CHAPTER IV

EFFECTS OF LH-FSH-RH AND METHALLIBURE (I.C.I. COMP, 33, 828) ON THE GONADS AND INTERRENAL GLAND OF CLARIAS BATRACHUS.
Introduction:

The presence of gonadotropin releasing hormone (GnRH-FSH-LH-RH) that controls the release of both FSH and LH from the pituitary in mammals has been extensively studied (Schally et al., 1973). It is reported to stimulate the gonadotropin(s) release in fish, amphibians and birds (reviews by Peter, 1978; 1982; Schally, 1978). It is suggested that this decapeptide appears to be non-species specific in its action (Schally, 1978; Mazzi et al., 1974; Licht, 1974). It has been reported that synthetic LH-RH induces ovulation in chickens (Tienhoven and Schally, 1972) and elevates LH levels in the plasma of fowls (Furr et al., 1973). Perfusion of the pituitary, in situ with LH-RH is shown to cause ovulation in winter *T. cristatus carnifex* (Vellano et al., 1974) and spermiation with elevation of testosterone levels in male newts (Andreoletti et al., 1980). A single dose of LH-RH induces spermiation in intact frog (Licht, 1974).

Gn-RH activity has been demonstrated in crude extract of the hypothalamus and telencephalon of some species of teleosts (Breton et al., 1971; Crim et al., 1976; Crim and Evans, 1980). It is well established that hypothalamic extracts from both teleosts and mammals,
synthetic LH-RH and the analogues of LH-RH that are superactive in mammals can stimulate GtH release from the teleost pituitaries in vivo and in vitro (Ball, 1981; Peter, 1982). King and Miller (1980) have suggested that Tilapia GnRH has the potency to stimulate LH release from sheep pituitary cells in vitro. It has been suggested that synthetic LH-RH can be used in studies on fish to gain information on the actions of endogenous GtH (Peter, 1982).

In recent years, the studies on the effect of mammalian and piscine gonadotropins on the gonads have revealed that the mammalian gonadotropin does not act as a complete gonadotropin in fish (Nath, 1978; Nath and Sundararaj, 1979). Nath and Sundararaj (1981) have reported that SGG-100 and c-GtH stimulate vitellogenin and yolk granule production, but mammalian gonadotropins stimulate only vitellogenin production without yolk granule deposition. Recently even piscine gonadotropin is reported to exhibit species specificity (Burlakov and Lapedewa, 1978, Epler et al., 1979; Goetz and Bergman, 1978). Hence a study on the effects of native gonadotropins or using hormones/drugs to stimulate/inhibit the release of endogenous gonadotropin(s) might shed some light on the role of endogenous gonadotropin(s) in the regulation of gonadal functions.
Since last ten to twelve years the effects of LH-RH on the gonadotropin release, oocyte maturation and ovulation has been studied in some species of teleosts. Breton and Weil, (1973) were able to induce gonadotropin release by LH-RH injection in the carp Cyprinus carpio. The first study of the effects of LH-RH on ovulation was conducted on the mature female ayu Plecoglossus altivelis (Hirose and Ishida, 1974). Since then, induced ovulation by LH-RH has been reported in goldfish (Lam et al., 1975), Oryzias latipes (Chan, 1977), Grass carp (Anonymous, 1978), Coho Salmon (Van der Kraak et al., 1983), Atlantic Salmon Salmo salar (Crim et al., 1983). It is reported that LH-RH and its analogues are now routinely used in induced breeding of fishes in China. Successful induction of ovulation has been reported in Grass carp (Ctenopharyngodon idellus), black carp (Mylopharyngodon piceus), Silver carp (Hypothalamichthys molitrix) and spotted silver carp (Aristichthys nobilis) by the Chinese workers (Anonymous, 1977a, b; 1978; Jiang et al., 1980).

There are only a few reports of the effects of LH-RH on spermiation. In mature goldfish, injection of LH-RH or LH-RH \( N^{6} \)-D-Ala caused an increase in plasma gonadotropin concentration but spermiation was not reported (Peter, 1980). Intraperitoneal administration of LH-RH or its analogues...
induced spermiation in Chum Salmon (Oncorhynchus keta) (Donaldson et al., unpublished cited in Donaldson and Hunter, 1983) and atlantic Salmon Salmo Salar (Weil and Crim, 1983). From these studies it is evident that most of the studies of the effect of LH-RH are related to the induction of oocyte maturation and ovulation and there are a few studies on spermiation. However, there are no studies on the effect of LH-RH on the changes in the structure and steroidogenic potential of the ovaries and studies on males are restricted to a very few species, and also there seem to be no reports on the effects of LH-RH on spermatogenesis and Leydig cells, to the best of our knowledge.

Methallibure, a non hormonal, non-steroidal dithiocarbamoyl hydrazine derivative (I.C.I. Comp. 33,828) is known to cause regression of the gonads in vertebrates (Paget et al., 1961; Sainapur et al., 1975; Mohanty and Naik, 1979; Nayak et al., 1983). Hoar et al. (1967) were the first to demonstrate the antigonadotropic effects of the compound in three species of teleosts. Since then it has been shown that methallibure treatment results in a decrease of gonosomatic index, blockage of vitellogenesis and atresia of yolky oocytes in the females (Van Ree, 1976). Oogenesis is blocked by methallibure treatment in Carassius auratus and Gasterosteus aculeatus (Hoar et al., 1967). Cymatogaster
aggregata (Weibe, 1969b) and Tilapia (Hyder, 1972). In males methallibure causes retardation of spermatogenesis and atrophy of the Leydig cells resulting in the regression of the testis (Martin and Bromage, 1970, Hyder et al., 1974; Chiba et al., 1978). It is reported that methallibure inhibits the transformation of spermatogonia to spermatocytes in the teleost testis (Hoar et al., 1967; Billard et al., 1970; Martin and Bromage, 1970; Mackay, 1971, 1973; Hyder, 1972). The studies of the effect of methallibure at the pituitary level have revealed that it blocks the synthesis and/or release of gonadotropin (Leatherland, 1969; Pandey and Leatherland, 1970; Mackay, 1971; Breton et al., 1973; Van Ree, 1976). Moreover species specificity of methallibure action is reported in mammals (Paget et al., 1961; Kar et al., 1968) and teleosts (Pandey, 1970). From these studies it is evident that methallibure affects the structure and function of the testis and the ovary by inhibiting the pituitary gonadotropin secretion.

