be removed from the tank, and secondly, it is advantageous in long term experiments where complete exchange of the water every day may not be practical. In fish less tolerant to metabolic waste, however, more frequent water exchange may be necessary. Although samples were taken in the present experiment only up to 7 days after immersion, Eales (1974) found plasma $T_4$ levels in starved brook trout did not change significantly between 7 and 27 days of immersion in 10 $\mu$g $T_4$/100 ml water.

Feeding resulted in small elevations of plasma $T_4$ levels in the 10 ppm group, and at this dosage oral administration appears to be an effective means of creating sustained increases in circulating $T_4$. Treatment with the higher dosage (100 ppm), however, produced a major surge in plasma $T_4$ levels comparable to that observed by Eales (1974) after intraperitoneal injections,
and hence feeding of the hormone at this level was considered unsuitable for creating chronic physiological plasma T₄ elevations. Plasma T₄ concentrations at both dosages peaked 4 hours after feeding; this correlates well with a study by Hayes (1968) which showed that intestinal absorption of T₄ in man was complete 4 hours after oral administration of the hormone. Hayes (1968) concluded that T₄ which was not absorbed within this period becomes firmly bound in the intestine and is not available for later absorption. In the present study, variability in both treatment groups was large perhaps as a result of variations in the amount of diet consumed by individual fish.

The finding that feeding of T₄ effectively elevates plasma T₄ levels in a teleost is of importance, since it has been suggested that this is the most practical procedure for T₄ administration in fish culture (Higgs et al., 1976).
SECTION II. EFFECTS OF THYROID HORMONES ON THE PITUITARY, GONADS AND LIVER OF INTACT FISH
INTRODUCTION

While mild hyperthyroidism may stimulate gonadal function in vertebrates, severe hyperthyroidism appears to inhibit gonadal development in several groups studied, including teleosts (Hurlburt, 1975), birds (Chandola et al., 1974), and mammals (Leatham, 1973). This inhibitory influence is possibly due to a negative feedback of thyroid hormones on pituitary gonadotropin production. In mammals, it is well established that changes in thyroid activity affect plasma gonadotropin levels. Both diurnal LH peaks and pituitary response to luteinizing hormone-releasing hormone (LH-RH) administration are significantly increased in hypothyroid rats, although basal plasma gonadotropin levels remain unchanged (Dunn et al., 1976; Kalland et al., 1976). In addition, plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations rise to higher levels in hypothyroid than in euthyroid rats following gonadectomy; replacement with T$_4$ results in a reduction of LH and FSH levels to those normally found in euthyroid-gonadectomized controls (Larochelle and Freeman, 1974) or even euthyroid-intact controls (Kalland et al., 1976). Metabolic clearance rates of LH and FSH were unaffected by thyroid state, suggesting that these changes result from a direct affect of T$_4$ on the hypothalamo-hypophyseal axis (Larochelle and Freeman, 1974).

Midcycle peaks in LH release may also be affected by thyroid hormones. LH surges in hyperthyroid rats were depressed to 25% of control levels, and the amount of estrogen needed to induce an LH surge in ovariectomized-thyroidectomized animals was much greater if they had received T$_4$ replacement than if no T$_4$ replacement was given (Freeman et al., 1976).
Evidence of the effects of thyroid hormones on gonadotropin production in man conflicts with the above findings. Two studies (Akande and Anderson, 1975; Clyde et al., 1976) report elevated gonadotropin concentrations in hyperthyroid patients; in both cases, treatment with anti-thyroid drugs restored serum levels to those found in euthyroid individuals. These results, however, may not be due to a direct effect of thyroid hormones on the pituitary. T₄ stimulates the formation of sex hormone binding globulins in man, decreasing the level of unbound sex steroids in the circulation. Negative feedback of the sex steroids on the pituitary is consequently reduced; hence, gonadotropin production is increased (Akande and Anderson, 1975).

Thyroid hormones appear to inhibit gonadotropin production in birds as well. Plasma LH levels in the Pekin duck were depressed following thyroxine treatment (Jallegeas et al., 1974) and, in the chick, testicular ³²P uptake was elevated following goiterogen treatment, suggesting that endogenous plasma gonadotropin levels were increased (Lehman and Frye, 1976). In addition, cytological studies of the avian pituitary indicate a suppression of gonadotropin cell activity in hyperthyroid individuals (Thapliyal, 1969; Chandola et al., 1974).

Studies of the effects of thyroid hormones on gonadotropin production in teleosts are limited. Sage and Bromage (1970b) found gonadotropic cells of Poecilia reticulata and Xiphophorous spp. to be inhibited by T₄ in vitro, and the same effect was evident in immature goldfish in vivo (Hurlburt, 1975). In the latter experiment, sample sizes were relatively small, and the fish were sampled only after 17 weeks of treatment. In addition, the two dosages used (100 and 1000 ppm orally) may both be considered pharmacological. The present study was designed to investigate the effects of known physiological
and supra-physiological doses of $T_4$ on gonadotropin cell activity in mature goldfish. The effects of induced hypothyroidism, with or without $T_4$ replacement, on the pituitary were also investigated, and liver weights, an easily measurable metabolic parameter, taken in this experiment.

Thyroid tissue in goldfish and other teleosts does not form a discrete glandular body as is the case in mammals, but rather consists of scattered follicles lying along the ventral aorta and in the head kidney. Hence, thyroidectomy is not possible, and hypothyroidism must be induced by the administration of goiterogenic drugs (chemical thyroidectomy) or $Na^{131}I$ (radiothyroidectomy). The latter method was not considered in the present study, since $Na^{131}I$ must be administered in large doses to effect destruction of the thyroidal tissue; extra-thyroidal tissues may also be affected, as the isotope is detectable in significant concentrations in the viscera and other body components following injection in the goldfish (Chavin, 1956). Instead, the goiterogen, propylthiouracil, was used. This drug was chosen as it has several times the goiterogenic activity of related anti-thyroid compounds (Liberti and Stanbury, 1971), and thus may be used in relatively small dosages. Clearance of these substances from the circulation and their concentration in thyroidal tissue (see Tausk, 1975) would thus be more efficient than when large dosages are administered. This is important, as most goiterogens act as anti-oxidants and may exert pharmacological effects on other metabolic functions.
MATERIALS AND METHODS

a. Effects of Thyroxine in Mature Fish

Sixty maturing female goldfish were divided among three tanks and maintained under long photoperiod (16L:8D) at 12°C. On day one of the experiment, the three groups were implanted with cholesterol pellets containing T₄ in amounts of nil (controls), 60, or 150 µg. Control fish and those of the 60 µg group were reimplanted on days 20, 40, and 60, while fish of the 150 µg T₄ group were reimplanted on days 30 and 60. All groups were fed regularly on brine shrimp throughout the experiment.

One-half of the fish in each group was killed by decapitation on day 40 and the rest on day 80. The pituitary regions and ovaries were fixed for subsequent histological examination. Unfortunately, several of the fish implanted with 60 µg T₄ died from bacterial infection early in the experiment, so sample sizes for this group are relatively small.

b. Effects of Propylthiouracil and Thyroxine in Immature Fish

Sixty immature goldfish were divided among four tanks and maintained under long photoperiod (16L:8D) at 13-14°C for three weeks. The groups were immersed in 50 ppm 6n-propyl-2-thiouracil (PTU-Sigma) or implanted with cholesterol pellets containing 60 µg T₄ as follows:

Group 1: Controls
Group 2: PTU
Group 3: T₄
Group 4: PTU and T₄
Groups 1 and 2 were implanted with pellets containing cholesterol only, and all groups were reimplanted on day 20. The PTU was partially dissolved in 100 ml 0.01 N NaOH before addition to the tanks. Half the water in all tanks was exchanged once a week, and either 100 ml 0.01 N NaOH (groups 1 and 3) or 100 ml 0.01 N NaOH + 50 ppm PTU (groups 2 and 4) added with the fresh water.

The fish were killed by decapitation 40 days after the start of the experiment. The gonads and livers of all fish were weighed, and the ovaries fixed for subsequent histological examination. Pituitary regions and thyroidal tissue of females were also preserved.

RESULTS

Pituitary

Effects of thyroxine. Table 1 summarizes the results of measurements of gonadotropin cell and nuclear diameters in maturing, T$_4$-treated fish. Analysis of variance showed a significant effect of T$_4$ on both cell and nuclear size. After 40 and 80 days of treatment, both values were significantly smaller in the 150 µg T$_4$ group than in the controls, while the 60 µg T$_4$ group differed from controls only after 80 days. Mean cell volumes (calculated from mean diameters) were reduced to 70% and 55% of the control value in the 60 µg and 150 µg T$_4$ groups respectively after 80 days of treatment; nuclear volumes of both treatment groups were 70% of control values at this time. Within each group, changes in mean cell and nuclear diameters between the two sample times were small, and both analysis of variance and comparison among means found no significant differences.
TABLE 1. EFFECTS OF THYROXINE ON GONADOTROPIN CELL AND NUCLEAR SIZE IN MATURING FISH.

