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GROWTH, MATURATION, AND PITUITARY ACTIVITY IN *TILAPIA* SPECIES

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by

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The work presented in this thesis is the result of my own investigations and has neither been accepted nor is being submitted for any other degree.

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ABSTRACT

The endocrine control of reproduction and aspects of growth in mouthbrooding cichlids Tilapia mossambica (Peters) and Tilapia aurea (Steindachner), have been investigated.

Comparative studies on the processes of development of the gonads have revealed no differences, on the broader aspects, in the two species. In the females, germ cells proliferate through mitotic divisions and become transformed into oocytes. Growth of the oocytes, as a result of protoplasmic, and then, trophic inclusions in the oocytes, results eventually in maturation. In the males, maturation is brought about as a result of a series of divisions of the germ cells, giving rise, eventually, to ripe spermatozoa.

Using histological and cytochemical techniques, eight cell types have been identified in the pituitary gland of the two species; eta or prolactin cells and α_1-, or ACTH cells in the pro-adenohypophysis; α - or STH cells, B_1 - cells, B_2 - cells (or two types of gonadotrophs), and γ - or chromophobic cells in the meso-adenohypophysis; and two acidophilic cell types, the functions of which are not clear, in the meta-adenohypophysis.

Studies of the cyclical activities in the meso-adenohypophysis and gonads have revealed the presence of a correlation in development. During the reproductive cycle, there is a gradual increase in the quantity of the meso-adenohypophyseal basophils, reaching a peak at the prespawning period.

Maintenance of fish under low temperatures of 13° - 15° C and in continuous darkness results in an inhibition of the development of the pituitary and gonads, and in an abolition of somatic growth.

Total gonadectomy in Tilapia results in degranulation, vacuolization and depletion of the basophils. At the same time, hypertrophy, hyperplasia and granulation of the meso-adenohypophyseal acidophils (STH cells) are observed. Significant weight increases

in the gonadectomized fish are recorded. The operation also results in the abolition of the secondary sexual characters.

Administration of testosterone propionate, restored the secondary sexual characters and spawning behaviour in gonadectomized males. Evidence of masculinization was found in the TP - treated females. Oestradiol benzoate stimulated the growth in size of the genital papilla in fish of both sexes but nuptial coloration was not restored in the males. Dependence of the secondary sexual characters and spawning behaviour on sex steroids has been inferred.

Treatment of fish with a dithiocarbamoylhydrazine derivative (Methallibure) reveals an inhibition of the pituitary gonadotrophic function. A dradual depletion in the basophilic cells of the meso-adenohypophysis is observed, with concomitant regressive changes in the gonads. In the ovary, all yolky oocytes undergo atresia but oogonia and protoplasmic oocytes remain. In the testis, germ cells beyond the spermatogonial stage become necrotic. However, ripe sperm present in the testis at the time of the treatment, persist for some time. Methallibure also abolishes the secondary sexual characters and spawning behaviour, but the feeding habits and consequently, growth, are not interrupted. The compound also has a secondary, favourable effect on growth in female Tilapia.

Unilateral ovariectomy results in compensatory hypertrophy of the remaining ovary, but Methallibure suppresses the compensatory hypertrophy.

Replacement therapy experiments have revealed that the chorionic gonadotrophins (HCG and PMS) have stimulatory effects on the regressed gonads and the appearance of the secondary sexual characters, and spawning behaviour of Methallibure-treated Tilapia. On the other hand, the gonadotrophic hormones (FSH and LH), when used singly were less effective. However, the gonads, the secondary sexual characters, and spawning behaviour, were all stimulated when the two hormones were administered in

combination. Evidence is presented on the regulation of maturation directly by the pituitary. The possibility of the elaboration of two gonadotrophic hormones in the pituitary gland of Tilapia is discussed. Extracts of carp pituitary suppressed gametogenesis and gonadal weights. In the testis, the treatment led to an abnormal development.

Testosterone propionate also suppressed gametogenesis and gonadal weights, but restored the secondary sexual characters and spawning behaviour. Evidence is presented on the control of the secondary sexual characters and spawning behaviour by the gonads (androgens) and its mediation through the pituitary.

The sites of steriod production in the gonads were investigated histochemically. 3B - hydroxysteriod dehydrogenase ac ivities were detected in the follicular cells of the ovary and the interstitial cells of Leydig in the testis.

A report on fish culture in Israel, with special emphasis on Tilapia is also presented, and discussed in the light of the experimental findings.

CONTENTS

INTRODUCTION

Tilapia mossambica (Peters) and T.aurea (Steindachner) are members of the Family Cichlidae which belongs to the Teleostean Order Percomorphi. Until about two decades ago these fish had received only minor attention as a staple article of food in its native environment - Africa and the Middle East.

As world food statistics show, a substantial portion of the human population has an inadequate intake of protein. It is hardly surprising, therefore, that many agencies concerned with the world food problem, particularly the Food and Agriculture Organisation of the United Nations, have been very much concerned with the cultivation of some species of fish as potential sources of cheap, abundant and palatable protein. Among the possibilities canvassed by these agencies is the culture of Tilapia species because they possess many of the desirable characteristics of pond fish and they are readily adaptable to culture (Atz, 1954). The potentialities of various species of Tilapia were demonstrated by W.H. Schuster before a gathering of inland fisheries experts at Surabaja in 1939 (Vaas and Hofstede, 1952).

Pond-reared Tilapia start breeding at a small size and breed all the year round at frequent intervals. This prevents an increase in weight of the individual fish, and this is most undesirable from a fish culture point of view. In a pond stocked with Tilapia the maximum standing crop is soon attained, not by the growth of the original stock, but by the proliferation of thousands of fry and fingerlings (Hickling, 1963). Moreover, Tilapia's habit of mouth-incubating (the female takes the fertilized eggs into her mouth where incubation and some period of post-embryonic development take place) undoubtedly contributes to the high survival rate of the young. A case has been recorded of 150 adult Tilapia producing 15,000 fry in less than four months, and another case is known in which 14 fish produced 14,000 fry in only two-and-a-half months.

The task in Tilapia culture, has therefore, for the past twenty years or so, been trying to raise large marketable-size fish. The methods adopted so far by workers have been purely empirical, entailing mostly ecological observations. Recently, a genetical approach has also been adopted to achieve this aim, but at present, this problem remains unsolved.

The objects of this research were to study the factors controlling gametogenesis with the view to manipulating them in order to retard the onset of maturation and breeding, allowing more time for somatic growth. A comprehensive review of literature relating to the Biology of Tilapia and the endocrine control of reproduction in fish was prepared. The process of development of the gonads and the pituitary was studied; laboratory experiments were performed upon a breeding stock of Tilapia in an attempt to postpone spawning, and the results of these investigations were tested on fish in ponds at the Fish Culture Research Station in Israel. The Economics, Management and Methods of the culture of fish in Israel was also studied.

The research work was done under particular conditions. The Food and Agriculture Organisation (financing the work) required that a substsntial part of the project be done at the Fish Culture Research Station in Israel. This resulted in (among other things) experiments being, to some extent, divided between two species of Tilapia - T. mossambica and T. aurea. Time and resources did not allow for duplication of experiments on both species and therefore one was used to compliment the other. The species are closely related and in my view (which is supported by other workers in the field) the results may be interpreted together, although in this thesis I indicate which species were involved.

REVIEW OF LITERATURE

BIOLOGY OF TILAPIA

In recent years many scientists in various parts of the world have studied the biology of Tilapia and its culture in ponds and rice paddies. The works of Vaas and Hofstede (1952), Chen (1953), Panikkar and Tampi (1954), and Swingle (1960) are of interest in this field of study. A wealth of information can be gathered from the bibliographies and reviews by Chimits (1955, 1957a,b) on the culture of Tilapia. The behaviour of the cichlids has been widely studied by Baerends and Baerends Van Roon (1950, 1967). Other sources of information on various aspects of Tilapia culture and biology have been given by Brock (1954) on spawning in salt water, Fish (1955) and Le Roux (1956) on feeding habits, and Lowe (1955) on fecundity. The culture of Tilapia for use as a possible supplement to natural bait supplies has been investigated by Uchida and King (1962).

Many investigators have reported on the feeding habits of the various species of Tilapia. Generally, T. mossambica is considered to be omnivorous (Schuster, 1952; Chen, 1953; Atz, 1954; Panikkar and Tampi, 1954; Brock and Takata, 1955; Van Pel, 1955). Vaas and Hofstede (1952) stated that most Tilapia species are herbivorous, but they will feed on planktonic crustacea "if such kind of food is more plentiful than vegetable food"; when a mixture of the two is present Tilapia shows a preference for vegetable food.

Maturation and spawning has been described in many species of Tilapia. Uchida and King (1962) stated that T.mossambica has been reported to spawn first at the age of 2-3 months and at the length of 8-9 cm. The frequency of spawning of Tilapia varies considerably, depending on environmental factors. Aronson (1951) observed up to 14 spawnings per annum in T.macrocephala. Lowe (1955) stated that at least nine East African Tilapia species have three or four successive breedings while three and possibly five breedings have been recorded by Fryer (1961) in T.variabilis. Cridland (1961) gives evidence that T. esculenta spawns seven times in 24 months with 39 days between spawning periods. Reidel (1965) recorded

four to five spawnings per season in T. mossambica with an interval of about 30 days between spawning periods, and Welcomme (1967) reports that T.leucosticta breeds more than once per season. Other investigators who have reported on the frequency of spawning of Tilapia are Chen (1953), Panikkar and Tampi (1954), Chimits (1955). These investigators reported that the frequency of spawning of various species of Tilapia ranges between 6 and 16 times a year.

That Tilapia spawns several times a year is clearly established by the experiments and observations of the above-mentioned workers, but the reason for the different spawning frequencies in different species have not been reported anywhere. In the present study, an attempt will be made to analyse the functional mechanism of the oocytes and testes of Tilapia in Ontogenesis in order to clarify this problem.

Chen (1953) reported that a mature fish of about 8 cm may produce less than 100 eggs at the first spawning. At subsequent spawnings, however, it produces from 100 to 150 eggs at each spawning. He stated further that the number of eggs spawned increased with successive spawnings, so that a fish more than six months old may produce in excess of 1,000 eggs per spawning. The same conclusion was arrived at by Cridland (1961) that for T. esculenta in the laboratory, the number of eggs in successive broods increases.

The developmental period of the eggs is likewise variable, but, generally, the eggs hatch after 2-5 days and the young are carried in the mouth of the female parent for another 5-8 days before they are released. (Chen, 1953; Panikkar and Tampi, 1954; Chimits, 1955; Dadzie, unpublished data).

In the opinion of Chen (1953) it is probable that Tilapia will spawn on bare floor, though Uchida and King (1962) recorded negative results until white beach sand was provided in the tanks. In the present study Tilapia mossambica has been

observed to spawn on a stony substrate, but not sandy. The fish spawned on the bare floor of the tank in between the stones. This confirms Chen's observation. With regard to T. aurea, it spawns either on a bare floor or on a sandy substrate.

Newly-hatched fry of T. mossambica measure 5 mm. in length, 5.8 mm. on the second day, and 8.0 mm. at the end of the fifth day (Uchida and King, 1962). On about the fifth day, they begin to spend less time in the mouth of the female parent or leave it altogether and swim about in a tight school near the surface of the water, feeding on tiny food particles (Panikkar and Tampi, 1954). Within the first few days the newly-hatched fry will always dash back into the females mouth for protection.

Chen (1953) reports that 20° - 35°C is the optimal temperature for rearing Tilapia but Cridland (1961), as a result of his experiments on the effects of light and temperature on the growth of Tilapia species has proved that within limits, higher temperatures are always more favourable to the growth of Tilapia.

It has been reported by Vaas and Hofstede (1952) and Brock (1954) that T. mossambica will spawn and the young will grow in salt water. According to these authors, spawning has occurred in Tilapia in a period during which the salinity of the water ranged from 3 - 4.8‰. They pointed out, however, that "according to the subsequent findings of the Extension Service in Indonesia, good growth is limited by a salinity of 4‰ and spawning by one of 3‰". Brock (1954) reported spawning of Tilapia in sea water of a chlorinity of 19.29‰ (equal to a salinity of about 34.85‰). Chervinski (1961 a,b) and Krayushkina (1965) both gave evidence that Tilapia can survive in waters of increased salinities.

GAMETOGENESIS

The study of the process of development of gonads in fish and the main factors regulating this process are of great scientific as well as practical interest. The increasing importance of fish farming, coupled with the great morphological diversity displayed by teleosts, reflected in the range of reproductive phenomena seen in these animals, make it clear why the problem of reproduction and the histophysiological studies of the gonads and their seasonal changes have been the subject of studies by numerous workers.

His (1873) was one of the pioneer workers in this branch of research and gave information regarding the structure of the ovaries of the salmonids. This author observed an asynchronous development of the oocytes.