The role of interrenal during sexual maturation and spawning has been studied in some fishes. Hypertrophy and hyperplasia of the interrenal tissue and also rise in plasma corticosteroid levels have been observed in the salmonids during the spawning season (Robertson et al., 1961, Schmidt and Idler, 1962). In the Indian catfish Heteropneustes fossilis, hyperactivity of the interrenal cells during the
breeding season is reported (Singh et al., 1974). Investigations in fish have demonstrated that certain steroids are capable of inducing oocyte maturation in vivo (Kirshenblat, 1959; Sundararaj and Goswami, 1966). In several species corticosteroids are effective in inducing final oocyte maturation and ovulation (Goswami and Sundararaj, 1971; 1974; Hirose, 1972; Van Ree, 1977). In the male catfish *H. fossilis* it is observed that the interrenal cells are stimulated following castration or LH treatment accompanied by the hyperactivity of the seminal vesicles (Sundararaj and Nayyar, 1969; Nayyar and Sundararaj, 1969), furthermore a high dose of cortisone (1000 mg/kg) induced spermiation in goldfish (Billard, 1976). All these studies indicate that there is a correlation between the interrenal and gonadal cycles and that the corticosteroids are involved in follicular maturation and ovulation. Therefore it is reasonable to assume that the interrenal steroids might affect/influence the gonadal activity. However, it is not known whether the stimulation or inhibition of gonadal activity is also accompanied by any changes in the interrenal cells. Considering the fact that gonadotropins influence the interrenal function, it was felt necessary to study the effect of two compounds, one which releases the gonadotropin from the pituitary gland and the other which inhibits its release, on the interrenal gland.
In view of these facts, the present study was designed to study the effects of LH-FSH-RH and methallibure on the testis, the ovary and the interrenal tissue in *Clarias batrachus*.

**Material and Methods:**

Adult *Clarias batrachus* of both the sexes were collected from the areas surrounding Mangalore city during the breeding season (June/July) and were transported live by road to Dharwad. They were acclimatized initially in cement cisterns for about 4 to 5 days. Fish of comparable size and weight were selected and again acclimatized in glass aquaria for 3 to 4 days. The detailed acclimatization procedure is already described in Chapter II. The fish belonging to the first group, 6 males and 6 females were autopsied on the day of the commencement of the experiment, as the initial controls. The experimental design is shown in Table 4.1. The remaining fish were divided into three groups of males and females each containing 8 fish. The second group of males and females received 0.1 ml of 0.6% saline and served as the parallel controls. The third group fish received 1 μg of synthetic lutening-hormone releasing hormone (LH-FSH-RH, NICHD, U.S.A., Batch#2) in 0.1 ml saline. Males and females of the fourth group were administered with 1 mg methallibure in 0.1 ml saline, as a suspension. All the
injections were administered through the intraperitoneal route every day for 8 days (a total of 8 injections). Every day before the injection the fish were gently stripped by stroking the abdomen to observe ovulated eggs or milt, if any. The fish were autopsied one day after the last injection.

Before autopsy, the fish were weighed and sacrificed by severing the spinal cord. The testes or the ovaries were dissected and weighed to the nearest milligram and fixed in aqueous Boun's fluid. The head kidneys containing the interrenal tissue were also fixed in aqueous Boun's fluid. The tissues were processed in graded series of ethanol, cleared in Benzene and embedded in Paraffin wax. 5 to 7 μm thick sections of the testis and the ovary and 4 to 5 μm thick sections of the head kidneys were cut and stained with Hematoxylin and Eosin.

The spermatogenetic stages were identified as spermatogonia, spermatocytes and spermatids as described earlier (Rao, 1980). The cell nests of different spermatogenetic stages in a lobule were counted. In addition, the presence, abundance or depletion of sperms were noted by eye estimations. A minimum of 5 lobules per cross section of the testis were randomly selected for counting the cysts of different spermatogenetic stages. Five such sections
were considered for each animal (a total of 25 lobules/fish). The Leydig cell morphology was noted and nuclear diameter of the Leydig cells was measured using an ocular micrometer under 1000x magnification which was later calibrated against a stage micrometer for the conversion of the ocular reading into micrometers (µm).

The oocytes were identified as Stage I, Stage II and Stage III as described by Sundararaj and Sengal (1970). The number of oocytes belonging to the different stages and the atretic oocytes were counted. A minimum of 25 sections per animal were counted. In addition, the presence of post-ovulatory follicles was noted. In the interrenal tissue, the nuclear and cellular diameter of the interrenal cells and the number of interrenal nuclei in a unit area were recorded under 1000x magnification. Means of controls and experimental groups were compared using Student's t test (Steel and Torrie, 1960).

For the histochemical localization, $5\Delta^{3\beta}$-HSDH and $17\beta$-HSDH enzyme activities, pieces of the testis, ovary and head kidney were quickly frozen over dry ice vapours and 14 to 16 µm thick sections were cut on a "Pearse-Slee" cryostat maintained at -18°C. The sections were thawed at room temperature and then incubated in appropriate media. The preparation of the incubation media is already described
in Chapter I. The intensities of the reactions were judged by the amount of diformazan granule deposition and visually graded from (+) trace reaction to (+++) intense reaction. All chemicals used were of analytical grade obtained from Sigma Chemical Co., U.S.A.

Observations:

I. Histology of the testis:

Initial Controls: The testis were enlarged and opaque with a few whitish patches. Histology of the testis revealed the presence of irregular seminiferous lobules and interstitial Leydig cells (Fig. 4.1). The enlarged seminiferous lobules contained mainly sperms and cysts of spermatogonia, spermatocytes and spermatids. The mean number of different spermatogenetic stages are presented in Table 4.3.

The interstitial tissue was compressed due to the enlargement of the seminiferous lobules and contained Leydig cells, either singly or in groups. The cell boundary of the Leydig cells was not clear, but their nuclei were round, vesicular and hypochromatic with distinct nucleoli (Fig. 4.1).

Saline Controls: The gonosomal index and the structure of the testis in the saline treated group did not differ.
from that of the initial control fishes. The mean number of spermatogonia, spermatocytes and spermatids did not significantly differ from those of the initial controls (Table 4.3, Graph 4.2). However, there was a significant increase in the diameter of the Leydig cells when compared to that of the initial controls (Table 4.2, Graph 4.1).

**LH-FSH-RH treated fish:** The testis of the fish treated with LH-FSH-RH were enlarged. There was a further enlargement of the seminiferous lobules. The relative weight of the testis also increased, but was not statistically significant (Table 4.2, Graph 4.1).

The seminiferous lobules contained the cell nests of different spermatogenetic stages. The testis predominantly contained sperms (Fig. 4.2). Spermiation was observed in all the fish with milt oozing out on gentle stripping after 3 to 4 injections. The quantitative analysis revealed that there was a significant decrease in the number of spermatocytes when compared to that of the saline injected controls (Table 4.3, Graph 4.2). The number of spermatogonia were also reduced, however, the decrease was not statistically significant when compared to corresponding stages in the saline injected controls (Table 4.3, Graph 4.2).
The nuclei of the Leydig cells were enlarged in size, round, vesicular and the nucleoli were distinct (Fig. 4.2). There was a significant increase in the nuclear diameter of the Leydig cells when compared to that of the controls (Table 4.2, Graph 4.1). Therefore, it was quite evident that the Leydig cells were stimulated following LH-FSH-RH treatment.