<table>
<thead>
<tr>
<th>DAYS OF TREATMENT</th>
<th>GROUP</th>
<th>n</th>
<th>CELL SIZE</th>
<th>NUCLEAR SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DIAM(µ) ± S.E.</td>
<td>VOLUME(µ³)</td>
</tr>
<tr>
<td>40</td>
<td>Control</td>
<td>8</td>
<td>9.0 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>382</td>
</tr>
<tr>
<td></td>
<td>60 µg T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>5</td>
<td>8.3 ± 0.2</td>
<td>299</td>
</tr>
<tr>
<td></td>
<td>150 µg T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>9</td>
<td>7.5 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>220</td>
</tr>
<tr>
<td>80</td>
<td>Control</td>
<td>9</td>
<td>9.6 ± 0.4</td>
<td>463</td>
</tr>
<tr>
<td></td>
<td>60 µg T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>5</td>
<td>8.5 ± 0.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>321</td>
</tr>
<tr>
<td></td>
<td>150 µg T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>9</td>
<td>7.8 ± 0.3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>248</td>
</tr>
</tbody>
</table>

<sup>a</sup>ANOVA p < 0.001 for effect of T<sub>4</sub> on diameter, NS for effect of time
<sup>b</sup><i>c</i> control (40 day) p < 0.01
<sup>c</sup><i>c</i> 60 µg T<sub>4</sub> (40 day) p < 0.05
<sup>d</sup><i>d</i> control (80 day) p < 0.05
<sup>e</sup><i>e</i> control (80 day) p < 0.01
Morphologically, GTH cells of control fish appeared quite active (Fig. 4); they contained large vacuoles, and large granules were frequent. In T4-treated fish, however, the vacuoles were reduced in size (Fig. 5), although large granules were still numerous.

**Effects of propylthiouracil and thyroxine replacement.** Results of pituitary measurements in immature control and treated fish (Exp. IIb) are recorded in Table 2. Mean cell and nuclear sizes of the controls are less than those of controls in the previously described experiment (IIa); this is probably due to the differences in gonadal development in the two groups, since GTH cell activity (hence cell and nuclear size) increases as the gonads mature (Naga­hama, personal communication).

Values for PTU-treated fish are significantly elevated over those of both the controls and fish receiving a combination of PTU and T4, suggesting gonadotropin production is stimulated in hypothyroidism.

GTH cells of all groups in this experiment were morphically less active than those of fish in Experiment IIa and both vacuolation and frequency of large granules were reduced (Fig. 6, 7, and 8). There was little difference in gonadotroph cytology between control and treated fish, however, and the main difference among the groups appeared to be the slight increase in cell and nuclear size in PTU-treated fish.

Since plasma samples were not taken for analysis in experiment IIb, thyroid epithelial cell height and TSH cell activity were studied to determine whether the PTU was indeed inhibiting thyroid hormone production. PTU is concentrated by the thyroid gland in mammals and acts to suppress both thyroid hormone synthesis, by blocking organic binding of iodine (Liberti and Stanbury, 1971), and peripheral conversion of T4 to the more physiologically active T3 form (Tausk, 1975). As a result of reduced plasma thyroid
TABLE 2. EFFECTS OF PROPYLTHIOURACIL (PTU) AND THYROXINE ON GONADOTROPIN CELL AND NUCLEAR SIZE IN IMMATURE FISH.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>CELL SIZE</th>
<th>NUCLEAR SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DIAM (μ) ± S.E.</td>
<td>VOLUME (μ³)</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>7.0 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>180</td>
</tr>
<tr>
<td>PTU</td>
<td>4</td>
<td>7.8 ± 0.1&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>248</td>
</tr>
<tr>
<td>PTU + T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>6</td>
<td>7.2 ± 0.1</td>
<td>195</td>
</tr>
</tbody>
</table>

<sup>a</sup>ANOVA p < 0.05

<sup>b</sup> > control p < 0.01

<sup>c</sup> > PTU + T<sub>4</sub> p < 0.05

<sup>d</sup> < PTU + T<sub>4</sub> p < 0.01
Figure 4. Pituitary gonadotrophs (G) and thyrotrophs (T) of untreated maturing female goldfish. Alcian blue-PAS-orange G. X610.

Figure 5. Pituitary gonadotrophs (G) of maturing female goldfish implanted with T₄ (150 µg) for 80 days. Alcian blue-PAS-orange G. X610.

Figure 6. Pituitary gonadotrophs (G) and thyrotrophs (T) of untreated immature goldfish. Alcian blue-PAS-orange G. X610.

Figure 7. Pituitary gonadotrophs (G) of immature goldfish immersed in propylthiouracil for 40 days. Alcian blue-PAS-orange G. X610.

Figure 8. Pituitary gonadotrophs (G) of immature goldfish treated with propylthiouracil and T₄ for 40 days. Alcian blue-PAS-orange G. X610.

Figure 9. Pituitary thyrotrophs (T) of immature goldfish immersed in propylthiouracil for 40 days. Alcian blue-PAS-orange G. X610.
hormone levels, pituitary TSH production rises dramatically and thyroid epithelial cells consequently hypertrophy. In the present study, only one of the PTU-treated fish had greatly elevated epithelial cells after 42 days, while in another, enlarged follicles were evident (active follicles are generally small in the goldfish (Chavin, 1956)), and the mean epithelial cell height in this group did not differ significantly from the controls (Table 3). Chavin (1956) also found little evidence of histological change in the goldfish thyroid following goiterogen treatment, yet thyroidal activity as measured by $^{125}$I uptake was significantly suppressed. In the present study, pituitary TSH production was apparently increased following PTU treatment, since cell frequency was greater and affinity for basic stains less than in control pituitary glands (Table 3; Fig. 9); both these changes are thought to be associated with an increase in TSH production in goldfish and other teleosts (Nagahama, 1973). In fish receiving a replacement dose of $T_4$, both thyroid epithelial cell height and TSH cell frequency were reduced over control values.

Ovary

Thyroxine treatment of maturing females had no effect on either GSI or maximum oocyte diameter (Table 4). In all groups, the variability in both values was large, and no significant differences were evident after 40 or 80 days.

Results of the effects of $T_4$ and PTU treatment of immature fish are given in Table 5. Ovaries of control fish showed little development over the experimental period, and no significant differences were evident in the mean GSI values of control and treated fish. The mean maximum oocyte size of the
TABLE 3. EFFECTS OF PROPYLTHIOURACIL AND THYROXINE ON THYROID EPITHELIAL (TH EPITH) CELL HEIGHT AND PITUITARY TSH CELL FREQUENCY

<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>TH EPITH CELL HEIGHT ($\mu$m) ± S.E.</th>
<th>TSH CELL FREQUENCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>4.0 ± 0.2$^a$</td>
<td>++(+)</td>
</tr>
<tr>
<td>PTU</td>
<td>4</td>
<td>5.4 ± 1.5</td>
<td>++++(+)</td>
</tr>
<tr>
<td>PTU + $T_4$</td>
<td>6</td>
<td>3.2 ± 0.1$^b$</td>
<td>+(+)</td>
</tr>
</tbody>
</table>

$^a$ANOVA p < 0.01

$^b$<control p < 0.01
### TABLE 4. EFFECT OF THYROXINE ON OVARIAN DEVELOPMENT OF MATURING FISH.

<table>
<thead>
<tr>
<th>DAYS OF TREATMENT</th>
<th>GROUP</th>
<th>n</th>
<th>BODY WT (g) ± S.D.</th>
<th>GSI ± S.E.</th>
<th>MAX OOCYTE DIAM(μ) ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>Control</td>
<td>9</td>
<td>11.8 ± 2.5</td>
<td>5.70 ± 1.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>495 ± 63&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>60 μg T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>5</td>
<td>15.1 ± 3.8</td>
<td>5.16 ± 1.00</td>
<td>465 ± 79</td>
</tr>
<tr>
<td></td>
<td>150 μg T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>9</td>
<td>13.1 ± 2.3</td>
<td>6.93 ± 2.47</td>
<td>565 ± 57</td>
</tr>
<tr>
<td>80</td>
<td>Control</td>
<td>8</td>
<td>12.5 ± 2.1</td>
<td>6.93 ± 2.47</td>
<td>550 ± 51</td>
</tr>
<tr>
<td></td>
<td>60 μg T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>5</td>
<td>13.1 ± 1.2</td>
<td>5.02 ± 2.14</td>
<td>470 ± 93</td>
</tr>
<tr>
<td></td>
<td>150 μg T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>9</td>
<td>13.6 ± 1.7</td>
<td>10.10 ± 1.90</td>
<td>603 ± 24</td>
</tr>
</tbody>
</table>

<sup>a</sup>ANOVA NS
TABLE 5. EFFECT OF PROPYLTHIOURACIL AND THYROXINE ON OVARIAN DEVELOPMENT OF IMMATURE FISH.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>BODY WT (g) ± SD</th>
<th>GSI ± SE</th>
<th>MAX OOCYTE DIAM (μ) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial control</td>
<td>10</td>
<td>9.3 ± 2.2</td>
<td>1.70 ± 0.14	extsuperscript{a}</td>
<td>146 ± 6	extsuperscript{b}</td>
</tr>
<tr>
<td>Final control</td>
<td>8</td>
<td>11.3 ± 3.2</td>
<td>1.66 ± 0.13</td>
<td>157 ± 4</td>
</tr>
<tr>
<td>T	extsubscript{4}</td>
<td>10</td>
<td>10.3 ± 3.1</td>
<td>2.07 ± 0.18</td>
<td>195 ± 21	extsuperscript{c}</td>
</tr>
<tr>
<td>PTU</td>
<td>5</td>
<td>11.7 ± 5.2</td>
<td>1.95 ± 0.26</td>
<td>175 ± 20</td>
</tr>
<tr>
<td>PTU + T	extsubscript{4}</td>
<td>8</td>
<td>11.0 ± 3.2</td>
<td>2.12 ± 0.24</td>
<td>185 ± 12</td>
</tr>
</tbody>
</table>

\textsuperscript{a} ANOVA NS

\textsuperscript{b} ANOVA p < 0.01

\textsuperscript{c} > initial control p < 0.05
The mean hepato-somatic index (HSI) was calculated for each group in Experiment IIb, and the results are given in Figure 10. PTU treatment of female fish results in HSI values which were significantly lower than those of both control and T_4-treated fish. In the groups receiving T_4 alone or PTU + T_4, HSI values were similar to controls. In males, however, relative liver weights were significantly elevated in the T_4-treated fish, while values for the PTU and PTU + T_4 groups remained unchanged from controls. The mean HSI of male controls is significantly less than that of female controls, although treatment values for the two sexes are very similar.