Owsiannikow (1885) gave a comparative description of the structure of the oocytes and the chorion of the eggs of the salmon, perch, burbot (Lota lota), smelt (Osmerus) and other teleosts. The author observed migration of the nucleus towards the micropole during maturation of the eggs.

Cunningham (1898) described the process of resorption of the remants of the eggs and observed the presence of droplets of yolk and fat in the ovaries of Trigla gurnadus.

Wollace (1903) described the histological structures of the ovaries, oocytes and the chorion of the eggs in Zoarces, and also the cytoplasmic formation in the eggs of some teleosts. Credit for the discovery of the yolk nucleus in the oocytes of fish goes to this author. He even observed the disintegration of the yolk nucleus into minute parts and granules.

Maréchal (1907) gave a fairly detailed description of the synaptic phase of development of the oocytes in a large species of teleosts and studied the changes which took place in the oocytes during maturation.

Other early information on the various aspects of gametogenesis are contained in Zalenskii (1878), Barfurth (1886), Scharff (1889), Jungersen (1889), Kastschenko (1890), Buhler (1902), Loewe (1903), Lams (1903, 1904), Waldejer (1906). Almost all these authors conducted either histocytological studies of certain specific phenomena connected with maturation of the ovaries and individual stages of the seasonal changes or tried to extend the information which had then been gathered from the higher vertebrates to fish. During those investigations fish were used only as objects for histological examinations. The ecology of the species, the character and biology of reproduction were not taken into account.

Over a decade later the attention of scientists was directed towards the description of the formation of cytoplasmic inclusions in the oocytes of teleosts and their role. Such descriptions were given by Champy et Gley (1923), Parat (1926), Hibbard and Parat (1927, 1928, 1930) and Gerbilskii (1939). The authors also treated the questions of the origin of the germ cells and the seasonal changes in the ovaries of fish. The works of Gerbilskii (1939) are of great interest. The author analysed the influence of temperature on the seasonal changes of the oocytes of the mirror carp. According to Gerbilskii (1939) temperature is the most important factor influencing the seasonal changes in the gonads of this fish.

The annual changes in gonads of many fish of economic importance have been studied by Maien (1927, 1939 a,b, 1944). The author suggested the use of the terminology "phase" for the description of the oocytes at the stage of growth. He spread the whole process of growth of the oocytes into three periods:

1) The phase of synaptic growth :- this period stretches from the oogonial to the diplotene phase;

2) The phase of Minor Growth :- starts from the diplotene

phase and is characterized by the growth of the oocyte as a result of protoplasmic inclusions. The appearance of numerous nucleoli in the nucleus marks the end of this period, which is now called the period of protoplasmic growth. Oocytes of the juvenile phase, or phase "B" and those of the single-layered follicular phase, or phase "C" are both included in the above period.

3) The Period of Major Growth :- this is the last stage of the development of the oocytes and is characterised by a great increase in the size of the oocytes as a result of inclusion of "definite" trophic substances, such as yolk and fat. This period is popularly described as vitellogenesis. The oocyte attains its maximum size at the end of this period, which is immediately followed by polarization of the nucleus, marking the beginning of processes connected with maturation of the egg.

The chorion of the egg appears distinctly at the beginning of vitellogenesis. Later phases of this period, according to Maien (1927) differ greatly among fishes and depend on the ecology of reproduction of different species. The micropile forms at this time. The whole period includes the phase of the first appearance of yolk or fat in the oocyte, (phase "D) the phase of considerable increased accumulation of trophic substances, (phase "E") and the phase of ripe oocyte, (phase "F").

In defining the stages of maturation in fish, Maien (1939a) established the six-stage system which is represented by the Roman numerals I - VI. This, amongst many other stages adopted by authors, seemed to be the most convenient. Stage I was described as the phase when the oocyte was filled basically with oogonia, though a few primary oocytes could also be present. When the majority of the cells of the ovary were in the phase of the protoplasmic growth, it was described as Stage II. Stage III marked the beginning of vitellogenesis and

Stage IV was the phase when the oocytes had attained their maximal size. The period of ovulation was marked as Stage V, and the post-spawning period, Stage VI. After Stage VI the ovary, after the first spawning, never returns to Stage I, because the latter was characterised only in juvenile fish. Depending on the sexual cycle of the particular species, the ovary, immediately after spawning, will return to Stage VI - II or Stage VI - III.

In his third work, Maien (1939b) gave a classification of different types of spawning. He studied the annual cycle of the perch, Caspian roach (Vobla) and a few other species of fish. The author, making use of the data gathered from his investigations on these species, tried to establish a general principle covering the other species which are synchronous spring-spawners. During his investigations, the author noted the presence of fat only in the oocytes of pelagic fish or fish with pelagic eggs. This latter view, however, has been proved to be wrong by numerous authors. However, all the present investigators on this subject would agree that fat is not to be found only in the fish with pelagic eggs.

In 1944 Maien produced more information on the changes in the sexual cycle of fish under different ecological conditions. The author remarked that the rate of maturation even in one species could change, depending on the time which the fish took to go through the Stage II of maturation, because it is at this stage that fish are most sensitive to environmental factors. Persov (1966) proved that various species of the young Salmonidae spend the greater part of their life span with oocytes in this stage of maturation.

The works of Kulayev (1927, 1928, 1939, 1944) are also of great interest. In one of his early publications the author described the changes in the generative part of the testis of

the perch and gave a general pattern of the development of the male sexual cells. The second work of the author was on the annual cycle of the testis of sexually mature roach, Rutilus rutilus. In 1939, he described his version of the six-stage system of maturation in the perch and roach. He described the difference in the cyclical changes in the testis of these fish by pointing out that perch spends the winter with sexual cells nearer to maturation, whereas the testis of the roach are far from maturation at this season.

The structure and cycle of development of the testis in sexually mature sheat-fish (Silurus glanis) was studied by Kulayev (1944) who came to the conclusion that spermatogenesis in teleosts differed even in close species, and so future research in this direction would be of great interest and importance, especially in economically valuable fish.

Summary of various aspects of gametogenesis is given on Table 1.

Recent information on oogenesis in five species of grey mullets (Mugil) has been given by Abraham et al (1966) who noted that a high Gonado-Somatic Index (GSI) in the fish in the prespawning season. M. capito and M. cephalus confined to freshwater did not ovulate and the authors explained that the high GSI in these species was due to accumulation of atretic oocytes. Histograms showing percentages of stages of oocytes of the various species of mullets were compared and the factors responsible for the infertility of the specimens confined to freshwater discussed. Wiebe (1968b) described the seasonal reproductive cycle of the viviparous seaperch, Cymatogaster aggregata, including the development of the Sertoli cells and the intestitial cells of Leydig. The testicular cycle was described in six phases by Rastogi (1968 a,b) in the freshwater spiny eel, Macrognathus aculeatum and the teleost, Amphipnous cuchia, and the origin of

the germ cells also reported. Occasional "lobule boundary cells" and intestitial cells were described.

The presence of a persisting yolk nuclei in the oocytes of Mugil cephalus has been noted by Abraham et al (1968) who observed their migration from a juxtanuclear position to the periphery of the ooplasm. The latest information on gametogenesis in fish was given by Dadzie (1969) in his studies on spermatogenesis and the stages of maturation in the male Tilapia mossambica. For the sake of convenience, the author divided the scale of maturity into five stages only, as compared with the existing scales (Hjort, 1910; Maien, 1939 a; Kulayev, 1939).

Finally, our limited knowledge on the histophysiology of the gonads of Tilapia acts as a great barrier in attempts to solve the long-standing problem of growth in these fish. A close study of the bibliography of publications concerning T.mossambica prepared by St. Amant and Stevens (1967) and another one by Van den Audenaerde (1968) on all Tilapia species, coupled with a study of all significant papers dealing with this species has revealed that up to January 1970, nothing had been reported on the ecological histophysiology of this fish. The first information on the morphology of the egg appeared only recently (Dadzie, 1968). Unpublished data and unfinished experiments of the same author, together with the research in reproductive endocrinology, which is the main project of the present study, when completed, would perhaps help to establish a clear picture of the reproductive phenomena in this species.

Table 1. Summary of various aspects of gametogenesis

Author	Species	Observations
Franz (1910)	Pleuronectes platessa	Origin and maturation of oocytes and different stages of oogenesis.
Turner (1919)	Perca	Spermatogenesis.
Wheeler (1924)	Pleuronectes limanda	Formation of oocytes from cells of the follicular epithelium.
Foley (1927)	Umbra limi	Spermatogonial chromosomes and the first maturation division.
Hickling (1930)	Merlucious merlucious	Biology with brief data on oogenesis.
Sivertsen (1935)	Gadus callerius	Cyclical changes in ovaries.
Gerbilskii (1937)	Cyprinus carpio	Adaptation of oocytes to low temperatures with formation of the circumnuclear ring.
Matthews (1938)	Fundulus heteroclitus	Seasonal cycle in males and females.
Gerbilskii (1939)	Cyprinus carpio	Influence of temperature on seasonal changes.
Trusov (1947)	Lucioperca lucioperca	Lengths of individual stages and periods during maturation of ovaries.
Gokhale (1957)	Gadus merlangus and G.esmarkii	Gametogenesis, intestitial tissue and corpus luteum formation.
Tampi (1957)	Chanos chanos	Stages of maturation and spawning seasons.
Swarup (1958)	Gasterosteus aculeatus	Maturation of testis and ovaries.

Table 1 continued

Author	Species	Observations
Nair (1959)	Hilsa kisha	Peaks of gametogenesis - March and August.
Galkina (1959)	Clupea pallasii	Degeneration and resorption of eggs.
Chicewicz (1959)	Lucioperca lucioperca	Stages of gametogenesis - five in oogenesis and four in spermatogenesis.
Nawar (1959)	Clarias lazera	Seasonal changes in gonads.
Bara (1960)	Scomber scomber	Origin of oocytes, behaviour of chromosones, formation of zona radiata, ovulation and formation of corpora lutea.
Sundararaj (1960)	Heteropneutes fossilis	Presence of sperm in testis throughout the year.
Bowers and Holliday (1961 a,b)	Clupea harengus	Synchronous ovulation.
Quasim and Qayyum (1961)	Cirrhina, Barbus, Labeo, Crela, Ophiocephalus, Mugil, Rynchobdella	Types of spawning frequencies and seasons - short 2-4 month seasons, successive spawnings, and without definite spawning season.
Sathyanesan (1961)	Barbus stigma	Presence of spawning periodicities.
Koshelov (1961a,b)	Perca fluviatilis Esox lucious Abramis brama Rutilus rutilus Acerina cernua	Histological changes in ovaries - all spawn once a year except last species which spawns twice a year.
Kubota (1961)	Misgurnus anguicaudatus	Gametogenesis.

Table 1 continued

Author	Species	Observations
Yamamoto & Yamazaki (1961)	Carassius auratus	Seasonal and ontogenetic changes in the ovary.
Hisoka and Firlit (1962)	Brachydanio rerio	Gametogenesis
Henderson (1962)	Salvelinus fontinalis	Gametogenesis
Mathur (1962)	Barbus stigma	Gametogenesis
Yamamoto (1962)	Oryzias latipes	Origin of oocytes through division of germinal cells.
Yamamoto & Shirai (1962)	Rhodeus amarus	Origin of oocytes through division of residual oogonia
Rajalakshmi (1966)	Gobius giuris	Atresia and formation of corpora atretica.

THE PITUITARY GLAND

a) GENERAL DESCRIPTION OF THE TELEOST HYPOPHYSIS

Stendell (1913) the author of one of the most valuable books on the structure of the pituitary in different groups of vertebrates emphasized that the most complicated structure of this organ can be found in fish. This is especially true of the teleost pituitary, the complex structure of which is apparent even during the embryonic development of this organ.

The hypophysis, which is of ectodermal origin, arises as an invagination of the roof of the pharynx. It is a very compact organ and, unlike the pituitary of the elasmobranchs, has no Rathke's pouch. According to Prosser & Brown (1962) Rathke's pouch gives rise to the three major divisions of the adeno-hypophysis as follows: the posterior cell-wall remains thin, becoming the pars intermedia, the anterior wall becomes greatly thickened to form the pars distalis, and a dorsal portion of the pouch extends towards the infundibulum forming the pars tuberalis; while Gorbman and Bern (1962) in the textbook of Comparative Endocrinology divide the adenohypophysis into the pars intermedia, rostral pars distalis and proximal pars distilas. These three zones are histologically distinct, containing recognizable callular types. A further differentiation between the three zones may be made on the basis of neurohypophyseal relationship. The pars intermedia is the most extensively penetrated by sheets of cords of neurohypophyseal tissue. The admixture of the two tissues is often so complete that the combined organ is called, as in the elasmobranchs, the neuro-intermediate lobe. The proximal lobe of the pars distalis contains much less, and the rostral zone the least neurohypophyseal tissue.