**Methallibure treated fish:** Eight days after methallibure treatment, a marked reduction in the size of the testis with a significant decrease in the relative testis weight when compared to that of the saline treated controls was observed (Table 4.2, Graph 4.1). Administration of methallibure induced regressive changes in the testis.

No spermiation was observed on stroking of the abdomen. The size of the seminiferous lobules was reduced. The seminiferous lobules contained degenerating sperms and cell debris (Fig. 4.3). There was a significant decrease in the number of spermatocytes and spermatids when compared to those of the controls, but spermatogonial number remained unaffected (Table 4.3, Graph 4.2).

The interstitium was enlarged and the Leydig cells were atrophied. The Leydig cells contained dark, solid hyperchromatic nuclei and the nucleoli were indistinct.
(Fig. 4.3). There was a significant decrease in the
diameter of the Leydig cell nuclei when compared to that of
the saline treated controls (Table 4.2, Graph 4.1).

II. Steroidogenic activity of the testis:

A moderately strong $^5\Delta^-3\beta$-HSDH (Fig. 4.4) and a
moderate $17\beta$-HSDH activities were observed in the Leydig
cells of the control fish. The intensities of the two enzyme
activities were increased after LH-FSH-RH treatment (Fig.4.5).
Administration of methallibure resulted in a decrease in the
intensities of both $^5\Delta^-3\beta$-HSDH (Fig. 4.6) and $17\beta$-HSDH
activities (Table 4.2).

III. Histology of the ovary:

Initial controls: The ovaries were highly enlarged,
brownish-red in colour, and well vascularized. Stage III
oocytes were found in large numbers, however, Stage I and
Stage II oocytes were also observed (Fig. 4.7). In addition,
the ovary contained vitellogenic atretic follicles in various
stages of resorption. The Gonosomatic Index and percentages
of different stages of the oocytes is presented in
Table 4.5 and Graph 4.3 & 4.4.
Saline controls: The general structure of the ovary in the saline treated fish was essentially similar to that observed in the initial controls. The gonosomatic index, the number (%) of the different stages of oocytes and the atretic follicles did not significantly differ, when compared to the corresponding parameters of the initial controls (Table 4.4, Graph 4.3 & 4.4).

LH-FSH-RH treated fish: Treatment of 1 μg of LH-FSH-RH for 8 days resulted in an increase in the ovarian weight, however, the relative weight of the ovary was not statistically significant when compared to that of the saline control fishes (Table 4.4, Graph 4.3). Light stroking of the abdomen after 3 to 4 injections resulted in the oozing of the eggs suggesting that ovulation has taken place. Per cent counting of the different oocyte stages revealed that there was a significant decrease in the percentage of Stage I and a significant increase in the percentage of Stage III oocytes when compared to those of the saline controls (Table 4.4, Graph 4.4). The increase in the percentage of Stage II oocytes was not statistically significant (Table 4.4, Graph 4.4), the number of atretic oocytes was reduced significantly (Table 4.4, Graph 4.4). In addition, postovulatory follicles were observed (Fig. 4.8). However, the number of postovulatory follicles were not counted.
Methallibure treated fish: Administration of methallibure resulted in a significant reduction of the gonosomal index. There was a drastic reduction in the size of the ovary. There was an extensive follicular atresia of the vitellogenic follicles. The incidence of atresia was so severe that hardly any normal Stage II and Stage III oocytes were encountered (Fig. 4.9) (Table 4.4). There was a significant increase in the number of Stage I oocytes when compared to that of the saline treated controls.

IV. Steroidogenic Potential of the ovary:

Histoenchemical localization of $\Delta^5$-3$\beta$-HSDH and 17$\beta$-HSDH enzyme activities revealed that there was a strong reaction for $\Delta^5$-3$\beta$-HSDH (Fig. 4.10) and a moderate 17$\beta$-HSDH enzyme activity in both the special thecal cells and the interstitial gland cells. The intensities of the two enzyme activities were similar in the saline controls and the initial controls (Table 4.5). Administration of LH-FSH-RH resulted in an increase in the steroidogenic potential of the ovary. The intensities of the two steroid converting enzymes, both in the special thecal cells and the interstitial gland cells were markedly increased (Fig. 4.11) (Table 4.5). In some preovulatory follicles the reaction was seen in the granulosa cells as well. In methallibure
treated fish there was decrease in the localization of the diformazan granules, suggesting a reduction in the activity of steroid dehydrogenases. Enzyme activity was difficult to visualize in the normal follicles in methallibure treated fish because of the presence of large number of atretic follicles in the ovary. However, the intensities of the two steroid dehydrogenases seemed to be decreased in the preovulatory follicles (Fig. 4.12). The intensities of the two enzyme activities appeared to be more in the atretic follicles (Table 4.5)

V. Histology of the interrenal gland:

**Initial controls:** The interrenal cells were elongated and contained basal nuclei with distinct nucleoli (Fig. 4.13). The mean diameter of the interrenal cells and its nuclei and the number of interrenal nuclei per unit area are presented in Tables 4.6 and 4.7 & Graph 4.5 and 4.6.

**Saline controls:** The interrenal cells of the saline controls were similar to those observed in the initial controls, but in the female fish there was a significant increase in nuclear and cell diameter when compared to that of the initial controls. However, there was no statistical difference in the males in these parameters. The number
of interrenal nuclei per unit area were not significantly different from those observed in the initial controls in both the sexes (Tables 4.6 & 4.7, Graph 4.5 & 4.6).

**LH-FSH-RH treated fish:** Administration of LH-FSH-RH resulted in a stimulation of the interrenal cells. There was a significant increase in the nuclear diameter and a significant decrease in the number of nuclei per unit area when compared to that of the saline controls (Table 4.6 & 4.7, Graph 4.5 & 4.6) (Fig. 4.14) but the increase in the cell diameter of the interrenal cells in the female was significant while it was not significant in the males (Tables 4.6 & 4.7, Graph 4.5 & 4.6).

**Methallibure treated fish:** Methallibure treatment resulted in marked regressive changes in the interrenal cells (Fig. 4.15). There was a significant reduction in the nuclear diameter of the interrenal cells and a significant increase in the number of interrenal nuclei per unit area when compared to the corresponding parameters in the saline controls (Tables 4.6 & 4.7, Graph 4.5 & 4.6). However, the increase in the cell diameter in the interrenal cells was statistically significant in the females but not in the males (Tables 4.6 & 4.7, Graph 4.5 & 4.6).
VI. Histochemical study of the interrenal gland:

Histochemical study of the interrenal gland revealed a strong $\Delta^5-3\beta$-HSDH (Figs. 4.16) and a moderate $17\beta$-HSDH enzyme activity in the interrenal cells of the control fishes in both the sexes. There was a appreciable increase in the intensities of the two enzyme activities after LH-FSH-RH treatment (Fig. 4.17). Metnaillbure administration resulted in a reduction in the intensities of activity of the two steroid dehydrogenases (Fig. 4.18) when compared to that of the controls (Tables 4.6 & 4.7). Out of the two substrates (pregnenolone and DHEA) used for the demonstration of $\Delta^5-3\beta$-HSDH activity, DHEA was better utilised than pregnenolone. Similarly, $17\beta$ estradiol was better utilised than testosterone for the demonstration of $17\beta$-HSDH activity in the interrenal cells.