DISCUSSION

Pituitary

It appears that, as in mammals, thyroid hormones may modulate gonadotropin production in teleosts. The present study supports the findings of Sage and Bromage (1970b) and Hurlburt (1975) which suggest that this influence is of an inhibitory nature. T_4 treatment of maturing goldfish resulted in a dose-dependent suppression of gonadotropic cell activity, while treatment of immature fish with the goiterogen, propylthiouracil, resulted in a slight stimulation of cell activity; the latter effect was reversed by T_4 replacement therapy.
Figure 10. Hepato-somatic indices of intact immature fish treated with thyroxine and propylthiouracil. Plain bars represent mean values of female fish, and stippled bars represent mean values of male fish. Vertical lines indicate standard errors, and numbers adjacent to each standard histogram are sample sizes.

Significant differences are evident between the HSI values of:

a. male controls and female controls (p < 0.05).
b. PTU-treated females and control females (p < 0.05).
c. PTU-treated females and T₄-treated females (p < 0.01).
d. male controls and T₄-treated males (p < 0.01).
e. T₄-treated males and both PTU-treated and PTU + T₄-treated males (p < 0.05).
The mechanisms whereby $T_4$ may affect gonadotropin production are uncertain, but it is most likely via an action on the hypothalmo-pituitary axis. In teleosts a direct effect on the pituitary was demonstrated by Sage and Bromage (1970b), who found that addition of $T_4$ to the medium of cultured pituitaries resulted in gonadotropin cell inhibition. Mammalian work suggests effects on both the pituitary and hypothalamus. The responsiveness of the pituitary to LH-RH was enhanced in hypothyroid rats (Kalland et al., 1976), and $T_4$ treatment of this species resulted in an increase in the estrogen receptor content of the anterior pituitary (Cidlowski and Muldoon, 1975). Since $17\beta$-estradiol alters sensitivity of the gonadotrophs to LH-RH (Cidlowski and Muldoon, 1975), a $T_4$-stimulated increase in estrogen binding would presumably decrease the gonadotropin cell response to endogenous LH-RH, as suggested by Larochelle and Freeman (1974). This may result from a decreased rate of estrogen receptor catabolism, rather than a stimulation of receptor anabolism, as the metabolic rate of the pituitary in mammals is, in contrast to most other tissues, diminished under the influence of thyroid hormones (Tonoue and Yamamoto, 1967; Lee et al., 1968; Cidlowski and Muldoon, 1975).

Freeman et al. (1976), however, found the LH response to LH-RH unaltered in hyperthyroid rats while LH production following hypothalamic stimulation was significantly reduced, indicating the hypothalamus must also be considered as a site of negative feedback of thyroid hormones on gonadotropin production.

**Ovary**

Thyroxine had no detectable effect on ovarian development of mature fish after 40 or 80 days of treatment. Variability in both GSI and maximum
Oocyte diameter in all groups was very large and, although both values were
greatest in the high dose $T_4$ group, statistical differences were not evident.
$T_4$ treatment of immature fish, however, resulted in a slight but statistical-
ly significant increase in ovarian maturation after 40 days. This is
consistent with the results of Hurlburt (1975) in goldfish and Higgs et al.
(1976) in coho salmon; $T_4$ in both cases stimulated ovarian development of
immature fish.

An inhibitory effect of high doses of $T_4$ on ovarian maturation was
described by Hurlburt (1975), and this was attributed to a thyroxine-induced
decrease in gonadotropin production. Retardation of gonadal development in
$T_4$-treated fish was not apparent in the present study. The dosages used,
however, were considerably less than the high dose of Hurlburt (1975)
(1000 ppm administered orally), hence this is perhaps not surprising.
Although gonadotropin cell activity was apparently inhibited in mature
treated fish (Exp. IIa), cell and nuclear diameters were still greater than
those values of immature controls in Exp. IIb. Any decrease in gonadotropin
production in the former case may have been compensated for by an increase in
plasma $T_4$ levels.

Previous studies have reported a retardation of oocyte maturation
following goiterogen treatment in teleosts (e.g. Barrington and Matty, 1952;
Grosso, 1961; Mukherjee, 1976). In the present study immersion in propyl-
thiouracil did not affect gonadal development; however, ovarian development
of control fish was disappointingly slight over the experimental period,
and any inhibitory effects of PTU would be difficult to demonstrate. It is
also doubtful that thyroid hormone levels were depressed for the full 40
days of treatment, since only synthesis and not release of stored thyroxine
is affected by goiterogens (Tausk, 1975). Alternately, the results of previous studies may have been due to a pharmacological action of the anti-thyroid drug directly on the ovary; dosages used in the present study were comparatively low, and MacKay (1973) found that, in the firetail gudgeon, a small dose of thiourea was effective in suppressing thyroid activity but had no effect on ovarian development while a higher dose inhibited both thyroid function and ovarian maturation.

Liver

The mean HSI of female fish was greater than that of male fish in Exp. IIb, suggesting a sexual difference in liver weights. This may be a result of estrogen-induced liver hyperplasia as has been described by Bailey (1957) in intact goldfish, since Khoo (1974) found previtellogenic and early yolk vesicle oocytes capable of steroid production in this species; however, Schreck and Hopwood (1974) report estrogen levels to be similar in immature goldfish of both sexes. Alternately, the differences in liver weights may represent a difference in endogenous $T_4$ secretion rates in males and females, since administration of exogenous $T_4$ increased the HSI of males to a value similar to that of female controls, while PTU treatment reduced the HSI of females to a value similar to that of male controls. Thyroid hormones have also been reported to increase liver weight in the trout, Salmo gairdnerii (Takashima et al., 1972) and in thyroidectomized water snakes, Natrix piscator (Gupta et al., 1975). The mechanisms by which thyroid hormones may affect liver metabolism in teleosts are uncertain, and will be discussed in a later section.
The absence of follicular hypertrophy in most of the propylthiouracil-treated fish is perhaps not surprising, since similar findings have previously been reported in the goldfish (Chavin, 1956). In addition, Subhedar and Rao (1975) found that immersion in thiouracil produced stimulatory changes in the thyroid follicles of the catfish, Heteropneustes fossilis, up to 30 days only; after this time, values for thyroid epithelial cell heights in treated fish dropped to within the control range. This suggests that the goiterogen, as well as inhibiting hormonal synthesis by the thyroid tissue, may perhaps reduce the sensitivity of the follicular cells to TSH stimulation after prolonged treatment.

Since the goiterogen dosage used in the present study was rather low (50 ppm), it might also be argued that thyroid hormone synthesis was not inhibited significantly. However, thiouracil, which has one-tenth the goiterogenic activity of propylthiouracil (Liberti and Stanbury, 1971), severely reduced $^{125}$I uptake by the goldfish thyroid at a dosage of 500 ppm in the absence of follicular hypertrophy (Chavin, 1956). In addition, morphological studies of TSH cell activity in the present experiment indicate that TSH production was increased somewhat in goiterogen treated fish, suggesting that thyroid hormone synthesis was indeed reduced.
SECTION III. EFFECTS OF THYROXINE AND SALMON GONADOTROPIN ON OVARIES AND LIVERS OF LONG TERM HYPOPHYSECTOMIZED FISH
INTRODUCTION

Hurlburt (1975) found that low doses of exogenous T₄ stimulated ovarian maturation in immature goldfish, while at high doses both stimulatory and inhibitory effects were evident. Preliminary studies of GTH cell activity in this study indicated that T₄ may inhibit GTH production, since at high (pharmacological) dosages GTH cell and nuclear size were considerably reduced and gonadal maturation retarded. These results suggested that thyroid hormones act synergistically with gonadotropin in their effects on ovarian development, and that in the absence of critical levels of gonadotropin, T₄ is ineffective in stimulating gonadal maturation.

To test this hypothesis, the effects of T₄ alone and in combination with salmon gonadotropin were studied in long term hypophysectomized fish.

MATERIALS AND METHODS

a. Effects of Thyroxine in Long Term Hypophysectomized Fish

Goldfish hypophysectomized for three months were divided among three tanks (20°C; 16L:8D). After a two-week acclimation period, the groups were immersed in T₄ at concentrations of nil (controls), 2, or 10 µg/100 ml. Half the water was exchanged three times a week and fresh T₄ added.

The experiment was terminated 25 days after the initial immersion. Fish were killed by decapitation, and the livers and ovaries removed and weighed; the ovaries were fixed for subsequent histological examination.
b. Effects of Salmon Gonadotropin and Thyroxine in Long Term Hypophysectomized Fish

Goldfish hypophysectomized three to five months were divided into three groups and maintained at 20°C and 16L:8D for two weeks. They were then injected intraperitoneally with saline (controls), or 3-4 µg/g salmon gonadotropin (SG-G100 - Nutrition and Applied Endocrinology Program, Fisheries and Marine Service, Research and Resource Services Directorate, West Vancouver, B. C., batch no. 4, May 1972). In addition to salmon gonadotropin, one group was fed T₄ (25 ppm) throughout the experiment; the control group and the group receiving SG-G100 alone were fed the unmodified meal-gelatin diet (see Section I). The SG-G100 was dissolved in fish saline (Wiebe, 1969) and 0.1 ml of the solution injected with a 1.0 ml tuberculin syringe and 30 gauge needle. Fish were injected three times a week for four weeks.