There are only three anatomic units involved in the nomenclature of the teleost adenohypophyseal structures.

The zones of the adenohypophysis, however, have been given various names by different investigators. Kazanskii and Persov (1948) gave a detailed description of the cytology, and a study of this region together with diagrams of the regions of the carp pituitary, leaves no doubt as to the confusion of terminology. For this reason, in the present study, for the purpose of convenience, the different areas of Tilapia's pituitary will be designated on a purely topographical basis free from any functional implication. Each area would therefore be identified with Pickford's terminology as well as with those commonly employed by previous workers. A fairly recent terminology was suggested by Pickford and Atz (1957) in which the three regions are designated as pro-adenohypophysis, meso-adeno-hypophysis and meta-adenohypophysis, and it is this terminology which will be employed to describe the various zones of Tilapia's pituitary.

Selected examples from the more familiar literature on the terminology of the various parts of the teleost pituitary are given on Table 2.

The hypophysial cavity is not retained in teleosts in general, but in young herring, Clupea harengus, up to the time of metamorphosis (Buchmann, 1940) and in young milkfish, Chanos chanos (Tampi, 1951; 1953) the pro-adenohypophysis communicates with the pharynx by an open hypophysial duct. In isospondylous teleosts such as the herring and salmon the pro-adenohypophysis has a follicular structure. In higher teleosts, such as the perch, the follicular structure or character of the pro-adenohypophysis is lacking.

The meso-adenohypophysis of teleosts appears to contain all the cell types that are associated with the mammalian pars anterior. There are at least two types of basophils (cyanophils) that may be differentiated by experimental treatment and the application of specific staining methods, and which have been identified as gonadotrophs and thyrotrophs

respectively (Atz, 1953 ; Olivereau and Herlant, 1954, Barrington and Matty, 1955). In some species, two types of acidophils have been differentiated by trichrome stains : fuchsinophils and orange G cells (Scruggs, 1939). Chromophobes are always present, and are usually abundant during the period of bodily growth, e.g. in salmonid parr and smolt. Maximal development of the basophils is usually associated with the maturation of the gonads.

Cell types of the meta-adenohypophysis have generally been described as chromophobes or weakly-staining basophils.

Table 2. Terminology of the Teleostean Pituitary (From Pickford and Atz, 1957)

Author	Neuro-hypophysis	Meta-adenohypophysis	Meso-adeno-hypophysis	Pro-adeno-hypophysis
Barrington & Matty(1955)	Neurohypo-physis	Posterior zone	Median zone	Anterior zone
Bell (1938)	Pars nervosa	Pars intermed-ia	Ubergangs-tiel	Pars anterior
Bock (1928b)	Hirnteil	Zwischenlappen	" Ubergangs-tiel	Hauptlappen
Bretschneider & Duyvene de Wit (1947)	Neurohypo-physis	Pars inter-medius	Pars anterior	Pars tuberalis
Buchmann (1940)	Hirnteil	Zwischenlappen	" Ubergangs-teil	Hauptlappen
Hagen (1936)	Hirnteil	Zwischenlappen	" Ubergangs-teil	Hauptlappen
Kazanskii & Persov(1948, 1949)	Neurohypo-physis	"Transitional lobe"	"Intermed-iate lobe"	"Main lobe"
Kerr (1943, 1949)	Pars nervosa	Posterior glan-dular region	Middle glan-dular reg-ion	Anterior glandular region
Lee (1922c)	Pars nervosa	Pars intermedia	Transition-al zone	Pars anterior
Miller,R.N. (1944)	Neurohypo-physis	Pars intermedia	Pars distalis	Pars tuberalis
Olivereau (1954b)	Pars nervosa	Pars intermedia	Region glanulaire moyenne	Pars anter-ior,or in some spp. partie follicu-laire
Potts(1942)	Pars nervosa	Pars intermedia	" Ubergang-steil	Pars anterior

Table 2. (Contd)

Author	Neuro-hypophysis	Meta-adenohypophysis	Meso-adeno-hypophysis	Pro-adeno-hypophysis
Rasquin (1949)	Pars nervosa	Intermediate lobe	Transitional lobe	Anterior lobe
Romeis (1940)	Hirnteil (Processus infundibularis)	Zwischenzone (Zona intermedia)	Vordelappen (Pars anterior)	Trichterlappen (Pars tuberalis)
Scruggs(1939)	Pars nervosa	Pars intermedia	Transitional zone	Pars anterior
Stendell (1914)	Hirnteil	Zwischenlappen	" Übergangsteil	Hauptlappen

b) CYCLICAL CHANGES IN THE CYTOLOGY OF THE PITUITARY GLAND

From the works of early investigators studying the seasonal changes in the pituitary of fish, it is clear that the cell components of the different parts of this gland undergo seasonal changes which could be expressed in major changes in the quantity of chromophobe, acidophil and basophil cells. (Stendell, 1914; Florentin, 1931, 1934, a.b) Bock, 1928; Matthews, 1936, 1939a; Evans Muir, 1940).

Bell (1938), for example, found that the pro-adenohypophysis of the goldfish, Carassius auratus consists mainly of basophil cells. In the same lobe of the pituitary of the stickleback, Gasterosteus aculeatus, Bock (1928) singled out a chromophobic part which, according to the author, consists of a reserve of chromophobe cells, which replace the chromophils after they are converted into eosinophils.

A clear picture of the cell components of the other parts of the pituitary is also not fully established. Matthews (1938, 1939) suggested that the meso-adenohypophysis of the pituitary of Fundulus consisted mainly of basophil cells. Eosinophil and chromophobe cells could be found only in small quantities in this zone.

Bell (1938) established that in the meso-adenohypophysis of the pituitary of Carassius auratus, in addition to the basophil elements, small polygonal cells could also be found at the boundaries of the connective tissue membrane which accompanies the sheets of cords of the neuro-hypophyseal tissue.

Bock (1928) denied altogether the presence of typical basophil cells in the meso-adenohypophysis of the pituitary of the stickleback. He suggested, however, that of the cells of this zone, chromophobes and basophils acquire basophilic characters during the supposed period of intensive formation of basophil cells. In this way, the author accounted for morphologically pronounced seasonal changes in the meso-adenohypophysis of the pituitary of this fish.

Hagen (1936) could not establish annual cyclical changes in the eel, Anquilla vulgaris. According to his views, the majority of the cells of the meso-adenohypophysis of the pituitary of this fish were basophilic. The cells adjoining the neurohypophysis were the only ones which were acidophilic.

Work on the cyclical changes in the fish pituitary has been carried out by quite a number of investigators recently. Sathyaneson (1958) during his studies on the morphology and seasonal histological changes in the pituitary gland of the freshwater teleost Cirrhina reba established that most prominent of all were changes in the basophils of the meso-adenohypophysis.

Beach (1959) correlated the seasonal changes in the maturation of the ovary of the goldfish with changes in the area of the pituitary basophils. In the same year, Scruggs described the seasonal changes in the cytology of the hypophysis of the goldfish. Honma (1959, a.b.c.) associated cyanophils with gonadotrophin production during his studies on the histomorphology of some endocrine glands of a cyprinid fish, Gnathopogon elongatus and also in a salmonoid fish, Plecoglossus altivelis.

In the carp, Cyprinus carpio, which is a spring spawner, the proportion of basophils drops from 70-80% in Spring to less than 50% in autumn, whereas in the rainbow trout, Salmo trutta irideus, an autumn spawner, basophils are more numerous at the height of the later spawning season (Parhon, Pittis and Dancasin, 1959). Statova (1959) carried out an investigation similar to that of Beach (1959) by correlating the appearance of the basophil cells in the pituitary of the zander, Lucioperca lucioperca, with changes in the ovaries of this fish, during the attainment of sexual maturity. Sundararaj (1959) suggested that the gonadotrophs of the pituitary gland of the Indian catfish, Heteropneustes, were the Periodic Acid-Schiff (PAS) positive cyanophils (basophils).

Critical analysis of the role of the pituitary gland in general, as well as the cellular origins of gonadotrophic

hormones, has been discussed by Ball (1960). In the Russian sturgeons, Acipenser guidenstadti and Huso huso, changes in the hypophysis have been studied by Barannikova and Polenov (1960). Many aspects of the relation of the pituitary of cyclostomes, elasmobranchs and teleosts to reproduction have been reviewed by Dodd (1960).

Sathyanesan (1960) gave the ratios of chromophobe-acidophil-basophil cells in his studies on the cyclical changes in the pituitary of Mystus seenghala and Barbus stigma. An increase in the quantity of pituitary basophils in Mugil cephalus, M. capito and M. auratus was observed by Stahl, Seite and Leray (1960). These basophil cells became vacuolized during maturation and may be assumed therefore to be associated with the secretion of gonadotrophins. The seasonal changes in the male pituitary of the Indian catfish were studied by Sundararaj (1960) who concluded that the changes he observed closely resemble those in the female. Sperm is present throughout the year, but the testis of this fish show seasonal variations.

Sokol (1961) gave a detailed account of the seasonal changes in the pituitary of Fundulus heteroclitus and those associated with gestation in Poecilia reticulata. During the late autumn and winter, the basophilic area of the meso-adenohypophysis of the killifish was in a depressed or inactive state. Not only were secretory grannules almost completely lacking, but in addition, the cells and their nuclei were in a shrunken condition. With the beginning of Spring more granules appeared in the meso-adenohypophyseal basophilis. Most of the nuclei were no longer compressed in appearance but were rounded, and their nucleoli were becoming obvious. By May, about half of the meso-adenohypophyseal basophils were filled with coarse, darkly-stained secretory granules. In June and July, the height of the spawning period, the basophils of the meso-adenohypophysis were solidly packed with secretory granules.

Only the unstained nuclei gave evidence of the cellular nature of this region, the cell boundaries being entirely obscured by the extreme density of the granules within the cells. After July, there was a steady decrease in the size of the deep-staining basophilic zone of the meso-adenohypophysis. The cells lost their granules and became smaller.

In the guppy Lebistes reticulatus a monthly-breeding viviparous fish, Sokol (1961) found that during the first few days after parturition, the ventral basophils were poorly granulated. There was a steady increase in basophilic granulation up to the beginning of the second week of gestation. From the middle to the end of the second week, there was, in general, a decrease in basophilic secretory material in the cells. The intervening decline in basophilia was followed by an immediate resurgence of granulation in the basophilic zone of the meso-adenohypophysis, during the third week of gestation, reaching its peak in the earlier part of the week. In the fourth and last week of gestation, with embryonic growth virtually complete early in the week, the pituitary of a female guppy revealed a profound decrease in the affinity of the meso-adenohypophyseal basophils for aldehyde-fuchsin (AF). This was a reflection of the reduced amount of secretory substance present in the cells. Chieffi (1962) described also the changes in the pituitary gland that accompany gestation in Torpedo marmorata and egg-laying in Scyliorhinus stellaris.

Robertson and Wexler (1962a) found identical results in their studies of the histological changes in the pituitary glands of the rainbow trout, Salmo gairdnerii and Pacific salmon, genus Oncorhynchus, accompanying sexual maturation and spawning. The pituitary glands of the sexually immature fish were characterized by a relatively small meta-adenohypophysis consisting principally of acidophils, and a large meso-

adenohypophysis. As the gonads developed, the dorsal lobe enlarged, the chromophils increased in number, with the basophils finally becoming predominant. Similar results were found by the same authors in the senile castrated kokanee Salmon, Oncorhynchus nerka kennerlyi (Robertson and Wexler, 1962b). In all the three fish, the investigators found degenerative changes in the glands, commencing some time before maturity.

Changes during individual development and seasonal changes in the pituitary have been described by Honma and Tamura (1963). Certain cyanophil cells were observed which seem to be gonadotrophs. Cyanophils of the maso-adenohypophysis of Hilsa ilischa, Barbus stigma, Heteropneustes fossilis and Mystus seenghala showed marked increases in secretory activity during pre-spawning and spawning seasons (Sathyanosan, 1963). In the pituitary of the catfish, Mystus vattatus Sathyanesan and Singh (1963) observed seasonal changes in the PAS positive cells. Gradual increases in the number of these cells occurs before spawning with drastic decreases afterward. During the sexual cycle, the authors believe that acidophils change into cyanophils. The meso-adenohypophysis of the pituitary gland of Gobius giuris exhibits profound cyto-morphological changes in its cyanophils during the reproductive cycle. Cytochemical studies indicated that a gradual accumulation of the acidophilic globules occurred within these cells until spawning, and that these globules were depleted during subsequent stages (Rajalakshmi, 1966).