Discussion:

Effects of LH-RH: In mammals, natural LH-RH as well as a synthetic decapptide corresponding to the structure of LH-RH is shown to induce the release of FSH and LH from the hypophysis and that the synthetic decapptide has no species specificity with regard to its action (Schally et al., 1973; Schally, 1978). In teleosts crude extracts of the hypothalamus and the telencephalon have been shown to possess
Gn-RH activity in several species (Breton et al., 1971; Crim et al., 1976; Crim and Evans, 1980). It is well established that the hypothalamic extracts from both teleosts and mammals, synthetic LH-RH and the analogues of LH-RH (those are superactive in mammals) can stimulate GtH release from teleost pituitaries in vivo and in vitro (Ball, 1981; Peter, 1982, 1983).

The synthetic gonadotropin-releasing hormone is known to induce the release of gonadotropin from the hypophysis in different species of non-mammalian vertebrates. It has been reported that synthetic LH-RH induces ovulation in chickens (Tienhoven and Schally, 1972) and elevates plasma LH levels in fowls (Furr et al., 1973). Perfusion of pituitary, in situ with LH-RH is shown to cause ovulation in females and mobilization of sperms cysts, a slight precocious activation of spermatogenesis and elevation of plasma levels of androgen in the males of the newt T. cristatus carnifex (Vellano et al., 1974; Andreoletti et al., 1980). In vitro addition of homogenates of hypothalamus or LH-RH to the incubation medium causes release of gonadotropin from the pituitary of the frog Rana pipiens (Thornton and Gesnwend, 1974). Administration of Gn-RH to PMS treated toad Xenopus laevis is shown to cause ovulation whereas treatment of 1 mg Gn-RH thrice a day for 4 days causes initiation of
vitellogenesis in the toads with initially regressed ovaries (Thornton and Geshwind, 1974). Administration of LH-RH causes spermiation in intact frog *H. regilla*, but not in hypophysectomized frogs (Lucht, 1974). These results indicate that LH-RH has the ability to induce the release of gonadotropin(s), which in turn influence the gonads in different groups of non-mammalian vertebrates.

LH-RH, (natural, synthetic and its analogues) has been used to induce ovulation in teleosts. The first study of the effect of LH-RH on ovulation in teleosts was conducted by Hirose and Ishida (1974), on mature female ayu, *Plecoglossus altivelis*, where with a single dose of intraperitoneal injection of 50, 100 or 200 mg/fish, 40%, 50% and 80% of the fish ovulated at low, medium and high dose respectively, after two days. Lam et al.,(1975) were able to demonstrate ovulation in female mature goldfish with intraperitoneal injection of 10 mg/kg or intra cranial injection at 2 mg/kg where, in the former case 75% fish ovulated while in the later the ovulation was 100%. The Japanese plaice *Limanda yokohame* responded to intra muscular injection of emulsified LH-RH, but not when injected with a similar dose in saline (Aida et al., 1978). In the common carp, repeated intrahypophyseal injection of 1 mg/kg of LH-RH caused germinal vesicle breakdown but not ovulation
(Sokolowska et al., 1978). In the Pacific Salmon, injection of LH-RH a D-Ala resulted in a dose related rise in plasma gonadotropin that was sustained for 96 hr (Van der Kraak et al., 1983) and in combination with 0.1 mg/kg SGG-100 was effective in inducing ovulation (Van den Kraak et al., 1982). These results indicate that ovulation can be induced in teleosts with natural, synthetic LH-RH and its analogues.

In the present study, 1 μg synthetic LH-RH/fish/day for 8 days induced ovulation. The appearance of Δ5-3β-HSDH activity in the granulosa cells of the preovulatory follicles indicates that these follicles were on the way to ovulation and might have ovulated in the course of time, as the appearance of Δ5-3β-HSDH activity in the granulosa cells just prior to ovulation is considered to be a good indicator of transformation of mature follicles from preovulatory to ovulatory follicles as a result of increase in the overall ovarian steroidogenesis. (Tikare, 1981, Lambert and Van den Hurk, 1982). Per cent count of the oocytes indicated that the Stage I oocytes decreased significantly and Stage III oocytes increased significantly in number. This indicates that LH-RH has accelerated the growth of the smaller follicles to the ovulatory size, and also induced ovulation. These observations indicate that LH-RH activates the process of
vitelloyensis, maturation and ovulation and also increases the steroidogenic potential, probably by increasing the release of endogenous gonadotropin(s) from the pituitary. Similar results have been obtained for the Japanese Medaka, Oryzias latipes (Chan, 1977) where in LH-RH treatment increased the gonosomatic index and the number of yolky oocytes.

The literature on induced spermiation in teleosts using LH-RH or its analogues is very sparse (Donaldson and Hunter, 1983). In the mature male goldfish, injection of LH-RH or LHRHa D-Ala⁶ induced an increase in plasma gonadotropin concentration, but did not induce spermiation (Peter, 1980). In the carp, Cyprinus carpio spermiation was induced following the injection of LH-RH or its alanine⁶-LH-RH ethylamide analogue. The response was maximum at the dose of 50 μg/Kg b.wt. of LH-RH and 10 μg/Kg of LHRH A (Billard et al., 1983 a, b). In the landlocked Salmon Salmo salar administration of LH-RH or LH-RH-ethylamide either by intraperitoneal, multiple injection in saline or propylene glycol or by single intraperitoneal implant in silicone tubes or a single Cholesterol pellet were effective in inducing spermiation (Weil and Crim, 1983), the volume of the sperm produced was correlated with plasma gonadotropin concentration (Weil and Crim, 1983).
In the chum salmon *Oncorhynchus Keta* two intraperitoneal injections of 0.5 mg/kg LH-RH a D-Ala₆, 72 hr apart induced spermiation (Donaldson *et al.*, unpublished cited in Donaldson and Hunter, 1983).

In the present study administration of 1 µg LH-FSH-RH to mature males of *C. batrachus* in the breeding season induced spermiation with milt oozing on light stroking of the abdomen. Quantitative study of the spermatogenesis revealed that the number of spermatocytes decreased significantly with increased number of spermatozoa and spermiation indicating a rapid conversion of the spermatocytes to spermatozoa. The Leydig cells were stimulated as indicated by the increased nuclear diameter and increased activity of the steroid dehydrogenases. These results suggest that even the low dose of 1 µg LH-RH can significantly stimulate spermatogenesis and spermiation as well.