The experiment was terminated 24 hours after the last injection. All fish were killed by decapitation, and both ovaries and lower jaws, containing thyroid tissues, removed and fixed for subsequent histological examination.

RESULTS

Ovary

Thyroxine alone had no apparent effect on ovarian development of long term hypophysectomized fish. Ovaries of both control and treated fish were histologically regressed, containing only pre-vitellogenic oocytes and, since the fish were hypophysectomized when immature, few old atretic follicles (Fig. 12 and 13). The GSI and maximum oocyte size values of all three groups were similar (Table 6).
TABLE 6. EFFECTS OF THYROXINE ON OVARIAN DEVELOPMENT AND LIVER WEIGHT IN LONG-TERM HYPOPHYSECTOMIZED FISH.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>BODY WT (g) ± SD</th>
<th>GSI ± SE</th>
<th>MAX OOCYTE DIAM(μ) ± SE</th>
<th>HSI ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>14.1 ± 3.0</td>
<td>0.92 ± 0.14</td>
<td>129 ± 8</td>
<td>7.65 ± 0.78</td>
</tr>
<tr>
<td>2 μg T₄</td>
<td>8</td>
<td>12.5 ± 1.5</td>
<td>1.15 ± 0.19</td>
<td>134 ± 7</td>
<td>11.69 ± 0.72&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 μg T₄</td>
<td>6</td>
<td>12.1 ± 2.3</td>
<td>1.23 ± 0.16</td>
<td>137 ± 5</td>
<td>9.60 ± 0.59&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> control p < 0.01
<sup>b</sup> 10 μg T₄ p < 0.05
<sup>c</sup> control p < 0.05
Salmon gonadotropin, with or without thyroxine supplement, stimulated a significant increase in ovarian weight over hypophysectomized controls (Table 7). While the GSI values of the SG-G100 and the SG-G100 + T₄ groups differed only slightly, the mean maximum oocyte diameter of the latter group was significantly greater than those of both the SG-G100 group and the controls. Significant differences in the frequency of developing oocytes and atretic follicles are also evident in this experiment (Fig. 11). As with the previous experiment, control ovaries were histologically completely regressed. Most of each ovary in this group consisted of old atretic follicles, with a relatively small number of intact oocytes (Fig. 14). Those oocytes which were present were pre-vitellogenic, and few exceeded 100 μm in diameter. Ovaries of fish receiving SG-G100 alone had a larger proportion of developing ova (Fig. 15) and 2 out of 6 fish had oocytes in the early yolk vesicle stage. In those fish receiving both SG-G100 and T₄, the number of atretic follicles was greatly reduced and vitellogenic oocytes were more frequent than in controls or in SG-G100-treated fish. Yolk vesicle formation was evident in five of the eight fish, and only in this group were developing follicles over 200 μm encountered (Fig. 16).

**Thyroid**

Thyroid epithelial cell heights were measured in Experiment IIIb to determine whether SG-G100 stimulated thyroidal function in treated fish (Table 7; Fig. 17 and 18). As is evident, follicular cell heights of the two treatment groups are significantly greater than those of the controls, suggesting that thyroid activity and perhaps also thyroid hormone production are indeed stimulated by the SG-G100 preparation.
### TABLE 7. EFFECTS OF SALMON GONADOTROPIN AND THYROXINE ON OVARIAN DEVELOPMENT AND THYROID EPITHELIAL CELL HEIGHT IN LONG-TERM HYPOPHYSECTOMIZED FISH.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>BODY WT (g) ± SD</th>
<th>GSI ± SE</th>
<th>MAX OOCYTE DIAM(μ) ± SE</th>
<th>THYROID CELL HT(μ) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>24.8 ± 11.3</td>
<td>0.56 ± 0.10</td>
<td>70 ± 13</td>
<td>2.2 ± 0.16</td>
</tr>
<tr>
<td>SG-G100</td>
<td>6</td>
<td>27.1 ± 11.8</td>
<td>1.57 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>165 ± 9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.8 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SG-G100 + T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>8</td>
<td>26.7 ± 8.7</td>
<td>1.66 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>214 ± 20&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>4.9 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> control p < 0.01
<sup>b</sup> control p < 0.05
<sup>c</sup> SG-G100 p < 0.05
Figure 11. Effects of salmon gonadotropin and thyroxine on oocyte composition of ovaries of long-term hypophysectomized fish.

The relative frequencies of atretic (A), pre-vitellogenic (PV), and vitellogenic (V) follicles are given for each group. Both treatment groups differ from the control group at $p < 0.01$, and the SG-G100 + $T_4$ group differs from the SG-G100 group at $p < 0.05$. 
Figure 12. Ovarian section of an untreated long-term hypophysectomized fish (Exp. IIIa) showing only pre-vitellogenic oocytes. Masson's trichrome. X140.

Figure 13. Ovarian section of long-term hypophysectomized fish immersed in $T_4$ (10 µg/100 ml) for 25 days. Only pre-vitellogenic oocytes are evident. Masson's trichrome. X140.

Figure 14. Ovarian section of an untreated long-term hypophysectomized fish (Exp. IIIb). Only small pre-vitellogenic (PV) and atretic (A) follicles are evident. Masson's trichrome. X140.

Figure 15. Ovarian section of a long-term hypophysectomized fish treated with SG-G100 for four weeks. Note early yolk vesicle stage oocyte (YV). Masson's trichrome. X140.

Figure 16. Ovarian section of a long-term hypophysectomized fish treated with SG-G100 and $T_4$ for four weeks. Note oocyte with well developed yolk vesicles (YV). Masson's trichrome. X140.
Figure 17. Thyroid follicles (F) from a control long-term hypophysectomized fish showing low epithelial cells. Masson's trichrome. X450.

Figure 18. Thyroid follicles (F) from a long-term hypophysectomized fish treated with SG-G100 for four weeks. Note hypertrophied epithelial cells. Masson's trichrome. X450.
Liver

Thyroxine treatment, especially at the lower dosage, resulted in an increase in relative liver weights in long-term hypophysectomized fish (Table 6). Since HSI values rise following pituitary ablation in untreated goldfish and other teleosts as a result of increased liver glycogen stores (Walker and Johansen, 1975; Ball and Hawkins, 1976), the effect of $T_4$ in the present study was to augment the liver response to hypophysectomy. Whether this was due to a further stimulation of glycogen accumulation could not be assessed; liver samples were taken for hepatic glycogen determination, but labels were lost during processing.
DISCUSSION

Ovary

The results of the present study demonstrate that T₄ alone has no effect on ovarian development in long-term hypophysectomized goldfish, but the hormone does augment the ovarian response to piscine gonadotropin stimulation. This suggests that the effects of T₄ are dependent on the pituitary, since the presence of gonadotropin is necessary for thyroxine to influence ovarian development. Thus, as suggested by Hurlburt (1975), thyroidal-gonadal interactions appear to be regulated by gonadotropin levels, and in the absence of the latter, thyroxine is ineffective.

A stimulatory effect of salmon gonadotropin on the ovary of goldfish hypophysectomized 6 weeks has been described previously by Yamazaki and Donaldson (1968). In the present study, SG-G100 was effective in re-initiating ovarian development 3 to 5 months post-operatively, indicating that the ovary does not lose its ability to respond to gonadotropin even after several months of pituitary deprivation.

Thyroid

Salmon gonadotropin stimulated thyroidal activity, as measured by histological criteria, in hypophysectomized fish. This likely resulted in an elevation in plasma T₄ levels in treated fish since, in a study on intact goldfish, plasma T₄ concentrations rose from a mean of 0.3-0.5 µg/100 ml to 0.9 µg/100 ml following SG-G100 treatment (unpublished results). Donaldson and McBride (1974) attribute this situation to TSH contamination in the SG-G100 preparation, rather than to an inherent thyrotropic activity of the
gonadotropin molecule itself.

Liver

Thyroxine treatment significantly elevated liver weights of hypophysectomized fish. This effect was greatest at the lower dosage, while at the higher dosage HSI values were intermediate between those of the control and low dose groups. Thyroxine in small concentrations may promote the accumulation of nutritional stores in the liver, perhaps via a stimulation of general metabolic processes in the animal, while administration of the hormone at high concentrations may result in a depletion of liver glycogen as is apparent in *Poecilia latipinna* (Ball and Hawkins, 1976) and mammals (White et al., 1973). Whether these effects reflect direct or indirect actions of thyroxine on the liver in the goldfish is uncertain.
SECTION IV. EFFECTS OF THYROXINE AND LH ON OVARIIES, THYROID, AND LIVER OF SHORT-TERM HYPOPHYSECTOMIZED FISH
INTRODUCTION

The effects of mammalian gonadotropins on gonadal development have been studied extensively in fish (see reviews by Pickford and Atz, 1957; Hoar, 1966; de Vlaming, 1974; M. Fontaine, 1976). LH and HCG (human chorionic gonadotropin) have been more successful in accelerating gonadal maturation than FSH in teleosts (de Vlaming, 1974), although all are considerably less effective than any of their piscine equivalents (M. Fontaine, 1976).