Recently, descriptions of the pituitary of the African lungfish Protopterus sp. have been given by Kerr and Van Oordt (1966). The authors, employing different staining techniques, distinguished three types of basophils and two types of acidophils in the meso-adenohypophysis. In the cichlid fish Aequidens portalegrensis Metuzals, et al (1968) described the general histology and the histo-cytological changes in the pituitary. Two types of acidophils were distinguished in the pro-adenohypophysis

as well as chromophobe cells; the first type of acidophils occurring also in the meso-adenohypophysis. In the latter region two types of basophil cells were recognised. Faintly staining basophils differing from the basophils of the meso-adenohypophysis were discovered in the meta-adenohypophysis. These basophil cells, according to the authors lack granules.

To designate the hypophyseal cells by their tinctorial affinities is successful, to some extent, in describing the general histology of the pituitary gland and the cyclical changes occurring in it in connection with maturation. For cytological and histophysiochemical studies, however, a much more identification criteria and a logical terminology is necessary. Romeis (1940) proposed a terminology based on Greek letters. In his terminology, the alpha (α-) cells corresponded to the classic acidophils, the beta (β-) cells, to the basophils and the gamma (γ-) cells corresponded to the chromophobes. Having developed various cytological and histochemical techniques Herlant (1960; 1964) described the α-cells of Romeis containing simple proteins as Serous cells. The author grouped the β- and γ- cells together for being mucoproteinaceous in nature and described them as mucoid cells. Herlant stated that α-cells are responsible for the secretion of somatotrophin (STH), prolactin (LTH) and corticotrophin (ACTH); while the mucoid cells comprise the β- and γ- cells or FSH and LH gonadotrophic cells as well as the δ- or thyrotrophic (TSH) cells. A report of the International Committee for Nomenclature of the adenohypophysis calls attention to the necessity for improving the present chaotic situation in the nomenclature of the hormone-producing cells in the adenohypophysis (Van Oordt, 1965).

Cytochemical and histochemical studies on the pituitaries of fish have been carried out by a few workers. Ball (1965) studying the histology of the pituitary remnant that was found among hypophysectomized killifish, Fundulus heteroclitus, in a

series of experiments carried out by Pickford, et al (1965), observed that the regenerated pituitary consisted almost entirely of the erythrosinophilic cells that in the normal pituitary occupy the pro-adenohypophysis, and also that the regenerated remnant secreted the prolactin-like hormone necessary for freshwater survival in the killifish. Kerr and Van Oordt (1966) described five cell types in the adenohypophysis of the African lungfish and equated the first three (basophils) with Thyrotrophic or TSH cells, FSH cells and ICSH cells respectively, depending on their time of appearance, staining affinities, abundance and distribution. Olivereau (1963, 1967) using several histochemical techniques, subjecting fish to various experimental conditions and conducting series of hormonal injections identified prolactin cells, ACTH cells and TSH in the pro-adenohypophysis of the female yellow or silver eels. From the meso-adenohypophysis STH and two types of gonadotrophs were identified. Two cell types were distinguished from the meta-adenohypophysis but their functional significance, the author added, remains uncertain. In the pro-adenohypophysis of the European eel, Anguilla anguilla Hanke, Bergenhoff and Chan (1967) described the ACTH cells.

During their histological studies on the pituitary of the cichlid fish Aequideus portalegreusis, Metuzals and his collaborators (1968) described an α-cell type, two β-cell types and a γ-cell type. The authors hinted about the possibility of the production and storage of LTH by the α-cells, the production and release of FSH by the β_1-cells and the production and release of LH by the β_2-cells. Molnàr and Szabo' (1968) ascertained that in the pituitary of Transylvanian lamprey, Eudontomyzon danfordi; the ACTH cells were not located in the pro- but in the meso-adenohypophysis.

Olivereau (1969) by employing histological techniques identified eight cell types in the pituitary of the roach. The pro-adenohypophysis consisted of three cell types; eta

or erythrosinophilic cells which were similar to the prolactin-secreting cells of other teleosts, ACTH cells and possibly TSH cells. Two cell types in the meso-adenohypophysis were suggested to be gonadotrophs. The third cell type of this region tended to enter the ramifications of the neurohypophysis. Its significance was not established. Two cell types were identified from the meta-adenohypophysis.

The cytological changes of the eta ("prolactin") cells in the pituitary of T. mossambica due to changes in environmental salinity have been described by Dharmamba and Nishioka (1968). In fish maintained in fresh water, the eta cells in the well-developed pro-adenohypophysis were large and contained many large secretory granules. The same region of the pituitary was found to be smaller in the fish kept in seawater and the eta cells appeared condensed.

In the present study an attempt would be made to describe the histocytology of the hormone-producing cells in the pituitary of Tilapia.

PITUITARY INFLUENCE ON THE GONADS

Numerous experiments on fish have employed the technique of introducing gonadotrophins into the body through a physiological solution of the pituitary itself or extracts thereof or through other physiological fluids such as pregnancy urine or serum.

Calvet (1932) tried to accelerate oogenesis in ammocoete larvae of Lampetra planerii by bathing the animals in a solution of pregnancy urine. Morotality was high, but considerable increase in size of the gonads was found in the two animals which survived. As a result of continuous injection on the eel, Anguilla volgaris, using prolan, Boucher, Boucher & Fontaine (1934) observed a relative increase in the weight of the ovaries and testis, and also an increase in the spermatogenic activity. By the injection of prolan, Morozova (1936), Skadovski and Parfenova (1937) observed ovulation in the perch, Perca fluviatilis.

The use of fresh pituitaries or those dehydrated in acetone for injection was first adopted by Brazillian and Russian specialists. Gerbilskii and Kashenko (1937) injected a suspension of hypophysis in the skull of Osmerus eperlanus and observed a transformation into spawning condition, and obtained ripe sexual products a month before the spawning period of Osmerus in nature. Ripe eggs and sperm have been received also from the pike-perch, Lucioperca lucioperca, the sturgeons, Acipenseridae and other fish of commercial importance (Gerbilskii, 1938 a, b, 1939, 1940 and 1941) using pituitary injection.

Table 3 gives a summary of the effects of Pregnant Mare Serum (PMS) and Chorionic Gonadotrophin (CG) on the gonads of fish. A substantial review of literature on the effects of mammalian pituitary preparations (among other sources of gonadotrophins) on the reproduction of fish has been prepared by Pickford and Atz (1957) and Dodd (1960).

Fish pituitaries are now being used extensively in the breeding of Indian Carps (Chandhuri, 1960; Ramaswami, 1962), and in the induced spawning of the grey mullet, Mugil cephalus reared in captivity in freshwater ponds (Yashouv, 1969).

Table 3. Effects of Pregnant Mare Serum (PMS) and Chorionic Gonodotrophin (CG) on reproduction in fish.

Author	Recipient	Material injected	Observation
Berkowitz (1941a)	Lebistes reticulatus	PMS and HCG separately	No increase in testicular weights.
Egami (1954a)	Misgurnus anguillicaudatus	PMS and HCG separately	Considerable increase in ovarian weights.
Egami (1954 b,c)	"	"	"
Hasler, et al (1939 b,)	Salmo gairdnerii	PMS	No ripening of eggs during four weekly injections. Once a week injection had no effect either. However, one injection toward end of spawning season accelerated maturation.
Johnson and Riddle (1939)	Salmo gairdnerii	PMS	Spermiogenesis occurred in three of the seven males. No female ripened.
Regnier (1937,1938 a,b)	Xiphophorus helleri	PMS	Twelve out of 20 females matured after 20 days of injections.
Vivien (1950 b)	"	"	No effect on ovaries

Table 3 (Continued)

Author	Recipient	Material injected	Observation
Baldwin & Li (1942)	*Xiphophorus helleri*	HCG	Spermatogenesis was exhibited in four out of 20 fish.
Butler (1940)	*Carassius auratus*	HCG	Slight increase in number of smaller eggs but gravimetric increase was very significant.
Brunn & Moller Christensen (1941)	*Anguilla anguilla*	HCG	Acceleration of spermatogenesis.
Boucher *et al* (1934)	"	"	Increase in testicular weights and incomplete maturation.
Ramaswami & Sundararaj (1957 a)	*Heteropneustes fossilis*	"	Successful breeding.
" (1957 b)	*Clarias Batrachus*	"	"

EFFECTS OF HYPOPHYSECTOMY AND REPLACEMENT THERAPY

A second approach has been made in an attempt to elucidate the influence of the pituitary gland on the gonads. Hypophysectomy, followed by replacement therapy has been the classic experimental method used by various authors to demonstrate pituitary control in vertebrates. The pioneer work of Vivien (1938, 1941) and Matthews (1939a) firmly established the pituitary regulation of the gametogenetic activity in teleost fish. Vivien (1938) found that spermatogenesis was inhibited by hypophysectomy in Gobius paganellus and that only spermatogonia eventually remained in the testis. His results indicate that gonadotrophin withdrawal (hypophysectomy) had different effects on spermatogenesis at different seasons of the year. The author, however, gave little information on the specific stages of cell development during which the effects of hypophysectomy are observed.

That ovulation ceases as a result of removal of the pituitary in fish was also demonstrated by Vivien (1941) who removed the gland from female gobies shortly before spawning and thereby prevented ovulation. During this period the control fish spawned. He also showed that pituitary injections of hypophysectomized fish would restore ovulation, and that glands from other species of fish were also effective in this respect.

It is now clear that many workers have been able to induce premature ovulation in bony fish by administering fish pituitary material, usually as injections. These workers have all come to the same conclusion as Vivien, that ovulation is directly dependent on the pituitary. After injections of pituitary extracts into Fundulus failed to have any stimulatory effect on the testis, and very little effect on the ovaries, Matthews (1939a) removed the pituitary gland and observed regressive changes which were particularly striking in the males. The author concluded that the pituitary gland exerted a controlling influence on the seasonal cycle which the testis of Fundulus exhibited. He, however, did

not make any attempt to inject pituitary material into the hypophysectomized fish.

The general point on the administration of fish pituitary material, usually as injections, is that glands from any one species may be effective in stimulating ovulation in any other teleost, but as with follicle-stimulation there is evidence of relative specificity correlated with phylogenetic relationships (Kazanskii, 1940).

The dosage relationship has not been the concern of most workers, but Kazanskii (1949) established the minimum dosage of an acetonized preparation of gonadotrophic hormone of many fish, using, especially the loach, Misgurnis fossilis, as the test object. He found that the Cyprinids were universal donors, while the pituitary hormone from the pike-perch would not provoke ovulation in any other fish. Ramaswami and Sundararaj (1957) found that pituitary glands from the gravid catfish Mystus were as effective as homoplastic glands in causing ovulation in another catfish, Heteropneustes; whereas glands from Ophiocephalus belonging to a different suborder, were ineffective at the same or greater dosage. There was evidence too that the female gland had a greater ovulation-inducing potency than that of the male, in sturgeons, carps, catfishes and herring (Barannikova, 1949a; Ball and Bacon, 1954; Ramaswami and Sundararaj, 1957; a,b c [illegible]).

Preovulational nuclear changes in the oocyte have been described for sturgeon by Russian workers (reviewed by Pickford & Atz, 1957) and for loach by Kawamura and Motanaga (1950). These changes can be accelerated and their time relationship altered by pituitary treatment (Kazanskii, 1950, 1954; Kawamura and Motanaga, 1950). Just before ovulation, too, the granulosa cells of the follicle increased in height and their nuclei enlarged (Kazanskii, 1950; Ball, 1960; Dadzie, 1968). These changes have also been induced by hormone treatment (Kazanskii, 1950; Kawamura and Motanaga, 1950; Greep and

Chester Jones, 1950). These changes suggested the possibility that pituitary gonadotrophins induced processes in the granulosa cells which resulted in ovulation. Greep and Chester Jones (1950) have demonstrated that LH produced pre-ovulatory follicular swelling and the zona granulosa became greatly increased in size.

More evidence is being obtained in support of Vivien's conclusion on the dependence of the normal function of the teleost gonad on the presence of the pituitary gland.

Changes in the gonads after hypophysectomy have been examined in Anquilla anquilla (Olivereau, 1954), in Fundulus heteroclitus (Burger, 1941, 1942). Bretschneider and de Wit (1947) studied the effect of hypophysectomy on the gonads of Rhodeus amarus. In Xiphophorus helleri (Vivien, 1952 a,b). Bathygobius soporator (Tavolga, 1955) and Ophiocepalus punctatus (Belsare, 1965) evidence of the effects of hypophysectomy on the gonads are given.