Androgens are known to stimulate spermatogenesis and spermiation (Lofts *et al.*, 1966; Pandey, 1969; Yamazaki and Donaldson, 1969; Sundararaj *et al.*, 1971; Billard, 1976, De - Clerq *et al.*, 1977 and Rao, 1980), and it is suggested that the gonadotropic influence on spermatogenesis may be through the production of a specific steroid by the testis rather than the direct effect of gonadotropin(s)
The increased hydroxysteroid dehydrogenase activity of the Leydig cells after LH-RH treatment in the present study in *C. batrachus*, suggests probable increased steroid output. It is known that testosterone stimulates spermatogenesis and spermiation in intact *C. batrachus* (Rao, 1980) and the results obtained in the present study lend further support to the hypothesis that in teleosts, gonadotropin(s) regulate spermatogenesis and spermiation through androgens.

**Effects of methallibure:** Methallibure, a non steroidal, non hormonal compound is shown to have antigonadotropic effects in mammals (Paget et al., 1961), birds (Nayak et al., 1983), reptiles (Mohanty and Naik, 1979) and amphibians (Saidapur et al., 1975). Administration of methallibure resulted in selective inhibition of pituitary gonadotropic functions in rats of either sex; in dogs and monkeys, it prevents the appearance of castration cells in the pituitary of castrated males and inhibits menstruation in monkeys (Paget et al., 1961). Methallibure inhibits ovulation in hens (Imai, 1972). In the pigeon, administration of methallibure to five day old nestlings resulted in a reduction in \[ \Delta-3\beta\text{-HSDH} \] and G-6-PDH activity in the Leydig cells (Nayak et al., 1983). It causes, regression of B₂ (FSH) and B₃ (ICSH) cells of the pars distalis in the lizard *Calotes versicolor* (Mohanty and Naik, 1979).
reduction in relative testis weight and regression of androgen dependent thumbpads in the frog *Rana cyanophlyctis* (Saldapour et al., 1975), decrease in the spawning rate and secretion of jelly in the oviduct of female *R. cyanophlyctis* (Kanakaraj and Gangadhar, 1967).

In teleosts, Hoar et al. (1967) first demonstrated the antigonadotropic effects of metalliboure in *Carassius, auratus, Cymatogaster aggregata* and *Gasterosteus aculeatus*. Oogonogenesis is blocked in *Carassius* and *Gasterosteus* (Hoar et al., 1967), *Cymatogaster aggregata* (Wiebe, 1969b) and *Tilapia* (Hyder, 1972) following the administration of metalliboure. Metalliboure is reported to cause a decrease in the proportion of yolky oocytes and increase in non yolky and atretic follicles in the ovary of *Hypreliotris galli* (Mackay, 1973). It inhibits ovulation, blocks vitellogenesis and induces atresia with decreased steroidogenic potential in female Zebra fish *Arachydano rerio* (Van Ree, 1976).

In the present study, metalliboure treatment for 8 days resulted in a decrease in the gonosomatic index. The ovary was characterized by the presence of large number of atretic follicles in different stages of resorption. The incidence of atresia was so drastic that hardly any
normal Stage II and Stage III oocytes were present. There was an increase in the number of Stage I oocytes. The steroidogenic potential of the ovary was diminished as indicated by decreased steroid dehydrogenase activity. These observations indicate that methallibure induces regressive changes and decreases the steroidogenic potential of the ovary. The observations are in agreement with those reported earlier in other teleosts (Mackay, 1973; Van Ree, 1976; Cho et al., 1978).

It is known that the synthesis of steroid hormones in the gonads is regulated by the pituitary (Hoar, 1969; Pickford et al., 1972; Lambert and Oordt, 1974; Van Ree, 1976) and it is reported that $5\Delta-3\beta$-HSDH activity in the ovary increases after gonadotropin treatment (Tikare, 1981; Lambert and Van den Kurk, 1982). Similarly Van Ree (1977) has reported enhanced activities of $5\Delta-3\beta$-HSDH and G-6-PDH in the ovary following LH administration to methallibure treated Brachydanio rerio. On the other hand, in Cyprinus carpio a decreased $3\beta$-HSDH activity is observed in the ovary after the treatment with methallibure, an antigonadotropic agent (Kapur and Toor, 1978).

It is known that in mature Clarias, during the prespawning and spawning periods, certain thecal cells of the preovulatory and ovulatory follicles and the interstitial
gland cells show the presence of a moderate activity of  
$\Delta^5$-3$\beta$-HSDH and 17$\beta$-HSDH which increases as the follicles  
are just about to ovulate (Tikare, 1983; Lambert and  
Van den Hurk, 1982). In the present study, there was a  
reduction in the activities of the two hydroxysteroid  
dehydrogenase in the thecal cells and the interstitial  
gland cells in the fish treated with methallibure. It is  
suggested that the reduction in the hydroxysteroid dehydro-  
genases in the ovaries may be due to inhibition of gonado-  
tropin secretion as a result of antigonadotropic activity  
of methallibure. Our observations are in agreement with  
the earlier report in Cyprinus carpio (Kapur and Toor,  
1978) and also provides additional support to the concept  
that steroidogenesis in the teleost ovary is regulated by  
pituitary gonadotrophin(s).  

It is reported that methallibure treatment in males  
duces retardation of spermatogenesis and atrophy of the  
androgen producing cells resulting in the regression of  
the testes (Hyder et al., 1974; Chuba et al., 1978). It  
has been shown that methallibure inhibits the transforma-  
tion of spermatogonia to spermatocytes in the teleost  
testes (Hoar et al., 1967; Wiebe, 1968; Billard et al.,  
have shown that type B spermatogonia, meiosis and spermatation
are gonadotropin dependent in the goldfish which are inhibited after methallibure treatment. Billard et al., (1971) have shown that all the stages except type A spermatogonia are affected by methallibure treatment.

In the present study, methallibure caused a significant reduction in the gonosomatic index in the male fish. There was a significant decrease in the number of spermatocytes and spermatids and also inhibition of spermiation. The degeneration of the sperms and cell debris in the lumen of the seminiferous lobules were observed. These observations suggest that the conversion of spermatogonia to spermatocytes and spermatocytes to spermatids are blocked by methallibure treatment, indicating that the conversion of spermatogonia to spermatocyte, and spermatocytes to spermatids might be dependent on pituitary gonadotropin(s). The inhibition of spermiation may also be consequence of decreased gonadotropin secretion. However, the spermagonial number is not affected and this observation is in agreement with the report of Billard et al. (1971).

It is well established that the Leydig cells in the testis are the primary source of testosterone (Guraya, 1976a; Callard et al., 1978). It is reported that $\Delta^5$-3P-HSDH activity in the Leydig cells of teleost is gonadotropin dependent (Yamazaki and Donaldson, 1969;
de Valming, 1974; Guraya, 1976). Kapur and Toor (1978) have demonstrated a decrease in the 3β-HSDH activity in the testis of the carp *Cyprinus carpio* after metnallibure treatment in a time dependent manner, further they have reported an increase in the enzyme activity after gonadotropin administration to metnallibure treated fish (Kapur and Toor, 1979), thus providing evidence, albeit indirect, that the steroidogenic activity of the Leydig cells is gonadotropin dependent.