LH and HCG have usually stimulated vitellogenesis in intact fish (e.g. stickleback, Ahsan and Hoar, 1963; *Gillichthys mirabilis*, de Vlaming, 1972; *Cytomagaster aggregata*, Wiebe, 1969). Treatment of hypophysectomized females, however, has produced conflicting results. LH was ineffective in the catfish and HCG ineffective in the goldfish in initiating vitellogenesis following pituitary ablation (Yamazaki, 1965; Sundararaj and Anand, 1972). LH did, however, restore steroidogenic enzyme activity in hypophysectomized goldfish (Khoo, 1974), and HCG prolongs *in vitro* survival of vitellogenic oocytes in this species (Remacle et al., 1976).

Mammalian gonadotropins are more consistently effective in stimulating gonadal development in male than in female teleosts (M. Fontaine, 1976). LH stimulated testicular development in *Couesius plumbeus* (Ahsan, 1966), *Fundulus heteroclitus* (Pickford et al., 1972), and *Heteropneustes fossilis* (Sundararaj and Mayyar, 1967) following pituitary ablation. Mammalian gonadotropins were also successful in accelerating testicular maturation in several species of intact fish studied (see reviews by de Vlaming, 1974; M. Fontaine, 1976). This, coupled with the fact that these hormones
appear to be more effective in intact than in hypophysectomized females, suggests that additional pituitary factors are necessary for mammalian gonadotropins to influence oocyte development.

Hence the effects of LH and T\textsubscript{4}, alone or in combination, on the maintenance of vitellogenic oocytes following hypophysectomy were investigated. In addition, since mammalian LH is known to have an intrinsic capacity to stimulate the teleost thyroid (Wallis, 1975), plasma samples of treated fish were analyzed for T\textsubscript{4} content. A preliminary experiment, designed to test the ability of LH alone to stimulate ovarian maturation in intact goldfish is included.

MATERIALS AND METHODS

a. Effects of LH in Intact Fish

In July, 1976, goldfish were divided into three groups, and placed in tanks supplied with flowing dechlorinated water at 12°C under natural photoperiod. Gonads of these fish had not matured as they do normally in this species through the late winter and spring months (Yamazaki, 1965), presumably as a result of poor environmental and nutritional conditions at the suppliers. Ten females were sampled at the start of the experiment to check the histological condition of the ovary (initial controls). These fish had very little or no fat, indicating that though the ovaries were immature they were probably not refractory to exogenous stimulation (Nagahama, personal communication).

The three experimental groups were injected three times a week for 21 days with either saline, 1 \( \mu g/g \), or 10 \( \mu g/g \) NIH-LH-S19. The LH was dissolved
in fish saline (Wiebe, 1969), and 0.1 ml of this solution was injected intraperitoneally with a 1.0 ml tuberculin syringe and 30 gauge needle.

The experiment was terminated 24 hours after the last injection. Fish were killed by decapitation after weighing, and portions of each ovary and of the lower jaw were fixed for subsequent histological examination.

b. Effects of LH and Thyroxine in Short-Term Hypophysectomized Fish

In September, 1976, 48 maturing goldfish were hypophysectomized. These fish were of the same stock as those used in Exp. IVa, and had been maintained at 12°C and long photoperiod (16L:8D) to stimulate gonadal development. Two of the fish died shortly after the operation, and the remainder were divided into four groups. The groups were treated with either ovine LH (NIH-LH-S19; 5 μg/g intraperitoneally), T4 (immersion; 10 μg/100 ml), a combination of LH and T4, or saline injection (controls). T4 treatment was begun immediately following hypophysectomy, and half the water was exchanged three times a week and fresh T4 added. LH injections were begun two days after the operation, and repeated three times a week for a total of 12 injections.

The experiment was terminated 24 hours after the last injection and water change. Fish were anaesthetized in MS 222 and blood samples taken from the caudal artery. The fish were then killed by decapitation, and their ovaries and livers removed and weighed; the ovaries were fixed for subsequent histological examination.

Plasma samples were analyzed for T4 levels using the Ames Tetralute kit as described in Section I.
RESULTS

Ovary

Mean values for GSI and maximum oocyte size in intact LH-treated fish are given in Table 8. Control ovaries matured little over the experimental period, while LH administration apparently stimulated ovarian development (Fig. 20 and 21). Differences in GSI and maximum oocyte size are, however, only significant when comparing the 10 μg/g LH group with the controls.

Hypophysectomy resulted in a decrease in both GSI and maximum oocyte size values (Table 9). Treatment with LH or T₄ alone had no effect on post-operative ovarian regression, while a combination of the two hormones appears to have retarded this process slightly but significantly (Table 9). Ovaries of hypophysectomized control and T₄-treated fish contained mainly pre-vitellogenic and atretic oocytes (Fig. 19, 25, and 27), although a few oocytes with small yolk vesicles (less than 8 μm in diameter) were evident in some of the fish studied (Table 9). Vitellogenic oocytes were found in ovaries of most fish treated with LH or LH + T₄, and yolk vesicles in these groups ranged up to 12 and 18 μm in diameter respectively (Table 9; Fig. 26 and 28). Only in the LH + T₄ group, however, did values for maximum oocyte size, GSI, and frequency of vitellogenic oocytes differ significantly from controls.

Thyroid

LH treatment of intact fish resulted in a significant increase in thyroid epithelial cell heights (Table 9; Fig. 22 and 23). This response was greatest at the highest dosage (10 μg/g), and values for this group
TABLE 8. EFFECTS OF OVINE LH ON OVARIAN DEVELOPMENT AND THYROID EPITHELIAL HEIGHT IN INTACT FISH.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>BODY WT (g) ± SD</th>
<th>GSI ± SE</th>
<th>MAX OOCYTE DIAM(µ) ± SE</th>
<th>THYROID EPITH CELL HT(µ) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial control</td>
<td>10</td>
<td>9.3 ± 2.2</td>
<td>1.54 ± 0.21</td>
<td>142 ± 6</td>
<td>-</td>
</tr>
<tr>
<td>Final control</td>
<td>15</td>
<td>10.8 ± 2.2</td>
<td>1.85 ± 0.12</td>
<td>188 ± 18</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>LH (1 µg/g)</td>
<td>12</td>
<td>10.3 ± 2.4</td>
<td>2.13 ± 0.29</td>
<td>266 ± 47</td>
<td>4.0 ± 0.06c</td>
</tr>
<tr>
<td>LH (10 µg/g)</td>
<td>11</td>
<td>11.1 ± 1.7</td>
<td>2.40 ± 0.42</td>
<td>320 ± 59&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4.4 ± 0.16&lt;sup&gt;c,d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>IC p < 0.01  
<sup>b</sup>FC p < 0.05  
<sup>c</sup>FC p < 0.01  
<sup>d</sup>LH (1 µg/g) p < 0.05
**TABLE 9. EFFECTS OF OVINE LH AND THYROXINE ON OVARIAN MAINTENANCE AND PLASMA THYROXINE LEVELS IN SHORT-TERM HYPOPHYSECTOMIZED FISH.**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>*No.</th>
<th>BODY WT * (g) ± SD</th>
<th>GSI ± SE</th>
<th>MAX OOCYTE DIAM (µ) ± SE</th>
<th>PLASMA T₄ ± SE (µg/100 ml)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact control</td>
<td>8</td>
<td>8</td>
<td>15.6 ± 2.77</td>
<td>3.20 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>475 ± 49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.60 ± 0.14 (4)</td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>2</td>
<td>2</td>
<td>9.5, 14.4</td>
<td>2.31, 5.14</td>
<td>310, 670</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPX-control</td>
<td>5</td>
<td>3</td>
<td>13.6 ± 3.3</td>
<td>1.21 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>134 ± 12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.48 ± 0.16 (5)</td>
<td></td>
</tr>
<tr>
<td>HPX-LH</td>
<td>9</td>
<td>7</td>
<td>13.7 ± 1.8</td>
<td>1.30 ± 0.13</td>
<td>151 ± 6</td>
<td>0.83 ± 0.14 (6)</td>
<td></td>
</tr>
<tr>
<td>HPX-T₄</td>
<td>8</td>
<td>3</td>
<td>12.7 ± 2.4</td>
<td>1.17 ± 0.10</td>
<td>140 ± 11</td>
<td>2.35 ± 0.23 (5)&lt;sup&gt;g,h&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>HPX-LH + T₄</td>
<td>9</td>
<td>8</td>
<td>13.6 ± 2.5</td>
<td>1.70 ± 0.13&lt;sup&gt;d,e,f&lt;/sup&gt;</td>
<td>177 ± 8&lt;sup&gt;f,g&lt;/sup&gt;</td>
<td>2.92 ± 0.24 (6)&lt;sup&gt;g,h&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

*Number vitellogenic; HPX-C + HPX-T₄ < HPX-LH + HYP-LH+T₄ p < 0.05 (χ² analysis).

<sup>a</sup>p < 0.01
<sup>b</sup>p < 0.025
<sup>c</sup>p < 0.05
<sup>d</sup>p < 0.05
<sup>e</sup>p < 0.05
<sup>f</sup>p < 0.05
<sup>g</sup>p < 0.01
<sup>h</sup>p < 0.01
Figure 19. Effects of ovine LH and thyroxine on oocyte composition of ovaries of short-term hypophysectomized fish.

The relative frequencies of atretic (A), pre-vitellogenic (PV), and vitellogenic (V) follicles are given for each group. IC = intact control; SH = sham operated; HPX = hypophysectomized. All hypophysectomized groups differ significantly from intact fish at p < 0.01, and the HPX-LH+T_4 group differs significantly from HPX-controls at p < 0.01.
Figure 20. Ovarian section of intact control fish (FC) in Exp. IVa. Only pre-vitellogenic oocytes are evident. Masson's trichrome. X140.