The critical experiments of Dodd and his collaborators (1960, 1963) have conclusively demonstrated pituitary gonadotrophic control in the most primitive class of living vertebrates, Lampetra fluviatilis, hypophysectomized in the late autumn and winter. The experimental fish were studied over a period of five months and the histological changes in the gonads of both males and females carefully described (Dodd, Evennett and Goddard, 1960). During this period, the normal and sham operated animals matured rapidly; ova increased in size, the ampullae of the testis became filled with spermatozoa; secondary sexual characters appeared and spawning occurred in early April. In female lampreys, hypophysectomy abolished the normal increase in size and dry-weight of the eggs. After hypophysectomy the ovary remained small and compact and the eggs were not released into the body cavity. However, Dodd and his associates did not observe any sign of atresia. The testis of lampreys hypophysectomized in November and December

contained sexual cells at all stages of spermiogenesis, from early spermatids to spermatozoa, the latter being loosely scattered, when the fish were examined in April of the following year. Examination in April of biopsy samples from the testis of lampreys hypophysectomized in October revealed the presence of primary spermatocytes in late prophase, together with meiotic prophases, secondary spermatocytes, spermatids and, rarely, spermatozoa. In every case the testis contained spermatozoa. The testis of those fish hypophysectomized in January and later, when examined in April, contained only spermatozoa. In all the hypophysectomized animals the maturation of the testis was greatly delayed although both spermatogenesis and spermiogenesis continued. The results of these experiments are significant, at least for two reasons. The first is a clear establishment of the fact that spermatogenesis and spermiogenesis in the lampreys, unlike other vertebrates, are autonomous processes, though they are appreciably catalysed by the pituitary, the absence of which delays the appearance of sperm by some ten weeks. The second point of interest is that in the female of other vertebrates, hypophysectomy is followed by atresia of all yolk-containing eggs, but in female lampreys no signs of atresia were observed after the withdrawal of the pituitary.

Secondary sexual characters never appeared in the operated animals, but after replacement therapy using lamprey pituitary extracts, mammalian gonadotrophins and steriods, they were restored in April. Sperm was present in the testis at that time and ovulation occurred in the females.

In the elasmobranch, Scyliorhinus caniculus, there was a complete pituitary dominance of gametogenesis (Dodd, Evennett and Goddard, 1960). Gonadotrophins were essential for the transformation of spermatogonia into spermatocytes. In the females, vitellogenesis ceased and eggs larger than about 4 mm. become atretic as well as the follicle.

The effects of hypophysectomy on the ovary, ovulation and oviposition and sexual behaviour in the goldfish Carassius auratus has been studied by Yamazaki (1961, 1962). The author observed atresia of all yolky eggs, while young oocytes failed to acquire yolk. Ovulation in the females was inhibited but not oviposition. Males showed normal sexual behaviour, but the emission of sperm ceased about five days after the operation. Spawning and fertilization took place in the recently hypophysectomized females and males, and normal offspring were produced.

Barr (1963 a,b,c) hypophysectomized both sexes of the plaice, Pleuronectes platessa and examined the effect of this operation on the sexual cycle of these fish. Barr's observations were in conformity with those of other workers. Vitellogenesis stopped in the operated animals and was followed by atresia of those oocytes in which yolk had already appeared; in the males, spermatogenesis ceased at the spermatogonial stage, although later stages could be completed in the absence of the pituitary and spermiation might occur. These data indicated that in fish the pituitary was necessary for the development and maturation of the sexual cells.

Replacement therapy was attempted by Barr (1963), using ripe fish pituitary material, CG and PMS. The administration to ripening fish of pituitary material obtained from ripe fish resulted in precocious maturation and ovulation of eggs. PMS did not provoke ovulation in the plaice. The fact that injection of C.G. but not PMS produced precocious maturation, the author said, suggested that the mechanism of ovulation was a response to "lutenizing" hormone (LH) rather than "follicle-stimulating" hormone (FSH). The author himself attached less importance to the results of his last experiments and stated that : "These results are highly suggestive, but it should be borne in mind that the animals were close to the normal spawning

season and that other stimuli, such as release of endogenous hormone at hypophysectomy, may have been responsible for the maturation of the eggs."

Recent reports on the effects of hypophysectomy have been, in most cases, in agreement with the findings of previous workers on the pituitary regulation of gametogenesis (Belsare, 1965; Ahsan, 1966; Sundararaj and Goswami, 1968 a, b; Yamazaki and Donaldson, 1968; and Pandey, 1969a). In all cases, hypophysectomy was followed by atresia of all yolky oocytes. Immature ova seemed not to be affected. In the males, regression of the testis coupled with abolition of mitosis in the spermatogonial cells into spermatocytes was also prevented. However, in the hypophysectomized adult male guppy, Poecilia reticulata Pandey (1969a) observed that spermatocytes, spermatids and sperm already present in the testis at the time of the operation develop into spermatophores.

Replacement therapy using partially purified salmon pituitary extracts (Ahsan, 1966; Yamazaki and Donaldson, 1968) and methyl testosterone (Pandey, 1969 b) had stimulatory effects on the gonads. The GSI of the hypophysectomized guppy receiving methyl testosterone was significantly increased, spermatogonial cysts divided rapidly and transformed into spermatocytes, but later stages of spermatogenesis did not occur. The author, however, did not give convincing explanation to his later observations.

The results under the present section of the review, especially those of Dodd and his associates (1960, 1963), Yamazaki (1962), Barr (1963) and Pandey (1969) suggest that there are still more facts to be uncovered in the study of the reproductive physiology of fish which falls within the scope of the present research.

The pituitary regulation of gametogenetic activities has been demonstrated by classical removal of the gland. This operation, however, is not always feasible in some fish because of

the anatomy of the head region (Hoar et al, 1967; Wiebe, 1968a). The latter workers applied Methallibure (ICI 33,838), a derivative of dithiocarbamoylhydrazine in inhibiting the pituitary of fish. Methallibure has the following chemical formula :

$$CH_2 = CH - \underset{\underset{CH_3}{|}}{CH} - NH - CS - NH - NH - CS - NH - CH_3$$

Treatment of fish with methallibure was first performed by Hoar et al (1967) who injected a suspension of Methallibure into the peritoneal cavity in three species: the gold fish, the three-spine stickleback, Gasterosteus aculeatus, and the surf perch, Cymatogaster aggregatus. Methallibure effectively blocked the pituitary gonadotrophic action in all the species studied. Vitellogenesis ceased in the ovary and spermatocytes were no longer formed in the testis. Proliferation of spermatogonia by mitotic division however, continued. In the sticklebacks, spermatocytes and sperm already present appeared healthy and normal. The authors also observed the reduction in gonadal steriodogenesis which resulted from an inhibition of pituitary gonadotrophins and evidenced by the decrease in kidney tubule height of male sticklebacks.

Wiebe (1968a) treated the viviparous seaperch, Cymatogaster aggregata with this nonsteroidal, nonhormonal compound. This time, the author added a suspension of Methallibure to the aquarium water five or six times per week. The pituitary gonadotrophic action on the gametogenetic processes was blocked. Spermatogenesis was halted after 4-6 weeks of treatment. Atrophy of secondary sex modifications on Cymatogaster male anal fins was observed, as well as a marked reduction of histochemically demonstrable steroid dehydrogenases in the intestitial cells.

The above mentioned authors, treating fish with methallibure, arrived at the same conclusion that the compound had the same effects on the gonads as the effects of surgical hypophysectomy.

L.H. treatment of methallibure treated Cymatogaster resulted in a significant increase in the number of dividing germ cells and stimulated the Leydig and epithelial boarder cells, resembling, once more the effect of replacement therapy on hypophysectomized fish, even though Wiebe (1968a) did not state the period of L.H. treatment.

The usefulness of this substance as a pharmacological agent for "hypophysectomy" Hoar et al. (1967) suggested, will depend on the extent of the blocking and the absence of side effects (either general toxicity or an action on other endocrine tissues).

Hypophysectomy in Tilapia mossambica, although it has been carried out by Handin, Nandi and Bern (1964) and Dharmamba, Handin, Nandi and Bern (1967) has apparently never been investigated in the context of reproduction. The reports of these authors on the technique of hypophysectomy are not very re-assuring. The survival rate of Tilapia mossambica after hypophysectomy is very depressing. The animals failed to survive more than six days in fresh water (Handin et al (1964). However, the same investigators report that this survival could be prolonged to 20 days in isotonic teleost Ringer's solution, although their mortality was somewhat higher than that of sham-operated controls.

EFFECTS OF GONADECTOMY AND HORMONE TREATMENT

Gonadectomy in fish has been performed by many research workers. The operation is often difficult to accomplish without a high mortality or a large proportion of unsuccessful attempts resulting from regeneration of the glands (see Pickford and Atz, 1957). Unless all specimens are finally examined under the microscope after being serially sectioned, the results of an experiment may be open to serious doubts (Atz, 1957).

Surgical castration has been employed most often to elucidate the role of the sex hormones in the development and maintenance of sex accessories or secondary sexual characters.

A close correlation between the secondary sexual characters and the gonadal secretions in animals has been demonstrated conclusively during the past decades. The work of numerous investigators has shown that removal or heterosexual transplantation of the sex glands was inevitably accompanied by marked disturbances of these characters. Experiments of this nature on mammals, birds, reptiles and amphibians have been so numerous and the results so uniform that the broader aspects of the problem are no longer open to question (Beach, 1948). In the case of fish, however, the relation between the sexual characters and gonadal secretions have been, until recently, assumed to be similar to that in other vertebrates, largely without the confirmation of experimental investigation. In fish, therefore, the relationship of the gonads to sex behaviour is not well known, and the evidence is, as yet, fragmentary and inconclusive.

The investigations of Bock (1928a), Tazowa (1923, 1929), Zahl and Davis (1932) and Ikeda (1933) provided the first evidence of a correlation between secondary sexual characters and gonadal secretions in fish. Males of stickleback, Gasterosteus (Bock, 1928) and Ikeda (1933), Japanese bitterling, Acheilognathus (Tazowa, 1929) and Amia calva (Zahl and Davis, 1932), have been

reported as losing all spawning and nesting behaviour after castration. The observations of numerous other investigators, some of which being inadequate in themselves as a basis for generalization, served to strengthen the conclusions drawn by the workers mentioned above, e.g. Glaser and Haempel (1932) in the bitterling, Rhodeus amarus, Kopec (1927) in the elritze, Phoxinus laevis; St. Amant (1941) in certain poeciliid fish such as Gambusia; van Oordt and van der Maas (1926) in the swordtail, Xiphophorus helleri; Hopper (1949a, b. 1951) in the guppy, Lebistes reticulatus.

Similar evidence has been obtained by hormone administration techniques for male bitterlings, Acheilognathus and Rhodeus (Wunder, 1931; Saito, 1936; Duyvene de Wit, 1940), male poeciliids, Gambusia (Turner, 1942 a, b, c,) Xiphophorus (Regnier, 1938; Sangster, 1948), male Fundulus (Burger, 1942) and the female gobiid fish, Chloea (Kinoshita, 1938). In these cases, the reproductive behaviour was similarly controlled by gonadal hormone.

The above observations, however, could not be found in the case of Tilapia macrocephala in which Aronson (1948) found that although castrated males lost their characteristic yellow opercular colours, they built normal nests. A second structure which was affected by castration in this fish was the genital tube, a small flaccid papilla that becomes erect and extended just before oviposition. The oviduct or sperm duct ends in this papilla as does the mesonephric duct. Aronson (1948) castrated young mature females of T. macrocephala and injected them on the same day. Injection continued every two weeks thereafter for 22 weeks. Genital tube lengths continued to increase in the controls and in the intact animals treated with sesame oil. In the castrates, the genital tube lengths dropped very rapidly and in some cases the genital tube disappeared almost completely. Following treatment with both

testosterone and estradiol, the genital tubes increased rapidly in size and continued to rise parallel to the controls.

The opercula were unaffected in the controls and in the intact sesame-oil treated animals. The castrate non-treated females developed silvery opercula similar to those of immatures. The castrate females receiving testosterone developed yellow opercula like those characteristic of the males. Intact females receiving testosterone also developed yellow opercula. The results to this point are what would be expected if the gonadal hormones of Tilapia were comparable in action to the mammalian hormones. However, Aronson observed that castrate females receiving estrogen did not redevelop the semi-transparent window in the opercula, as was anticipated, despite the fact that estrogen increased the size of the castrate genital tube. Also, intact females receiving estrogen lost the semi-transparent window in their opercula, which developed an appearance resembling that of castrates.

Aronson (1948) explained the results of his experiments with the suggestion that the hormone produced by Tilapia ovary may not be estradiol, but a steroid sufficiently similar so that estradiol stimulated growth of the genital tube. The author cited Bretschneider and Duyvené de Wit (1947) as having reported that in the bitterling, Rhodeus a variety of steroids will cause lengthening of the ovipositor and therefore suggested that it is likely that the genital tube of Tilapia and the ovipositor of the bitterling are homologous tissues having similar growth characteristics. In the intact estradiol-treated fish, the injected hormone may have maintained the genital tubes but at the same time may have inhibited the ovary from secreting a more specific hormone controlling the coloration of the female operculum. Such an inhibition might work through the pituitary and indirectly on the ovaries. That an inhibition of this kind actually occurs in T. macrocephala was verified by the fact that the ovaries of the intact estrogen-treated animals, according to Aronson were clearly underdeveloped.