In the present study, a marked decrease in the 5Δ-3β-HSDH and 17β-HSDH activities in the Leydig cells of metnallibure treated suggests a decreased androgen output and also that steroidogenesis in the testis of *C. batrachus* is dependent on gonadotropin and thus confirm the earlier report on *C. batrachus* (Rao et al., 1979). Our observations are also in agreement with those of Yamazaki and Donaldson (1969), de Valaming (1974), and Guraya (1976a).

There are evidences to show that the action of metnallibure are mediated through the pituitary. Paget *et al.* (1961) have noted that metnallibure treatment prevents the appearance of the castration cells in the pituitary of castrated rats. In teleosts, it is noted that metnallibure causes a decrease in the gonadotropin synthesis in *Cymatogaster* (Leatherland, 1969), *Poecilia reticulata*.
(Pandey and Leatherland, 1970) and Tilapia mossambica (Chuba et al., 1978). de Valming and Licht (unpublished cited in de Valming, 1974) have found that the pituitaries of the methallibure treated fish were more effective in stimulating in vitro ovulation in the ovarian fragments of Gillichthys and suggested that methallibure might inhibit the release of gonadotropin from the pituitary. So it appears that methallibure acts at the level of the pituitary, preventing the synthesis and/or release of gonadotropin in C. batrachus, however, a direct action of this compound cannot be ruled out as opined by Singh et al. (1977).

Interrenal gland: The adrenal cortex of mammals is controlled by the pituitary ACTH, however, recent studies have shown that gonadotropins can as well influence the adrenal cortex (Kime et al., 1981). In the marsupial Trichosurus, treatment with PMS or HCG elevated circulating adrenal 17β-hydroxy androgens levels (Kime et al., 1981). Phillips and Poolsanguan (1978), have shown that intravenous injection of ovine LH in female rats significantly increases corticosterone output within 1-2 minutes suggesting a direct action of the hormone at the adrenal level. There are a few reports wherein LH-RH is shown to affect the adrenal gland in mammals. A rise in plasma cortisol level
in response to a stimulus of LH-RH has been reported in some patients with pituitary dependent Cushing's syndrome (Matsakura et al., 1977; Pieters et al., 1979; Okai et al., 1981). Recently it has been shown that administration of LH-RH in normal dogs results in a significant increase in the plasma concentration of cortisol (Stolp et al., 1982). These results suggest that LH-RH has some effect on the secretory activity of the adrenocortical cells of mammals.

In the frog, *Rana cynamophlyctis*, LH-RH administration stimulated the interrenal cells (Yajurvedi, 1984). There are no report on the effects of LH-RH on the secretory activity of the adrenal gland in fish to the best of our knowledge.

In the present study it is observed that administration of LH-RH stimulated the interrenal cells with an increase in the intensities of the steroid converting enzymes. On the other hand, methallibure administration caused regression of the interrenal cells with decreased steroidogenic potential in both the sexes. Since gonadotropin secretion is stimulated by LH-RH in teleosts (Peter, 1980) and methallibure causes a decrease in its secretion (Breton et al., 1973), it is suggested that the increased titres of gonadotropin(s) might have stimulated the
interrenal cells and the decrease in gonadotropin secretion after methallibure treatment might have caused the regression of the interrenal cells. Our study further suggests that not only high titres of gonadotropin cause stimulation of the interrenal cells, but also that a fall in gonadotropin level causes regression. However, this suggestion needs further elucidation. It is not known whether the corticotrophs are affected after LH-RH or methallibure treatment. Further studies are obviously needed to find out the changes (if any) in the corticotrophs after LH-RH and methallibure treatment in teleosts.


<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Duration</th>
<th>Parameters studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Initial controls</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Testis</td>
</tr>
<tr>
<td></td>
<td>6 Males</td>
<td></td>
<td></td>
<td></td>
<td>Histology</td>
</tr>
<tr>
<td></td>
<td>6 Females</td>
<td></td>
<td></td>
<td></td>
<td>Histochemistry</td>
</tr>
<tr>
<td>2.</td>
<td>Saline controls</td>
<td>0.6%</td>
<td>0.1 ml</td>
<td>8 days</td>
<td>Ovary</td>
</tr>
<tr>
<td></td>
<td>8 Males</td>
<td></td>
<td></td>
<td></td>
<td>Histology</td>
</tr>
<tr>
<td></td>
<td>8 Females</td>
<td></td>
<td></td>
<td></td>
<td>Histochemistry</td>
</tr>
<tr>
<td>3.</td>
<td>IH-FSH-RH treated</td>
<td>IH-FSH-RH</td>
<td>1 µg in</td>
<td>&quot;</td>
<td>Interrenal</td>
</tr>
<tr>
<td></td>
<td>8 Males</td>
<td></td>
<td>0.1 ml</td>
<td></td>
<td>Histology</td>
</tr>
<tr>
<td></td>
<td>8 Females</td>
<td></td>
<td>saline</td>
<td></td>
<td>Histochemistry</td>
</tr>
<tr>
<td>4.</td>
<td>Methallibure treated</td>
<td>Methallibure</td>
<td>1 mg in</td>
<td>&quot;</td>
<td>Testis</td>
</tr>
<tr>
<td></td>
<td>8 Males</td>
<td></td>
<td>0.1 ml</td>
<td></td>
<td>Histology</td>
</tr>
<tr>
<td></td>
<td>8 Females</td>
<td></td>
<td>saline</td>
<td></td>
<td>Histochemistry</td>
</tr>
</tbody>
</table>

All injections were administered through the intraperitoneal route, every day for 8 days and the fish were autopsied 24 h. after the last injection.
Means of controls and experimental groups were compared using Student's t test and judged as significant if \( p \leq 0.05 \).

* Intensity of the reaction is visually graded as (trace), (+) minimum and (+++) maximum reaction.

All chemicals used are of analytical grade obtained from Sigma Chemical Co., USA.