Figure 21. Ovarian section of intact fish treated with LH (10 μg/g) for three weeks showing yolk vesicle (YV) and yolk granule (YG) stage oocytes. Masson's trichrome. X140.

Figure 22. Thyroid follicles (F) from intact control fish (Exp. IVa). Masson's trichrome. X450.

Figure 23. Thyroid follicles (F) from an intact fish treated with LH (10 μg/g) for three weeks. Note hypertrophied follicular epithelium. Masson's trichrome. X450.
Figure 24. Section of intact control ovary showing yolk granule (YG) stage oocyte. Masson's trichrome. X140.

Figure 25. Ovarian section of control fish hypophysectomized for four weeks. Only pre-vitellogenic (PV) and atretic (A) follicles are present. Masson's trichrome. X140.

Figure 26. Ovarian section of hypophysectomized fish treated with ovine LH for four weeks post-operatively. Note early yolk vesicle (YV) stage oocytes. Masson's trichrome. X610.

Figure 27. Ovarian section of hypophysectomized fish immersed in thyroxine for four weeks post-operatively. Masson's trichrome. X140.

Figure 28. Ovarian section of hypophysectomized fish treated with both ovine LH and thyroxine for four weeks post-operatively. Note mid yolk vesicle (YV) stage oocyte. Masson's trichrome. X140.
were significantly greater than those of both the controls and the low (1 μg/g) dosage group. Thus it appears that thyroid activity is stimulated by mammalian LH treatment. However, no conclusions can be drawn as to whether plasma levels of thyroid hormones are in fact elevated in treated fish, since thyroid follicular cell height is strictly a morphological index that does not necessarily reflect the rate of hormone production (Eales, 1964).

Hence, it was decided to measure plasma T₄ levels in experiment IVb to determine whether circulating T₄ levels are indeed elevated by LH treatment. T₄ is detectable in the plasma of hypophysectomized controls at a level not significantly different from intact controls, indicating that hormonal release from the thyroid is not completely abolished by pituitary removal. Administration of LH alone and in combination with T₄ elevated plasma levels over those values of control and T₄-immersed fish respectively; however, these results are not statistically significant, perhaps as a result of the small sample sizes.

Liver

HSI values for intact and hypophysectomized fish are given in Figure 29. In hypophysectomized controls, the mean HSI is elevated over that of untreated intact animals, and treatment with LH or T₄ alone had no effect on this post-operative increase. In those fish receiving a combination of LH and T₄, however, the increase in liver weight following hypophysectomy appears to have been prevented, and HSI values in this group are similar to those of intact controls.
Figure 29. Effects of ovine LH (5 µg/g) and thyroxine (10 µg/100 ml) on the hepato-somatic index of short-term hypophysectomized female fish.

Bars represent mean HSI values, and vertical lines represent standard errors. Numbers adjacent to each histogram represent sample sizes. IC = intact controls; HPX = hypophysectomized. Values for the IC and HPX-LH+T$_4$ groups are significantly less than those of all other groups at p <0.01. ANOVA is significant at p < 0.001.
DISCUSSION

Ovary

The results of Experiment IVa indicate that mammalian LH is capable of stimulating ovarian maturation in intact goldfish. Control ovaries matured only slightly over the experimental period, while ovarian development was accelerated significantly in fish treated with 10 μg/g LH.

LH alone had no effect on preventing ovarian regression following hypophysectomy. This is probably due to the absence of endogenous pituitary factors (gonadotropins), and indicates that LH has only a partial gonadotropic potency in goldfish. The β protein subunits of mammalian LH and piscine gonadotropins differ greatly (Pierce et al., 1976), and, since the β subunit of the glycoprotein hormones confers hormonal specificity in mammals at least (Wallis, 1975), the lack of effectiveness of LH alone in the present study may be explained by phylogenetic differences in structure between the ovine LH preparation and goldfish gonadotropin(s); gonadotropin receptors in the goldfish ovary are presumably more responsive to endogenous hormones. Studies of the goldfish with another mammalian gonadotropin, HCG, show similar results; HCG was unable to initiate vitellogenesis in long-term hypophysectomized fish (Yamazaki, 1965), and was only partially effective in maintaining yolky oocytes in vitro (Remacle et al., 1976).

The inability of T₄ alone to affect ovarian regression in hypophysectomized goldfish supports the hypothesis that thyroid hormones act in synergy with endogenous gonadotropins in their effects on ovarian development in intact fish.

A combination of both LH and T₄ apparently did retard post-operative
atresia. These results are somewhat surprising in light of the fact that 
T$_4$ was present in significant amounts in hypophysectomized, LH-treated fish. 
However, since ovarian development in the latter group was slightly greater 
than in untreated fish, ovarian regression may indeed have been retarded 
but the sampling time was perhaps too late to detect this phenomenon. In 
support of this, Anand and Sundararaj (1972) found treatment with 5 µg/g 
NIH-LH-S16 daily for 20 days partially effective in maintaining yolky 
oocytes in the catfish, Heteropneustes fossilis. In the goldfish, the high 
plasma T$_4$ levels present in the LH+T$_4$ group may have augmented the ovarian 
response to LH, resulting in significantly greater ovarian development in 
this group than in the controls after 28 days.

**Thyroid**

The ability of mammalian gonadotropins to stimulate thyroid function 
in teleosts is well documented (Wallis, 1975). These "heterothyrotropic 
factors", as they have been called, have been shown to induce thyroid 
follicular cell hypertrophy and increase $^{131}$I uptake by the gland in fish 
(Pickford and Grant, 1968; Y. Fontaine, 1969a,b), but the author knows of no 
studies measuring the actual changes in plasma T$_4$ in response to mammalian 
gonadotropins. In the present study, circulating T$_4$ levels were 
slightly but not significantly elevated in hypophysectomized fish following 
ovine LH treatment. This difference may have been greater earlier in the 
experimental period, since the thyroidal response to mammalian TSH, as 
measured by plasma T$_4$ analysis, drops after repeated injection in the brook 
trout (Chan and Eales, 1976); this may be due to a depletion of thyroid 
hormone stores if hormone secretion exceeds hormone production (Chan and 
Eales, 1976). The actual thyroid-stimulating capacity of the LH dosage
used in the present study was equivalent to about 3 mIU TSH/g body weight (this includes both TSH contamination of the LH preparation and the heterothyrotropic ability of the LH molecule itself as calculated from Y. Fontaine, 1969b). In a 15 g brook trout, a dose of 3 mIU/g would raise plasma T\textsubscript{4} levels by 0.5 μg/100 ml or less (Chan and Eales, 1976), and, since circulating T\textsubscript{4} levels in untreated goldfish and brook trout are very similar (see Section I), the LH dosage used in the present study might be expected to raise plasma T\textsubscript{4} levels by a similar amount in a 15 g goldfish. As the results indicate, this was indeed the case.

The fact that plasma T\textsubscript{4} levels in untreated fish hypophysectomized for four weeks fall within the range of values observed in intact controls is surprising, since Chavin (1956) found a severe reduction in thyroidal \textsuperscript{131}I uptake 24 days post-hypophysectomy in the goldfish; the latter would indicate that thyroid hormone synthesis was also greatly reduced. However, hormonal stores may have been released in the absence of pituitary stimulation or de novo synthesis in the present study. In addition, the metabolic clearance rate of thyroid hormones from the plasma is perhaps less in hypophysectomized than in intact fish, resulting in an accumulation of plasma T\textsubscript{4}.

Liver

As in Poecilia latipinna (Ball and Hawkins, 1976), liver weights of untreated goldfish increased following hypophysectomy. This is due to an accumulation of hepatic glycogen and water in the goldfish, with the absolute content of protein and lipid remaining unchanged after pituitary ablation (Walker and Johansen, 1975). Treatment with LH alone had no apparent effect, while T\textsubscript{4} administration augmented slightly the post-operative liver hyper-
trophy. A combination of LH and T₄, however, prevented this increase in hepatic weight, suggesting a synergistic action of the two hormones on liver function. It might be argued that this effect is a result of elevated T₄ levels in the LH+T₄ treated fish, since administration of high doses of T₄ can lead to a depletion of liver glycogen in teleosts (Ball and Hawkins, 1976); this is, however, unlikely, as plasma T₄ values in the LH+T₄ group were only slightly and not significantly greater than in those treated with T₄ alone.
The involvement of the thyroid in gonadal maturation of teleosts has been largely inferred from experiments involving chemical thyroidectomy (Barrington and Matty, 1952; Pickford and Atz, 1957; Matty, 1960; Raizada, 1974), from observations of increased thyroidal activity at the time of gonadal maturation (Berg et al., 1959; Hickman, 1962; Takashima et al., 1972; Singh et al., 1974), and from the stimulation of precocious development of secondary sexual development by thyroid hormones (Grobstein and Bellamy, 1939). In the early part of this study (Hurlburt, 1975), it was found that thyroxine administration in low dosages stimulated ovarian maturation in immature goldfish, while at high dosages a secondary inhibitory influence was evident. Preliminary data on pituitary gonadotropin cell activity in treated fish suggested that the thyroid may stimulate gonadal development through an action on general metabolism or, more specifically, ovarian metabolism, and also may modulate pituitary gonadotropin production via a negative feedback on the hypothalamo-hypophyseal axis (Hurlburt, 1975).

The present study confirms the above hypothesis, and also demonstrates that thyroxine, in the absence of the pituitary, has no effect on the initiation of vitellogenesis or on the maintenance of yolky oocytes. The hormone does, however, augment the ovarian response to gonadotropin stimulation, suggesting that thyroid hormones act synergistically with endogenous gonadotropin in their effects on gonadal development. No conclusions can be drawn as to the activity of gonadotropin in the absence of thyroxine, as both gonadotropin preparations used in this study have apparent thyroid-
stimulating activity.