It has already been mentioned that castration has been carried out in the three-spined Stickleback by Bock (1928a). Ikeda (1933) and also by Craig-Bennett (1931), and all the investigators found that, in the male, the secondary sexual characters (e.g. the red belly and the blue eye) gradually disappeared after the operation. Baggerman (1957) also observed that no sign of nuptial coloration returned in fully castrated males that were held in a healthy condition for over eight months before they had to be sacrificed. With regard to the effect of castration on behaviour, Baggerman's experiments showed that males gonadectomized in the sexual phase very soon ceased to perform sexual activities, though usually some aggressive behaviour remained.

That gonads were not essential for parental behaviour in the stickleback was demonstrated by Baggerman (1957) who found that when given eggs the castrated male sticklebacks started a normal parental cycle, though at a somewhat lower level. The result, the author suggested, might be an indication that parental behaviour was induced and maintained by prolactin secreted by the pituitary.

Hoar (1962) also observed gradual fading of the developing breeding colours of male sticklebacks, castrated just at the beginning of the appearance of the nuptial colours. These fish completely reverted to the immature silvery condition by three weeks after the operation. There was no evidence of nest-building or other breeding activities. The author further observed that gonadectomy during the pre-breeding phase did not seem to interfere with the aggressive behaviour when the fish were maintained under long photoperiods. Both the operated and unoperated fish were less aggressive under the short photoperiod regime. Since aggressive behaviour of the castrated fish was thus less intense under the short photoperiods, the author argued that factor(s) elaborated in the pituitary (stimulated by long photoperiods) were probably associated with

the initial phases of antagonistic behaviour during the establishment of breeding territories in the stickleback. This, however, was only a suggestion, and hypophysectomy would give a definite proof.

As a result of treatment of pre-spawning fish with reproductive hormones, Hoar (1962) came to a conclusion that in the male sticklebacks, the secondary sexual characters depended on the androgenic secretions of the testis but the breeding behaviour depends at least in part, on a hormone or hormones of the pituitary.

On the effect of castration on spawning fish, Hoar (1962 a,b) as a result of his experiments and personal communications with Baggerman concluded that "reproductive behaviour of the stickleback ceases within 24 hours (perhaps immediately) after castration. The gonad (presumably its hormone) is essential for the nest-building and sexual behaviour - including the defence of the nest site." On the effect of castration on the sex characteristics and general behaviour of the stickleback, Hoar's conclusion was similar to those on records that nest-building stops as a result of castration.

Androgen treatment of castrate male sticklebacks led Hoar to conclude that the gonad was essential for nest-building and associated reproductive behaviour. It has not yet been proved, so far, that androgen is the only hormone involved. Moreover, the refractoriness of androgen-treated fish under short photoperiods suggested a pituitary involvement.

Androgen treatment of female sticklebacks was less effective in the female (Oguro , 1956; Hoar, 1962) since 15 out of 27 of the castrated males built nests before two females showed this activity under the same conditions.

In castrate male loach, Misgurnus anguillicandatus, Kobayashi (1951) reported that the pearl organs and the swellings of the dorso-lateral region of the body just posterior to the dorsal fin never appear. In individuals, in which the pearl organs and the lateral swelling have already been formed at

the time of the operation, these characters disappeared within a short period. Following the implantation of pellets of methyl-dihydro-testosterone in the female, the author showed that the pectoral and abdominal fins were transformed into fins of the male type, and the pearl organs and lateral swellings appeared.

Gonadectomy was successfully carried out in five females and five male lampreys by Evennett and Dodd (1963). These lampreys developed no secondary sexual characters, and this, according to the authors, indicated that control by the pituitary of the development of these characters was mediated by the gonads since hypophysectomy, conducted during the same series of investigations, also abolished the appearance of the sex characters. Evennett and Dodd therefore concluded that even in the lampreys, the most primitive of living vertebrates, the pituitary gland was essential for the development of the secondary sexual characters.

Egami (1960) studied the morphology of the sex characters in several species of Japanese gobies, with reference to the effects of sex steriods on the characters. The author reported that castration and androgen administration showed that not all sexually dimorphic characters in various species were influenced by sex steroids. It is possible that further investigations would reveal the pituitary to be the source of production of hormone which influences the appearance of the other sexually dimorphic characters.

Apart from the elucidation of the role of the sex hormones in the development and maintenance of sex accessories or secondary sexual characters, gonadectomy has also been employed to identify the gonadotrophs in the pituitary. There is very little data on the latter topic, and it is in fact a subject which has only recently attracted the attention of research workers.

In his Ph.D. thesis, Schreibman (1962) studied the pituitary gland of the platyfish, Xiphophorus maculatus and

observed that the hypophysis of surgically castrated male fish was characterised by hyperplasia, hypertrophy, and prominent vacuolation of the peripheral meso-adenohypophyseal cyanophils. A description of "castration cells" in the peripheral meso-adenohypophysis was also presented.

Evennett and Dodd (1963) gave evidence that in gonadectomized lampreys examined at the height of the spawning period, i.e. in April, the basophil count in the meso-adenohypophysis remained low.

A detailed account of the histological changes in the pituitary of senile castrated kokanee salmon, Oncorhynchus nerka kennerlyi, was given by Robertson and Wexler (1962c). The authors reported the presence of large castration cells (basophils) in the mesoadenohypophysis some of which were multinuclear. Their cytoplasm was composed of densely packed granules. The relative number of basophil cells was considerably greater in the pituitaries of castrate kokanee than present in the pituitaries of immature kokanee. These castrate fish, however, showed at death regeneration of a small fragment of testis.

Degenerative changes were regularly observed by Robertson and Wexler (1962) in castrate Oncorhynchus: Vacuolization of the basophils with subsequent loss of nuclei; changes in the staining properties of the cytoplasmic granules of the basophils which normally stain green with the Masson technique, but in those old gonadectomized fish stain bright red, and an increase in the amount of connective-tissue stroma and occurrence of cyst-like spaces. As the deterioration progressed, the authors observed that cytolysis - especially of basophils - became more marked and was accompanied by the appearance of many "ghost cells", i.e. nuclear rims, which were seen lying free in the stroma reticulum (Robertson and Wexler, 1962b). In several pituitaries large areas of necrosis were present. According to the authors, cytological changes were most pronounced in the dorsal lobe (meso-adenohypophysis) but disintegration of cells also

occurred in other regions of the gland. Robertson and Wexler (1962b) however, pointed out that all these signs of deterioration are found in the pituitaries of spawning salmon.

Recent information on castration of fish was given by McBride and Van Overbeeke (1969). The authors studied the cytological changes in the pituitary of the sockeye salmon, Oncorhynchus nerka after gonadectomy, and reported that castration of fully grown, but sexually immature, fish appeared to prevent differentiation of any periodic acid-Schiff (PAS) positive cells. On the other hand, castration of mature stockeye induced degranulation of this cell type. The authors further noticed hyperplasia and hypertrophy in the acidophil cells of the gonadectomized fish and suggested that they might be connected with an increased production of growth hormone. The operation also resulted in the disappearance of the secondary sexual characters in the ripe fish . In the immature ones these characters failed to appear.

Unilateral castration has also been performed by several workers and all have reported some degree of compensatory hypertrophy in the remaining gonad (Bock, 1928, Craig-Bennet,1931). Robertson (1958) unilaterally castrated the rainbow trout and observed an accelerated development of the remaining testis. But this occurred only in trout more than 13 months old, below this age there was slight stimulation. In operations conducted on fish above 17 months of age, the author reported that the gonads did not develop faster than the gonads of unoperated controls. When pituitaries were implanted into those young fish below 13 months old, their testes increased greatly in size. He therefore concluded that the lack of response to the operation in young fish was due to the absence of gonadotrophin in their pituitary.

The compensatory hypertrophy response of the ovary after unilateral ovariectomy was studied at various periods of the annual reproductive cycle of the catfish Heteropneustes fossilis by Goswami and Sundararaj (1968 a,b). The authors observed that

the remaining ovary had the ability to hypertrophy and compensate for the loss of its contralateral counterpart in all periods of the reproductive cycle except the post-spawning period. They stated, however, that if the experiment was performed nearer to the spawning season, full compensatory hypertrophy was achieved quicker than in those in which the operation took place in an out-of-season period. Administration of high doses of EB effectively blocked ovarian compensatory hypertrophy whereas a low dose was only partially effective. HCG increased the compensatory hypertrophy response. HCG administered along with EB counteracted the inhibitory effects of EB, but no stimulatory effect on ovarian compensatory hypertrophy was noticed when FSH was injected. The authors concluded that in unilaterally castrated catfish gonadotrophins were involved in the ovarian compensatory hypertrophy response.

THE EFFECTS OF LIGHT AND TEMPERATURE ON THE REPRODUCTION OF FISH

It has been well known for a long time that environmental factors control reproduction among mammals, birds and other vertebrates. Bissonnette (1936) is a pioneer in this field of study; he demonstrated that light could initiate the sexual cycle of birds. Since then, very many studies on the same line have been carried out by a number of investigators, but only a few of them have used fish as their material in treating this problem.

Up to the present, the experiments on the effects of light upon fish reproduction have been confined to only a few species. This number may be too small for formulation of a general pattern. Moreover, almost all experiments of which the results have been published presented different evidence from different species, so that a generalization of experimental results is impossible. The latter statement is also true with regard to the effect of temperature on the reproductive cycles of fish.

By artificially alternating photoperiod Hoover and Hubbard (1937), Hazard and Eddy (1951) and Corson (1955) demonstrated the premature spawning of Salvelinus fontinalis.

For his Ph.D. thesis, Sargeant (1956) studied the effects and mode of action of external factors regulating reproduction in three species of fresh-water teleosts. In male sticklebacks long day length brought about early development of the secondary sexual characters, but did not hasten testicular maturation. In male minnows long day length brought about full spermatogenesis with development of spawning tubercles in late winter, a 24 hour day being more effective than a 16 hour day. A high temperature had the same effect, the optimum temperature being near 18°C. In female minnows high temperature and long days advanced oocyte ripening, but a great individual variation among the fish obscured the optimal conditions. In bitterling females, Sargeant reported that different levels of light durations

and intensity had no effect on the growth of the ovipositor at any stage of the reproductive cycle. A rise in temperature caused growth which was transient only.

Burger (1939, a, b, 1942) and Matthews (1939b) in Fundulus heteroclitus and Bullough (1939) in Phoxinus laevis have given information concerning the effects of light on the sexual cycle of these fish. Their information was in support of the theory, that light plays the main role in controlling the sexual cycles of fish.

Harrington (1950, 1956, 1959a, 1959b) gave evidence of the effects of day-length on the sexual cycles of cyprinid fish. The author, however, confined his observations to the relation between the time of exposure to light and the onset of the spawning cycle in out-of-spawning season. The period, during which the fish do not react to stimulus manipulations was named the "refractory period". In his work published in 1959(b) on the effects of four combinations of temperature and day-length on the ovogenetic cycle of a low-latitude fish, Fundulus confluentus, Harrington observed that high temperature induced later phases of maturation, but retarded earlier ones; low temperature accelerated earlier phases, but suppressed later ones. He suggested that retardation of oogenesis by high temperature seemed to be strongly reinforced by long days.

Morozova (1957) during her studies on the significance of external and internal factors in the sexual cycle of the perch Perca fluviatilis, kept males and females of this species under different environmental conditions, which could be briefly described as follows : males and females kept with and without light, heat, and a sandy substrate. She established that only too low a temperature prevented maturation and spawning.

In his review on the reproduction in male bony fish, Marshall (1960) discussed the environmental control of reproduction including the effect of light on the phenomenon of "runting" which is popular among populations of Tilapia.

John (1957) analysed the role of light, temperature and flooding in the regulation of the bimodal spawning season in Rhinichthys osculus in the chiricahua mountains, Arizona.

Baggerman (1957) experimentally analysed the effects of light and temperature on the timing of breeding and migration in the three-spined stickleback Gasterosteus aculeatus L. The conclusion drawn by the author on the rate of development at different day lengths was that a long day-length accelerated gonadal development, both at low and high temperatures. On the influence of different intensities of light on gametogenesis, Baggerman concluded that light of high intensity (± 300 f.c.) was no more effective in inducing maturation than light of a much lower intensity (25-30 f.c.). The author, however, pointed out that light intensity of about 15 f.c. may have a retarding effect on gonadal maturation compared to a light intensity of 25-30 f.c.

In the greenthroat darter, Etheostoma lepidum Hubbs and Strawn (1957) pointed out that the reproductive rate was controlled by temperature and the condition of the fish. No consistent differences were noted in the laboratory experiments between the fish exposed to different light durations held at approximately the same temperature. In the light of these results, the authors suggested that light intensity and duration did not appear to affect fecundity of greenthroat darters.

Seguin (1957) kept sexually mature brook trout, Salvelinus fontinalis in a dark hatchery and observed that spawning occurred two months later than usual. High temperatures accelerated maturation by nearly a year.