### Table 4.2

<table>
<thead>
<tr>
<th>SL No.</th>
<th>Group</th>
<th>Relative weight of the testis (mg + S.E.)</th>
<th>Nuclear diameter (( \mu )m) Pre MIA Teste E2-17β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Initial Controls</td>
<td>767.80 ± 121.80</td>
<td>4.12 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td>Saline Controls</td>
<td>916.00 ± 40.71</td>
<td>4.40 ± 0.10</td>
</tr>
<tr>
<td>3</td>
<td>IH-FSH-RH treated</td>
<td>1271.40 ± 316.93</td>
<td>5.29 ± 0.11</td>
</tr>
<tr>
<td>4</td>
<td>Methallibure treated</td>
<td>488.00 ± 73.94</td>
<td>3.70 ± 0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( p \leq 0.001 )</td>
</tr>
</tbody>
</table>

Judged as significant if \( p \leq 0.05 \).
TABLE 4.3

Effects of IH-FSH-RH and methallibure on spermatogenesis in G.batrachus.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Group</th>
<th>Mean number of cysts of different spermatogenic stage ± S.E.</th>
<th>Sperms*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spermatogonia</td>
<td>Spermatocytes</td>
</tr>
<tr>
<td>1.</td>
<td>Initial Controls</td>
<td>2.01 ± 0.29</td>
<td>3.59 ± 0.59</td>
</tr>
<tr>
<td>2.</td>
<td>Saline Controls</td>
<td>2.46 ± 0.31</td>
<td>3.78 ± 0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>3.</td>
<td>IH-FSH-RH treated</td>
<td>2.39 ± 0.32</td>
<td>1.28 ± 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>4.</td>
<td>Methallibure treated</td>
<td>2.01 ± 0.26</td>
<td>1.75 ± 0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

Means of controls and experimental groups were compared using Student's t test and judged as significant if p < 0.05.

* The presence and abundance of sperms were visually graded as (+) few sperms and (+++) full of sperms.
### TABLE 4.4

**Effects of LH-FSH-RH and methallibure on the ovary of C.batrachus.**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Group</th>
<th>Relative weight of the ovary (mg ± S.E.)</th>
<th>Percentages of different oocyte stages ± S.E.</th>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage III</th>
<th>Atretic follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Initial Controls</td>
<td>2099.4 ± 251.52</td>
<td></td>
<td>33.24 ± 4.83</td>
<td>14.63 ± 2.55</td>
<td>35.66 ± 3.12</td>
<td>16.46 ± 1.49</td>
</tr>
<tr>
<td>2.</td>
<td>Saline Controls</td>
<td>7990.0 ± 2687.20</td>
<td></td>
<td>32.90 ± 0.49</td>
<td>14.10 ± 1.32</td>
<td>39.51 ± 2.18</td>
<td>13.51 ± 1.52</td>
</tr>
<tr>
<td>3.</td>
<td>LH-FSH-RH treated</td>
<td>8369.0 ± 1495.16</td>
<td></td>
<td>22.37 ± 1.60</td>
<td>18.79 ± 1.55</td>
<td>49.95 ± 2.64</td>
<td>8.92 ± 1.10</td>
</tr>
<tr>
<td>4.</td>
<td>Methallibure treated</td>
<td>1476.5 ± 251.13</td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Many</td>
</tr>
</tbody>
</table>

Means of controls and experimental groups were compared using Student's t test and judged as significant if p < 0.05.
### TABLE 4.5

Effects of LH-FSH-RH and methallibure on the ovary of *C. batrachus*.

**Histochemical observations**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Groups</th>
<th>5-3β-HSDH*</th>
<th>17β-HSDH*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre</td>
<td>DHA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FW</td>
<td>Ig</td>
</tr>
<tr>
<td>1. Initial Controls</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2. Saline controls</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>3. LH-FSH-RH treated</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>4. Methallibure treated</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

* Intensities of the reaction are visually graded as (+) trace, (−) minimum to (+++) maximum activity.

FW - Follicular Wall, Ig - Interstitial gland cell, AF - Atretic Follicle

All chemicals used are of analytical grade obtained from Sigma Chemical Co., USA.
TABLE 4.6

Effects of LH-FSH-RH and methallibure on the interrenal cells of male *C. batrachus*.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Group</th>
<th>Nuclear diameter (μm ± S.E.)</th>
<th>Cell diameter (μm ± S.E.)</th>
<th>No. of interrenal nuclei/unit area (× ± S.E.)</th>
<th>( \Delta^5 \text{-} 3\beta\text{-}HSDH^* )</th>
<th>17β-17αSDH^*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Initial controls</td>
<td>5.21 ± 0.14</td>
<td>7.78 ± 1.30</td>
<td>38.80 ± 1.00</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>2.</td>
<td>Saline controls</td>
<td>5.62 ± 0.11</td>
<td>8.21 ± 1.30</td>
<td>40.10 ± 0.36</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>3.</td>
<td>LH-FSH-RH treated</td>
<td>5.95 ± 0.08</td>
<td>8.78 ± 0.80</td>
<td>30.66 ± 0.50</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>4.</td>
<td>Methallibure treated</td>
<td>4.99 ± 0.20</td>
<td>7.57 ± 0.05</td>
<td>49.49 ± 0.67</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

Means of controls and experimental groups were compared using student's *t* test and judged significant if *p* < 0.05.

* Intensities of the reactions are visually graded as (+) trace, (+) minimum to (++++) maximum reaction.

All Chemicals used are of analytical grade obtained from Sigma Chemical Co., U.S.A.
### TABLE 4.7

Effects of IH-FSH-RH and methallibure on the interrenal cells of female *C. batrachus*.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Group</th>
<th>Nuclear diameter ($\mu m \pm S.E.$)</th>
<th>Cell diameter ($\mu m \pm S.E.$)</th>
<th>Nr. of interrenal nuclei/unit area ($\pm S.E.$)</th>
<th>$\Delta^{5}$-3P-HSDH*</th>
<th>17P-HSII*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Initial controls</td>
<td>4.96 ± 0.06</td>
<td>7.92 ± 0.10</td>
<td>40.09 ± 2.44</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Saline controls</td>
<td>5.69 ± 0.05</td>
<td>8.66 ± 0.08</td>
<td>41.82 ± 1.19</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p &lt; 0.001</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>IH-FSH-RH treated</td>
<td>6.40 ± 0.07</td>
<td>9.66 ± 0.07</td>
<td>30.64 ± 1.64</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td></td>
<td>p &lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Methallibure treatment</td>
<td>4.74 ± 0.02</td>
<td>7.13 ± 0.36</td>
<td>48.63 ± 2.56</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.01</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Means of controls and experimental groups were compared using student's $t$ test and judged significant if $p < 0.05$.

* Intensities of the reactions are visually graded as (+) trace, minimum to (+++) maximum reaction.