Thus, thyroid hormones appear to influence gonadal maturation in teleosts through effects at both the pituitary and target organ levels. The following discussion will consider the evolutionary associations of the thyroidal and gonadal systems, and also several of the specific mechanisms by which the thyroid may regulate reproductive processes in teleosts. These include an inhibitory influence of thyroid hormones on pituitary gonadotropin production, and stimulatory effects of thyroxine on aspects of general or ovarian metabolism. In addition, the involvement of the thyroid in liver function will be considered, since liver metabolism is affected both by general metabolic processes (e.g. Walker and Johansen, 1975; Birnbaum et al., 1976; Lewander et al., 1976) and ovarian activity in teleosts.

Pituitary

In the present study, hyperthyroidism resulted in a decrease in gonadotropin cell activity as measured by histological criteria, while goiterogen-induced hypothyroidism stimulated gonadotropin cell function slightly. This supports the findings of Sage and Bromage (1970b) and Hurlburt (1975) and suggests that, as in mammals, thyroid hormones exert a negative feedback on pituitary gonadotropin production in teleosts (see Section II for discussion of possible mechanisms). These effects may be a result of evolutionary associations of the thyroidal and gonadal control systems, and, although a thyroxine-induced suppression of gonadotropin production may not be important physiologically in teleosts, it serves to emphasize the close involvement of the thyroid with reproductive processes in this group.

An inhibitory influence of thyroid hormones on pituitary gonadotropin production has been reported in several vertebrate classes including
mammals (e.g. Larochelle and Freeman, 1974; Freeman et al., 1976), birds (Chandola et al., 1974), and teleosts (Sage and Bromage, 1970b; Hurlburt, 1975). Thyroxine alters TSH production by negative feedback on the pituitary in several teleosts studied (Baker, 1965; Baker, 1969; Sage and Bromage, 1970b), including the goldfish (Peter, 1971), hence it is perhaps not surprising that GTH production is affected in the same way. Evidence points to both a close evolutionary link and a complex, functional interaction between the thyroidal and the gonadal systems. GTH and TSH are structurally related glycoprotein molecules and in mammals contain common α protein subunits, while variations in the β subunits confer hormonal specificity (Wallis, 1975). They may have arisen from a single molecule which performed gametogenic, steroidogenic, and thyrotropic functions by interactions with specific target organs (Hoar, 1975). Divergence of a TSH molecule distinct from GTH has not occurred in cyclostomes, and possibly not in elasmobranchs (see account of Sage [1973] summarized in the introduction to this paper). Although separate TSH and GTH molecules are evident in teleosts and higher vertebrates, their control mechanisms remain closely linked (Sage and Bern, 1971). Hormones regulating both GTH and TSH production originate from similar regions of the hypothalamus in goldfish (Peter, 1970) and, in mammals at least, the structure of hypothalamic thyrotropin-releasing hormone (TRH) is similar to part of the LH-RH molecule (Sage, 1973). A factor similar to mammalian TRH has been identified in the hypothalamus of teleosts (Jackson and Reichlin, 1974), although no substances immunoreactive with mammalian LH-RH has been identified in this group (Crim et al., 1976). Mammalian LH-RH does, however, stimulate gonadotropin production in goldfish, and a gonadotropin-releasing
substance has been identified in the hypothalamus of this species (Crim et al., 1976).

Thyroid hormones and sex steroids influence both GTH and TSH cells in teleosts via common pathways within the brain (Sage and Bromage, 1970b); a similar response occurs in birds (Chandola et al., 1974). In addition, evidence indicates that gonadotropin production is under inhibitory control of thyroid hormones in several avian species, this mechanism perhaps preventing unseasonal gonadal growth in tropical finches (Thapliyal, 1969; Weiseltier and Van Tienhoven, 1971; Chandola et al., 1974) and promoting testicular regression following the spring breeding season in the domestic duck (Jallegeas and Assenmacher, 1974). Thus, a close relationship between gonadal and thyroidal systems is found in teleosts, and this relationship is further supported by evidence from other vertebrate groups.

These results are significant in that they indicate that thyroxine stimulates gonadal maturation by some mechanism other than via a stimulation of pituitary gonadotropin production. Other pituitary effects of thyroxine cannot, however, be excluded. Production of growth hormone, which stimulated gonadal development in the female toad (Billeter and Jorgensen, 1976) and male killifish (Pickford et al., 1972), may be induced by thyroxine in some teleosts (Sage, 1967; Higgs et al., 1976) and mammals (Ishikawa et al., 1976). In addition, TRH appears to regulate prolactin and in some cases growth hormone production in mammals (Chihara et al., 1976; Dannies et al., 1976; Hirvonen et al., 1976; Jeppson et al., 1976). Whether this is true in teleosts as well is unknown; in this group, however, the hypothalamic thyrotropin-controlling factor appears to inhibit rather than stimulate pituitary TSH production (Peter, 1972; Bromage, 1975; Bromage et al., 1976).
Ovary

Thyroxine was effective in stimulating gonadal maturation in intact goldfish but was ineffective in hypophysectomized individuals, indicating that the actions of this hormone on gonadal development are pituitary-dependent. This is most likely explained by a synergistic activity of thyroxine with gonadotropin, rather than a stimulatory action of the former on pituitary function, since thyroxine did augment the ovarian response to SG-G100 in hypophysectomized fish, and its effects on the pituitary appear to be inhibitory in nature. Thyroid hormones and gonadotropin perhaps act synergistically in the stellate sturgeon as well; in this species T$_3$ administration restored the ovarian response to exogenous hypophyseal stimulation following prolonged captivity or cooling of the fish (Detlaf and Davydova, 1974).

A synergistic effect of thyroid hormones and pituitary gonadotropins on ovarian development is well established in mammals. Eartly and Leblond (1954) found thyroxine effective in stimulating testicular function in intact rats but ineffective in hypophysectomized individuals. Thyroidectomy of rats results in a reduction in ovarian weight (Leatham, 1973; Kovacs and Mess, 1976), and the characteristic weight increase of the ovary in response to gonadotropin stimulation is abolished (Johnson and Mites, 1950; Janes, 1954). Histologically, ovaries of thyroidectomized rats treated with gonadotropin several weeks post-operatively contain cystic, atretic, and luteinized follicles with few normally developing oocytes (Janes, 1954). In addition, T$_4$ or T$_3$ added to the medium of porcine granulosa cells stimulated an increase in progesterone production in response to FSH and LH (Channing et al., 1976). Thyroxine treatment of euthyroid rats, however, did little to
alter the ovulatory capacity of the ovary in response to gonadotropins (Dubin, 1974). The above suggests both that a normally functioning thyroid is essential for gonadotropins to stimulate ovarian development, and that gonadotropins in turn are necessary for thyroid hormones to influence gonadal function in mammals.

The nature of this synergistic effect is uncertain, but both indirect and direct actions of thyroxine on the ovary must be considered. Thyroid hormones play an important role in the regulation of general metabolism in mammals; if this is true in teleosts, they might serve to increase the availability of nutrients, metabolites, and yolk precursors necessary for ovarian development. In addition, thyroid activity may affect steroid biosynthesis within the ovary or the extra-ovarian metabolism of these hormones. Since steroids in the goldfish affect both yolk vesicle and yolk granule formation (Khoo, 1974), and appear to regulate liver yolk precursor production in this and some other teleosts (Bailey, 1957; Ishii and Yamamoto, 1970; Plack et al., 1971; Campbell and Idler, 1976), and involvement of the thyroid in steroidogenesis might affect yolk formation.

The influence of thyroid hormones on metabolism in teleosts is still poorly understood. It is known that thyroid hormones affect both growth and development (Novales et al., 1973) and influence liver metabolism (Hochachka, 1962; Ball and Hawkins, 1976; Ray et al., 1976). Regulatory effects of T₄ and T₃ on nitrogen excretion (Ray and Medda, 1976), erythropoiesis (Slicher, 1961; Srivastava, 1976), and plasma lipid components (Takashima et al., 1972) have also been reported in teleosts. However, metabolic responses as measured by changes in oxygen consumption following administration of thyroid hormones, TSH, or anti-thyroid drugs, have been inconsistent in fish
(Higgs and Eales, 1971). Hochachka (1962) suggests that whether or not thyroidal stimulation of metabolic activity is reflected in oxygen uptake could depend on the major metabolic pathway influenced by thyroxine, and upon the predominant oxidation scheme operating at the time. Higgs and Eales (1971) conclude that, although the evidence is still far from conclusive, an involvement of the thyroid in the regulation of metabolic rates in teleosts is likely.

The energy needs of the teleost ovary are great since the ovary may increase from less than 1% to over 20% of the body weight during maturation; hence, the importance of a thyroidal stimulation of general metabolism on ovarian development cannot be overestimated. In mammals, by contrast, this role might be less important, since there is no yolk associated with the ova and the energy requirements of the ovary are minor when compared with teleosts and other lower vertebrate groups.