The goldfish, Carassius auratus, proved indifferent to ration of light (Bjorklund, 1958). Keeping immature males in darkness ten months and females nearly four did not prevent maturation.

The control of light has been found to be essential for controlling the time of daily spawning and collecting of eggs of the zebra fish, Brachydanio rerio Legault (1958).

Combs et al (1959) observed that spawning of Oncorhynchus nerka was delayed by lengthening daily light exposure and advanced by shortening it. The authors stated that light was more important in the maturation of adult blue-back salmon than water temperature.

Histological examinations of oocytes of baunt whitefish Coregenus laveratus baunti by Anpilova (1959) revealed that relatively high temperatures (13.5° - 17.5°C) were required through summer to induce transformation from protoplasmic to trophoplasmic growth.

On the influence of photoperiodicity in ayu-fish, Plecoglossus altivelis, Shiraishi and Takeda (1961) observed that short days accelerated sexual maturation and long days delayed it.

Experiments on the effect of light and temperature on the growth of Tilapia zilli have been performed by Cridland (1962) who concluded that strong periodic illumination for 12-hour periods appears to delay sexual maturity. On the other hand, under periodic low illumination, the growth rate was markedly affected by temperature, being greater at higher temperatures up to 31°C, than at lower temperatures down to 19.8°C. Sexual maturity was also attained earlier at higher temperatures, lower temperatures regarded the onset of maturation.

Gokhale (1957) analyzed the influence of temperature in the seasonal histological changes in the gonads of the whiting, Gadus merlangus L. and the Norway pout, G. esmarkii.

Ioff (1958, 1960) suggested that a period of cold followed by a rise in temperature was necessary for the ripening of gonads in the minnow, Phoxinus phoxinus, and that maintaining the fish through winter at about 20°C inhibited sexual maturation.

Keeping Oryzias latipes at 4-8°C for 2 to 24 hours delayed egg-laying and almost completely inhibited growth of the oocytes (Egami, 1959).

Maturation of Acipenser and Gasterosteus aculeatus was delayed for long periods in the spring and Coregonus in the autumn by selecting definite values of light and temperature (Kazanskii, 1962).

Temperature played an important role in the annual spawning of the catfish, Mystus seenghala (Sykes) (Sathyanesan (1962)).

In the ovipositor, Rhodeus amarus, high temperature and lack of suitable substrate inhibited ovarian maturation (Shirai, 1962). In his studies on the reproduction of rainbow trout, Salmo gairdnerii, Nomura (1962) proved that spawning could be accelerated by control of light.

On the effects of day-length on the reproduction of the Japanese killifish, Oryzias latipes, Yoshioka (1962) gave evidence that short days ended egg-laying, while long ones advanced the reproductive season in both sexes. Eggs laid by females exposed to 18 hours of light were smaller than normal.

In the zebra fish, Brachydanis rerio, Hisaoka and Firlit (1962) observed that at 26°C. females exhibited precise ovarian cycle of mating and egg-laying every five days. Higher temperatures reduced the interval, lower ones inhibited ovulation. Spawning occurred soon after day-break.

Photoperiodicity has clearly been established in the sticklebacks by Wai and Hoar (1963) who performed their experiments on intact and gonadectomized animals. The dependence of behaviour on light was shown by these authors who demonstrated that sixteen hours of light induced aggressive and territorial behaviour in both male and female gonadectomized Gasterosteus aculeatus, and that administration of methyl testosterone does not intensify aggressive behaviour in either the 8-hour or 16-hour photoperiod fish.

An increased light regime hastened maturation of adult male and female eastern brook trout, Salvelinus fontinalis, but had no effect on fish maturing for the first time. Fish exposed to short or long day-lengths responded differently to high or low temperatures, but those in natural day-length did not (Henderson, 1963a).

A classic method has recently been introduced by Kuronuma (1963) to control the spawning of Ayu, Plecoglossus altivelis by means of light. The difficulty for Japanese workers to get enough natural food (algae, rotifers, crustaceans, etc.) to feed the fry in pond water during the autumn and winter initiated these experiments to advance and delay the spawning time of Ayu by regulating the length of day-light hours or exposure to artificial lighting. To effect earlier spawning the daylight hours were shortened by 19 hours by covering the pond by shading during May, then, daylight hours were returned to normal until mid-June, followed by sudden or gradual shortening by 5 to 6 hours from natural duration. The fish treated this way spawned about two months earlier than those in natural rivers in the same region.

To retard the spawning of the agu the daylight hours were extended to 14 to 18 hours from July or early August to October, by adopting electric lighting. In this way gametogenesis was inhibited until the period between February and April the following year.

Temperature and light regulation of the cyclical activities in the lake chub were studied by Ahsan (1966a) who stated that temperature was the major environmental factor controlling spermatogenesis. Higher temperatures (16-21°C) promoted proliferation of spermatogonia and hasten or terminate spermiation in prespawning fish; low temperatures (5-12°C) the author stated, were essential for normal gonadal proliferation and formation of primary spermatocytes. Photoperiod did not appear to dominate spermatogenetic processes at any stage. In the male viviparous

seaperch, Cymatogaster aggregata. Wiebe (1968c) observed that an increasing or long photoperiod in late winter, spring or early summer resulted in spermatogenesis, development of secondary sex structures, and reproductive behaviour. Warm temperatures, he said, enhanced those processes, but the cold temperatures and short photoperiod of the winter months promoted testicular restitution and growth of spermatogonia. In females, the author reported that oocyte formation was enhanced by warm temperature (and perhaps decreasing photoperiod) of late summer and early autumn, while oocyte maturation was fostered by the cold temperatures of early winter.

Hyder (1969) cited his paper in the press inferring that in Tilapia both light and temperature were critical in the gonadal development. In T. leucosticta breeding was also stimulated by - but could certainly proceed without - rainfall. Thus, the author added, Tilapia in equatorial ponds enjoying high intensities of both light and temperature have highly stimulated gonads and reproduce continuously without rainfall.

The results of some of the experiments are of great importance in regulating maturation in other fish. Kuronuma(1966) demonstrated on the ayu-fish, Plecoglossus altivelis for the first time that it was possible to retard maturation at least in some species of fish, not only in the laboratory but also in ponds. Moreover, Hyder (1969) has demonstrated the role of light and temperature in accelerating precocious spawning in Tilapia. By adopting Kuronuma's method and that of other workers, e.g. Gridland (1962), it could be possible to retard gametogenesis in fish such as Tilapia which have the tendency to "runt" very quickly. By retarding maturation by regulating the length of daylight hours and possibly temperature, it could be possible that large-size fish could be reared.

STEROIDOGENESES

It has been demonstrated by several workers that sex steroids in fish are produced by the endocrine tissues of the gonads. The inference that these tissues produce steroids is based on the following grounds : (1) prevention of the appearance of the secondary sex characters as a result of gonadectomy, and the abolition of these characters when development had occurred before the operation (Hoar, 1965, Wiebe, 1967). (2) Stimulation of the appearance of the sex characters and an increased reproductive behaviour after administration of mammalian steroids (Tavolga, 1955; Pickford and Atz, 1957; Dodd, 1960). (3) Changes in the histological appearance of the hormone-producing cells concomitant with the gametogenetic state of the fish (Henderson, 1962: Wiebe, 1968a) (4) Isolation of steroids from the gonads and blood of certain teleosts (Gottfried et al, 1962, Lupo and Chieffi, 1963; Eylath and Eckstein, 1969). (5) Demonstration of 3B - Hydroxy-steroid dehydrogenase activity in the gonads (Bara, 1966 ; Yaron, 1966; Simpson and Wardle, 1967; Simpson et al, 1968; Wiebe, 1969).

The existence of 3B- Hydroxysteroid dehydrogenase, a key enzyme in steroid hormone biogenesis was first demonstrated by Samuels et al (1951). The histochemical methods for visualizing this enzyme has been found useful in localizing the sites of steroid hormone production in vertebrates (Wattenberg, 1958; Levy et al, 1959; Galil and Deane, 1966).

According to present concepts 3B - Hydroxysteroid is one of the basic steps in the biosynthesis of almost all the active steroids. The latter are synthesized from the former by means of a convenient steroid substrate e.g. dehydroepiandrosterone (DH_A), diphosphopyridine nucleotide (DPN) - dependent hydroxysteroid dehydrogenases (the cofactor), and a tetrazolium salt (Samuels, 1960; Barker and Anderson, 1963; Baillie et al, 1966). The reaction for this enzyme depends on the reduction of a water-soluble tetrazolium salt to an insoluble formazan, the deposit of which, in the tissues, marks the site of hormone production.

Identification of Steroid-producing Tissues in the Gonads of Fish.

(a) The Testes

Two types of endocrine tissues are encountered in the testis of fish - the typical interstitial Leydig cells which are also found in higher vertebrates, and the lobule-boundary cells which are considered to be the only endocrine tissue in the testis of some teleosts (Marshall and Lofts, 1956).

The presence of 3B - hydroxysteroid dehydrogenase activity in the interstitial cells of fish has been demonstrated by a few workers (Lupo and Chieffi, 1963, in Morone lubrax; Stanley et al, 1965, in Gobius pagannellus; Yaron, 1966 in Tilapia mossambica; Bara, 1966, in Fundulus heteroclitus; Livni, 1969 in Mugil Wiebe (1969) in Cymatogaster aggregata observed the activity in both the Sertoli cells and in the interstitial cells of Leydig. In Squalus acanthias, however, Simpson and Wardle (1967) observed this enzyme activity in the semen, Sertoli cells sytoplasm and in the lumens of mature germinal ampullae. No trace of Leydig cells with 3B - hydroxysteroid dehydrogenase activity was found in the testis of the spurdog by the authors, even though Collemot and Ozon (1964) demonstrated their presence in the testis of Scyliorhinus canicula.

(b) The Ovaries

There is much less information available regarding the steroid-producing tissues of the ovaries in relation to 3B - hydroxysteroid dehydrogenase, Bara (1965) gave evidence of the occurrence of 3B - hydroxysteroid hydrogenase activity in the thecal cells of maturing, mature and spent follicles of Scomber scomber. The follicular cells were similarly identified as the sites of sex hormone production in the ovary of Cymatogaster aggregata (Wiebe, 1968).

For a better understanding of the location and function of the hormonal steroids and their mode of production in connection with maturation and the appearance of the secondary sex characters, further investigation on fish into this aspect of endocrinology will be necessary.

In the present study, an attempt will be made to locate the 3B - hydroxysteroid dehydrogenase activity in both the testis and ovary of normal T. aurea, and then subject the fish to some experimental conditions and treatments with the hope to study the steroid-hormone regulation (if any) of maturation and the appearance of the secondary sexual characters.

MATERIALS AND METHODS

(i) Source and Maintenance of Fish

A breeding stock of Tilapia mossambica was maintained in the laboratory. They were generally maintained in 100-litre glass tanks, at temperatures between 25° and 30°C, under a ~~normal~~ natural regime of light and recurring darkness. They were fed ad libitum on commercially dried food (Tetramin), containing 46% crude protein, 5% crude fat and 8% crude fiber, and on Tubifex. The diet was supplemented, from time to time, with fresh lettuce. Occasionally, when large numbers of young or mature fish of the same generation were required, according to the nature of the experiment, they were procured from tropical fish dealers in London, Bradford, and Falkirk.

For breeding purposes, adult males and females were kept together in separate tanks. After spawning, the males were removed from the spawning tank. The young were shaken from the mouth of the female parents shortly after the eggs hatched, and they were maintained in the same tanks until the population density of the tank called for adjustments. The female parents were also removed from the spawning tank soon after the eggs were removed from the buccal cavity.

All the tanks were well aerated and there was a continuous (closed) circulation of freshwater. The tanks were cleaned fortnightly but one-third of the aquarium water was replaced weekly with tap water.

Tilapia aurea were raised in ponds at the Fish Culture Research Station, Dor, Israel. For the purposes of laboratory experiments, the required number of fish were, each time, transferred into 50-, 100-, 200-, 400-, and 500- litre tanks in the laboratory. The stages of maturation of the fish were always checked, to make sure that they satisfied the requirements of the experiment.

A few days were allowed for acclimation to laboratory conditions, before experiments started. They were maintained in the same way as T. mossambica except that they were fed only on commercially prepared pellets, containing 69% maize, 15% fish meal and 16% soya bean.

(ii) Experimental details and techniques.

(a) Studies on Gametogenesis and cyclical activity in the Pituitary

Two to three generations of T. mossambica, and one of T. aurea were used for these investigations. Sampling was routinely carried out and where possible, processing of the tissues followed without delay.