All chemicals used are of analytical grade obtained from Sigma Chemical Co., U.S.A.
Graph 4.1: Vertical bars showing the mean relative weight of the testes (± S.E.) and the mean diameter of the Leydig cells (um±S.E.) in initial controls, saline controls, LH-FSH-RH treated and methallibure treated C. batrachus. Note the significant decrease in the relative testes weight in methallibure treated fish when compared to the saline controls, significant increase in the diameter of the Leydig cells in LH-FSH-RH treated fish when compared to the saline controls and significant decrease in methallibure treated fish when compared to the saline controls.
LE YD G C E L NUCLEAR DIAMETER μm ± S E

RELATIVE TESTES WEIGHT % E

INITIAL CONTROLS
SALINE CONTROLS
LH-FSH-RH TREATED
METHALLIBURE TREATED

*— SIGNIFICANT COMPARED WITH SALINE CONTROLS

GRAPH 4 1
Graph 4.2: Vertical bars showing the mean number of cell nests of spermatogenic stages (± S.E.) in initial controls, saline controls, LH-FSH-RH treated and methallibure treated fish. Note the significant decrease in the number of cell nests of spermatocytes in LH-FSH-RH treated fish and significant decrease in the number of cell nests of spermatids after methallibure treatment when compared to the saline controls.
NUMBER OF CYSTS PER LOBULE CROSS SECTION

INITIAL CONTROLS
SALINE CONTROLS
LH-FSH-RH TREATED
METHALLIBURE TREATED

* — SIGNIFICANT COMPARED WITH SALINE CONTROLS

GRAPH 4.2
Graph 4.3: Vertical bars showing the mean relative weight of the ovary (± S.E.) in initial controls, saline controls, LH-FSH-RH treated and methallibure treated fish. There was no significant variation in the relative weight of the ovary in any of the treatment groups.
RELATIVE WEIGHT OF THE OVARY (mg ± S.E.)

- INITIAL CONTROLS
- SALINE CONTROLS
- LH-FSH-RH TREATED
- METHALLIBURE TREATED

GRAPH 4.3
Graph 4.4: Vertical bars showing the per cent count of Stage I, Stage II, Stage III and atretic follicles (± S.E.) in initial controls, saline controls, LH-FSH-RH and methallibure treated fish. Note the significant decrease in the number of Stage I and atretic follicles and significant increase in the number of Stage III oocytes in LH-FSH-RH treated fish. Also note that in methallibure treated fish only Stage I oocytes and a large number of atretic follicles were present, Stage II and Stage III oocytes were absent hence not represented in the graph.
INITIAL CONTROLS
SALINE CONTROLS
LH-FSH-RH TREATED

*—SIGNIFICANT COMPARED WITH SALINE CONTROLS

GRAPH 4.4
Graph 4.5: Vertical bars showing the mean nuclear and cellular diameters (μm±S.E.) of the interrenal cells and the number of cells per unit area (μm±S.E.) in the initial controls, saline controls, LH–FSH–RH treated and methallibure treated male *C. batrachus*. Note the significant increase in the nuclear diameter and significant decrease in the number of cells per unit area of LH–FSH–RH treated fish and significant decrease in the nuclear diameter and significant increase in the number of cells per unit area in methallibure treated fish.
INITIAL CONTROLS
SALINE CONTROLS
LH-FSH-RH TREATED
METHALLIBURE TREATED
*— SIGNIFICANT COMPARED WITH SALINE CONTROLS
Graph 4.6: Vertical bars showing the mean nuclear and cellular diameters (μm±S.E.) of the cells per unit area (μm±S.E.) in the initial controls, saline controls, LH-FSH-RH treated and methallibure treated female *C. batrachus*. Note the significant increase in the nuclear diameter in saline treated controls when compared to the initial controls, significant increase in the nuclear and cellular diameters and significant decrease in the number of cells per unit area in LH-FSH-RH treated fish; and significant decrease in the nuclear and cellular diameters and significant increase in the number of cells per unit area in methallibure treated fish when compared to the saline controls.
Graph 4.6

- Initial Controls
- Saline Controls
- LH-FSH-RH Treated
- Methallibure Treated

- * Significant compared with initial controls
- ○ Significant compared with saline controls
Fig. 4.1: T.S. of testis of control fish during the breeding season showing lobules filled with spermatozoa (Stz) and well developed interstitium with Leydig cells (L.).

Fig. 4.2: T.S. of testis of LH-FSH-RH treated fish showing lobules filled with spermatozoa (Stz) and hypertrophied Leydig cells (L.).

Fig. 4.3: T.S. testis of methallibure treated fish. Note the reduction in the size of the lobules with derangement of spermatogenesis. The Leydig cells (L.) are atrophied.

Fig. 4.4: Fresh frozen section of the testis of control fish showing \( \Delta^{5}-3\beta\)HSDH enzyme activity in the Leydig cells.

Fig. 4.5: Fresh frozen section of the testis of LH-FSH-RH treated fish showing \( \Delta^{5}-3\beta\)HSDH activity in the Leydig cells. Note the increase in the intensity of the enzyme activity.

Fig. 4.6: Fresh frozen of the testis of metnallibure treated fish showing \( \Delta^{5}-3\beta\)HSDH activity. Note the marked reduction in the intensity of the enzyme activity.

Figs. 4.4 to 4.6 substrate used is DHEA

Scale line indicates 50 \( \mu \)m.
Fig. 4.7: T.S. of ovary of control fish during the breeding season showing Stage I (SI), Stage II (SII), Stage III (SIII) and atretic follicle (AF).

Fig. 4.8: T.S. of ovary of LH-FSH-RH treated fish showing a post-ovulatory follicle (POF) and Stage III oocyte (SIII).

Fig. 4.9: T.S. of ovary of methallibure treated fish. Note the large number of atretic follicles.

Fig. 4.10: Fresh frozen section of the ovary of the control fish showing $\Delta^5$-3\(^\beta\)-HSDH activity in the follicular wall (arrows).

Fig. 4.11: Fresh frozen section of the ovary of LH-FSH-RH treated fish showing increase in the enzyme activity (Arrows).

Fig. 4.12: Fresh frozen section of the ovary of methallibure treated fish showing $\Delta^5$-3\(^\beta\)-HSDH activity in the atretic follicle (AF).

Figs. 4.10 to 4.12 substrate used is DHEA. Scale line indicates 50 \(\mu\text{m}\).
Fig. 4.13: T.S. of head kidney of control fish showing the interrenal cells (IR) around the post cardinal vein (V) and chromaffin cells (C).

Fig. 4.14: T.S. of head kidney of LH-FSH-RH treated fish showing the interrenal cells. Note the hypertrophy of the interrenal cells (IR).

Fig. 4.15: T.S. of head kidney of methallibure treated fish showing the interrenal cells. Note the regression of the interrenal cells (IR).

Fig. 4.16: Fresh frozen section of the head kidney of control fish showing \( ^5 \Delta \text{-3} \beta \text{-HSDH} \) activity in the interrenal cells.

Fig. 4.17: Fresh frozen section of the head kidney of LH-FSH-RH treated fish showing an increased in the intensity of \( ^5 \Delta \text{-3} \beta \text{-HSDH} \) activity in the interrenal cells.

Fig. 4.18: Fresh frozen section of the head kidney of methallibure treated fish showing \( ^5 \Delta \text{-3} \beta \text{-HSDH} \) activity in the interrenal cells. Note the decrease in the intensity of the enzyme activity.

Figs. 4.16 to 4.18 substrate used is DHEA Scale line indicates 50 \( \mu \text{m} \).