A direct effect of thyroid hormones on general or specific aspects of ovarian metabolism is a likely possibility in mammals, and perhaps also in teleosts. From the results of the present study it appears that, as suggested by Myant (1964), thyroxine increases gonadal sensitivity to gonadotropic stimulation. Steroid production in mammals is influenced by thyroid hormones; this is possibly a result of increased levels of steroidogenic enzymes, since thyroxine is known to stimulate the formation of many enzymes (Hardy et al., 1960; Snedecor et al., 1972; Goodrich and Adelman, 1976) including those in the steroidogenic pathway (White et al., 1973). In hypothyroid rats, 3β-hydroxysteroid dehydrogenase activity and utilization of ovarian cholesterol for steroid biosynthesis are decreased (Leatham, 1973), and the ovulatory response to gonadotropin is inhibited, leading to the formation of
cystic (anovulatory) follicles (Janes, 1954; Callard and Leatham, 1965). Studies on the steroidogenic capacity of polycystic ovaries induced by treating hypothyroid rats with gonadotropin reveal a dramatic decrease in estrogen production, possibly due to a decreased conversion from androgen intermediates (Callard and Leatham, 1965). Hyperthyroidism, on the other hand, results in an increase in plasma estrogen levels (Southren et al., 1974; Akande, 1975; Olivo et al., 1975). Southren et al. (1974) suggest that thyroid hormones may stimulate ovarian androstenedione production and also increase the activity of 17β-hydroxysteroid dehydrogenase, which converts androstenedione to testosterone and estrone to estradiol. Whether these results are due to a specific action of thyroid hormones on steroidogenic enzyme activity, or to a more generalized stimulation of ovarian cell metabolism as suggested by Channing et al. (1976) remains to be elucidated.

Liver

Thyroxine treatment of both intact and hypophysectomized goldfish tended to increase relative liver weights except in Experiment IVb, while administration of a combination of LH and T₄ prevented post-hypophysectomy liver hypertrophy. The mechanisms mediating these responses in teleosts are uncertain, but may involve effects of thyroxine on hepatic glycogen, protein, and lipid metabolism (Baker-Cohen, 1962; Hochachka, 1962; Takashima et al., 1972; Ball and Hawkins, 1976).

Liver glycogen concentrations in hypophysectomized Poecilia latipinna were unaffected by presumed physiological levels of thyroid hormones (induced by TSH administration) but decreased dramatically by a pharmacological dose of T₄ (50 µg/100 ml immersion - Ball and Hawkins, 1976). This is consistent
with mammalian studies, which demonstrate a depletion of liver glycogen only when excessive dosages of thyroid hormones are administered (White et al., 1973). The latter effect may result from a direct action of thyroid hormones on cellular metabolism, or indirectly by, for example, increasing tissue sensitivity to glucagon (Stouffer and Dunaway, 1976) or adrenalin (Guttler et al., 1975).

However, physiological levels of thyroxine appeared to increase rather than decrease liver weights in the present study. This may result from increased general metabolic rates stimulating a build-up of nutritional stores in the liver. Takashima et al. (1972) found that liver weights increased but relative proportions of hepatic lipid and protein were unchanged following thyroid hormone treatment in rainbow trout, indicating that these constituents may increase in absolute content under thyroid stimulation. In addition, hyperthyroidism in mammals leads to increased ACTH and cortisol secretion, the latter promoting liver glycogen deposition (White et al., 1973). Whether this is true in teleosts is uncertain. Ball and Hawkins (1976) found cortisol production stimulated by mammalian TSH in hypophysectomized P. latipinna, but this was attributed to a direct effect of TSH on the interrenal rather than an indirect effect mediated by the thyroid, since thyroxine administration did not stimulate interrenal function histologically; cortisol levels were not, however, measured following thyroxine treatment.

Treatment of hypophysectomized goldfish with a combination of LH and T₄ prevented post-operative liver hypertrophy, suggesting a synergistic action of the two hormones, either directly or indirectly, on liver function. High physiological doses of T₄ are apparently necessary to elicit this synergistic
effect, since $T_4$ was detectable in the plasma of fish treated with LH alone but liver weight was unaffected.

Mammalian LH may affect liver metabolism in teleosts through a stimulation of ovarian steroid production. Androgen secretion in male fish is augmented by LH or HCG therapy (see review by de Vlaming, 1974), and steroidogenic enzyme activity in ovaries of hypophysectomized goldfish is stimulated by LH (Khoo, 1974) indicating estrogens are being produced. In teleosts, including the goldfish, estrogens appear to act directly on the liver to stimulate production of yolk granule precursors or "vitellin", which consist of phospholipoproteins; these are released into the circulation for transport to the developing oocytes (Bailey, 1957; Ishii and Yamamoto, 1970; Plack et al., 1971; Aida, 1973; Campbell and Idler, 1976). Takashima et al. (1972) suggest that the thyroid is also involved in regulation of circulating vitellin levels, since plasma lipid and lipoprotein components were increased following estrogen treatment and decreased following thyroid hormone treatment in rainbow trout.

Hence LH, through a proposed stimulation of estrogen production, and $T_4$ may have acted synergistically in regulating vitellin metabolism in the present study. The failure of the two hormones to do more than partially maintain vitellogenic oocytes following hypophysectomy is perhaps explained by the absence of endogenous pituitary factors. In amphibia, vitellin uptake by developing oocytes is directly controlled by gonadotropins (Follet and Redshaw, 1974). While studies on yolk uptake in teleosts are limited, Campbell and Idler (1976) demonstrated that a non-glycoprotein pituitary factor is involved in stimulating ovarian yolk accumulation in the winter flounder, suggesting that some substance other than gonadotropins regulate this process in teleosts.
Conclusions

The effects of the thyroid on gonadal maturation are complex, and involve actions at both the pituitary and target organ level. Thyroxine appears to both inhibit pituitary gonadotropin production by a negative feedback on the hypothalamo-hypophyseal axis, and stimulate ovarian development in synergy with gonadotropin. The latter effect may be mediated by a thyroidal stimulation of general metabolism, increasing the availability of nutrients, yolk and steroid precursors utilized in oocyte development, and perhaps through a regulation of plasma vitellin levels. Alternately, thyroid hormones may act directly on the ovary, increasing follicular sensitivity to gonadotropin stimulation or regulating certain aspects of steroidogenesis.

Thyroxine, in high physiological and pharmacological dosages at least, appears to inhibit pituitary gonadotropin cell activity. While this phenomenon may be an important mechanism regulating gonadal development in some avian species, its physiological importance in teleosts is uncertain. Since thyroid activity increases at the same time as gonadotropin cell activity in many teleost species, it is doubtful that the thyroid normally plays a significant role in the regulation of gonadotropin in this group. Ovarian maturation appears to be unaffected except when high pharmacological dosages are administered (see Hurlburt, 1975). At lower thyroxine dosages, gonadotropin production is perhaps not severely reduced, or alternately, thyroxine may increase gonadal sensitivity to gonadotropic stimulation thus compensating for reduced plasma gonadotropin levels.

A synergistic action of thyroxine and gonadotropin on ovarian development is evident in the goldfish. While ovarian maturation was stimulated
in intact fish by thryoxine treatment, the hormone was ineffective in initiating vitellogenesis or maintaining yolky oocytes following pituitary ablation. Ovarian response to exogenous gonadotropins was, however, augmented by thyroxine in hypophysectomized fish, indicating that the thyroid acts to stimulate aspects of gonadotropin-induced gonadal development. This may be via an action of the thyroid on general metabolism, increasing the availability of nutrients and metabolites necessary in oocyte growth, while a direct stimulation by thyroxine of steroidogenic processes within the ovary might enhance yolk vesicle formation and yolk granule precursor production in the liver. Thyroid hormones may also regulate plasma vitellin levels.

The effectiveness of gonadotropin on ovarian development in the absence of thyroxine could not be elucidated in this study. Piscine gonadotropin preparations, as a result of TSH contamination, and mammalian gonadotropins, due to inherent heterothyrotropic activity, both stimulate thyroid activity in teleosts. Surgical thyroidectomy is not possible in most teleosts, since the thyroid tissue is scattered along the ventral aorta and functional heteroptic (extra-pharyngeal) follicles are frequently found. In addition, complete chemical or radiothyroidectomy may have various pharmacological effects on extra-thyroidal tissue. Hence, the question of whether thyroid hormones are essential for gonadotropin to stimulate normal ovarian maturation is difficult to assess.

In conclusion, it is evident that the thyroid plays an important role in the reproductive processes in the goldfish and other teleosts. In view of the many interactions and probable evolutionary links between the thyroid and gonadal systems, however, the problem of the mechanisms mediating these
effects is obviously complex, and the diversity of physiological functions influenced by thyroid hormones leads to the possibilities of both direct and indirect involvement in reproductive processes.
SUMMARY

1. Thyroxine administration by cholesterol pellet implantation, immersion, or feeding raised plasma thyroxine levels in the goldfish, *Carassius auratus*. Immersion was found to be the most effective means of creating sustained physiological thyroxine elevations, while both cholesterol pellet implantation and feeding are practical for flowing water systems where immersion is not feasible.

2. Thyroxine administration to maturing fish resulted in a decrease in gonadotropin cell activity, while goiterogen treatment of immature fish stimulated gonadotropin cell activity slightly.

3. Ovarian maturation of intact mature fish was not affected by thyroxine treatment. In immature fish, thyroxine stimulated oocyte development.

4. Thyroxine alone was ineffective in initiating vitellogenesis or maintaining yolky oocytes in hypophysectomized individuals. The hormone did, however, augment the ovarian response to exogenous piscine or mammalian gonadotropins in both short- and long-term hypophysectomized fish.

5. Thyroxine treatment of both intact and hypophysectomized fish tended to increase relative liver weights. When administered with mammalian LH, however, post-hypophysectomy liver hypertrophy was prevented, indicating a synergistic action of the two hormones on liver function.

6. Piscine and mammalian gonadotropins stimulated thyroid function, as measured by both histological criteria and plasma $T_4$ analysis, in intact and hypophysectomized goldfish.
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