The terms "stage" and "phase" are commonly used in the text, especially in describing the process of development of the germinal cells and the cyclical changes in the gonads. In considering gametogenesis the term "stage" is used to describe specific periods of gonadal development. For instance, the whole period, starting from the transformation of oogonia into primary oocytes, and ending in the first evidence of the appearance of trophic substances in the oocyte, is called the period of protoplasmic growth or Stage II. However, this stage is further divided into two substages. These substages, are referred to in the text, as phases. Hence, primary oocyte and juvinile phases of protoplasmic stage. Similarly, the period of vitellogenesis, incorporating Stages III and IV are further divided into early-mid, and mid-late vitellogenetic phases. In considering the cyclical changes in the gonads in relation to the structure of the pituitary, the term "phase" was adopted, merely to harmonize with the popular terminology used by previous workers. However, to avoid confusion, a summary of the use of the terms "stage" and "phase" is given in Table 6.

(b) Studies on the Cytology of the Pituitary

The eta or prolactin cells.

Following reports by earlier workers on the identification of the eta cells by manipulation with the environmental salinities, and the inference of the osmoregulatory function of these cells, T. mossambica was also subjected to different saline environment, in an attempt to identify the eta cells in the pituitary. The experimental details are given with the results.

The α,- or Corticotrophin (ACTH) cells.

The only available information on the use of Metopirone (methyl - 2 bis (pyridyl) - 1,2 - propanone - 1 (CIBA) in the identification of the ACTH cells in teleostean pituitary was provided only recently (Olivereau, 1965). Apart from the ACTH cells, Metopirone, according to the same author, causes modifications in the thyrotrophs (TSH cells). This method was therefore preferred in the attempt to identify the ACTH and TSH cells. The experimental details are given with the results.

Upon the report of Castrejon (1949) on the effect of low temperature on the quantity of the ACTH cells in the pituitary after adrenalectomy, the pituitary of 10 fish maintained at 13° - 15° C (from another experiment) were examined for comparative studies.

For the studies of the other cell types in the pituitary and to elucidate their functional significance, numerous slides of pituitaries of fish under various experimental conditions falling within the scope of the present research programme, were examined.

(c) Studies on the Effects of Low temperature and Total darkness on Growth and Maturation.

All the details regarding the methods, sizes of fish used, and feeding regime in these experiments are given with the results.

(d) Tagging

For experiments requiring the identification of fish individually, the fish were tagged. The following technique was used: Nylon threads were used as tags. With the help of a needle one end of the thread was passed through the flesh of the fish at the anterior base of the dorsal fin. The ends were tied such, that a round figure made of the thread was obtained - several colours of the thread were used either singly, or in combination whenever necessary.

(e) Total Gonadectomy

Surgical techniques

The techniques of Aronson (1951) were adopted with slight modifications. Males and females were anesthetized by immersion in MS 222 (Sandoz) - ~~1:7000~~ 1:7000. The solutions were made up just prior to the operations. As soon as the fish became completely immobilized (2-5 minutes), it was removed from the solution and placed on the operating table, on a piece of wet paper towel.

A patch of skin was exposed in the region beneath which lie the gonads, by the removal of one scale. With the sharp point of a cataract needle, an incision was made through the skin into the peritoneum. The incision was then extended anteriorly, just ventral to the dorsal wall of the peritoneal cavity to the level of the pectoral girdle, and ventro-caudally, just anterior to the anal opening, with a pair of curved iris or capsular scissors.

The gonad was then traced anteriorly and freed from the mesorchium, with a pair of suture forceps. One gonad at a time was lifted and laid caudally over the tail of the fish. Then, the short gonaducts were freed from the surrounding tissues and the genital pore was carefully separated from the urinary pore before the gonads were transected. The incision was closed with individual stitches of No. 6 silk suture.

With experience, the whole operation lasts five minutes and it is usually bloodless. Getting to the end of the operation the fish usually showed signs of recovery from the effects of the anesthetics, and it was always preferred that it almost completely recovered before being placed in the aquaria. However, care was taken not to interrupt respiration for any length of time. This was achieved by maintaining a small pool of water, sufficient for respiration, around the mouth and on the body.

After the operation the fish were put back into the aquaria with freshwater. Since the trial fish survived in freshwater without the addition of salt ingredients and without any profilactic measures being taken, the experimental, operated groups were also maintained under the same conditions. The fish resumed feeding between 12 and 24 hours after the operation. The incision healed after 10-20 days, but in larger fish it took about 30 days to heal.

Unilateral Ovariectomy

The surgical procedures were the same as those employed in total gonadectomy, except that only a half-pair of the ovary was removed at the time of the operation. The removed gonad was weighed and preserved.

Sham Operation

The techniques for the sham-operations were also the same as those for the total gonadectomy except that the gonads were only exposed but not removed, and the incision was then closed with individual stitches.

(f) Methallibure Treatments

A suspension of Methallibure (a dithiocarbamoylhydrazine derivative; I.C.I Batch No. PD/AS5102/67) (My gratitude to Mr. B.D. Hoskin, Animal Health and Husbandry Dept., I.C.I., Macclesfield, for supplying the Methallibure.) containing 1.0 gm

per 100 ml distilled water was prepared. Tween 80 (about 1 drop per 10 ml) was used to maintain the suspension. Control solutions contained the same amount of Tween 80. Unless otherwise indicated, the suspension was added to the aquaria six times per week in doses of 1.0 ml. per 100 litres of water. This mode of treatment is also referred to as "external treatment". The controls received a similar volume of suspension medium.

In the "oral treatment" the same amount of commercially prepared pellets used as feed for the externally treated fish, was soaked in equal amount of Methallibure as those used for the external treatment. The pellets, dissolved in a suspension of Methallibure, were then repelletized and given to the fish. The fish accepted them readily since they were already used to eating the pellets.

(g) Pond Experiments

The area of the ponds used was 5 sq. metres. Apart from the natural food available in the ponds, commercially prepared pellets were also added as supplementary feed. Supplementary feeding was estimated at the rate of 4% of total weight of fish per pond in every 24 hours. Once a week, the ponds were fertilized with superphosphate to increase the growth of planktons, the natural food source of the fish. It was added at an estimated rate of 6 kg. per 25 sq. metres, plus the same amount of ammonium sulphate. An organic fertilizer (chicken manure) was also used.

(h) Hormone Treatments of Methallibure treated and Gonadectomized Fish

The following hormones were tested :

(i) Follicle Stimulating Hormone (FSH; Armour) Lot No. E.13803.
(ii) Luteinizing Hormone (LH; Armour) Lot No. D.13006.
(iii) Human Chorionic Gonadotrophin (HCG; Organon) Batch No. 48179.
(iv) Pregnant Mare Serum (PMS; Organon) Batch No. 48462.
(v) Extract of Carp Pituitary (CP; Collected from mature fish at the Fish Culture Research Station, Dor, Israel).

(vi) Testosterone Propionate (TP; Organon) Batch No. 48425.

(vii) Oestradiol Benzoate (OB; Organon) Batch No. 48657.

The diluent for FSH, LH, HCG, PMS and CP was sodium chloride while Arachis oil with 10% Benzyl alcohol served as the solvent for TP and OB.

The alcohol-preserved carp pituitaries were dried and homogenised before the appropriate amount was extracted for injection.

Further details, including individual doses of all the hormones used are given with the results.

Injection Techniques.

The fish were injected without anesthesia. They were placed on wet paper towels and injected intraperitoneally, using a 1.0 ml. syringe with a 26 g x 3/8" hypodermic needle. Some time was allowed before the needle was withdrawn from the body, a precaution which prevented loss of hormone from the fish after injection.

(i) Histological Techniques.

The usual fixatives used were Bouin and Bouin - Hollande with a sublimate. Occasionally, Zenker - formol fixative was also used. Sectioned tissues fixed in Bouin - Hollande were passed through Lugol's solution and then sodium thiosulphate before proceeding with the desired staining method. Sections of the ovary were cut at 5-7μ , and that of the testis - 3 to 5μ . Serial sections of the pituitary were also cut at 3-5μ .

Due to the minute size of the pituitary of Tilapia, the whole organ tended to fragment in the handling process. For this reason whole heads of the fish were fixed. Decalcification of the cranial bones became necessary in tissues fixed in Zenker-formol (in which the acetic acid is replaced by 5% formalin) and Bouin-Hollande (in which the acetic acid is substituted with a sublimate). The decalcifying agent consisted of 5 ml. Nitric acid

(HNO_3), 5 ml. formalin and 90 ml. of 70% alcohol. After decalcification, the regular routine of dehydration, infiltration, embedding, sectioning, staining and mounting was followed. Yolky oocytes, before infiltration with paraffin, were immersed in solutions of celloidin for about a week.

The following staining methods were employed : Heidenhain's iron haematoxylin, Heidenhain's azan, Cleveland-Wolfe, Crossmon's modification of Mallory's, Periodic acid Schiff, Thionin - PAS - Naphthol Yellow, Alcian Blue - PAS - Orange G, Aldehyde Fuchsin (Halmi) and Masson's Trichrome.

Methods of Cell Counting

In the testis : the degree of spermatogenetic activity was estimated by counting the total number of germinal cysts of each spermatogenetic stage found in six randomly selected cross-sections of testis lobules. The mean for each spermatogenetic stage was then estimated for each experimental group, and the percentage of the mean was plotted as a histogram.

In the pituitary : total counts of various cell types were made in six saggital sections. The mean for each cell type was then estimated for each experimental group, and the percentage of the mean plotted as a histogram.

(j) Histochemistry of the Steriod Producing Cells in the gonads.

Mature male and female T. aurea weighing between 24 and 60 gm and measuring 10.5 - 16 cm were used for these investigations. They were maintained at the Fish Culture Research Station in Dor, but were sent by car, on each day of the histochemical investigations, to the Hebrew University of Jerusalem (a three-hour journey) where the analysis took place.

Half of each gonad, soon after removal from the fish, was promptly frozen in liquid air, while the other half was fixed in Bouin's fixative for routine histology. The unfixed, frozen

tissue was sectioned at 12-15μ in a cryostat maintained at -25°C. The sections were dried at room temperature.

The techniques of Wattenberg (1958) with minor modifications, were used for locating the enzymes. The incubation medium consisted of 20 ml. of 0.2 M phosphate buffer (pH 7.4), 50 ml. of aqueous 0.2% solution Nitro blue tetrazolium (Nitro -BT; Sigma), 5 ml. Ringer's solution, 4.0 ml. distilled water containing 15 mg. B-nicotinamide adenine dinucleotide (NAD; Sigma) and 1.0 ml. acetone containing the substrate. The following substrates were tested : Δ^5 - androsten - 3B - ol - 17 one, 2 mg/1 ml. acetone; 11B dehydroxy - 5α - androsten - 17 - one, 1 mg/1 ml. acetone; 17 B - hydroxy - 4 - androsten - 3 - one, 1 mg/1 ml. acetone; and sodium succinate, 10 ml. of 0.06 M. The control medium lacked the substrate. The sections were incubated at 37°C.

(k) The Gonadosomatic Index (GSI)

The Gonadosomatic Index is a useful biological criterion and it reveals the relationship between the gonadal and somatic growth. It is defined as the weight of the gonad divided by the weight of the fish multiply by 100; i.e. GSI = $\frac{\text{Wt. of gonad}}{\text{Wt. of fish}}$ x 100.

Making use of this criterion, it is possible, in fisheries work, to compare either the rate of somatic, or gonadal growth, not only between species of the same generation, but also between species of different sizes belonging to a different generation.

In experimental work, GSI reveals either the increase, or decrease in gonadal weights of experimental animals, over controls, in relation to somatic growth. In the present work, GSI has been adopted for judging the gonadal weight differences in relation to the weights of fish under various experimental conditions, and the results have been compared with those obtained in controls. In this way, an idea of the effectiveness of a particular experimental technique or therapy, has been obtained.

RESULTS

OOGENESIS IN TILAPIA AUREA

The oocytes of the ovary of T. aurea undergo a series of morphological changes during development, and for descriptive purposes can be divided into various stages on the basis of : (1) the structure of the nucleus and nucleoplasm, (2) cell size, (3) the structure of the ovarian cell-wall, and (4) the presence of different inclusions in the oocyte. The whole process of growth of the oocyte is presented in Table 4.

Stage I - Oogonia

These are the primary germ cells from which the oocytes are formed, and are found either singly or in small nests (Plate 1(a). They are the smallest cells in the germinal tissue and measure 15 - 20μ in diameter. The cell is covered by a very thin sheet of connective tissue and has a large nucleus which measures 3 - 15μ . The nuclear wall, unlike the cell membrane, is sharply defined and the nucleus has a fine chromatin reticulum and a single prominent nucleolus (Plate 1(a).

Primary oogonia proliferate through mitotic divisions giving rise to subsequent generations of oogonia (Plate 1(a)).

Stage II - Primary oocytes.

After the last oogonial division there is a short period of growth during which the oogonia develop into oocytes. This transformation is accompanied by a reorganisation in the nucleus connected with meiotic division.

The chromosomes become conspicuous, and this marks the beginning of meiotic prophase. From the leptotene to the pachytene stages there is a small increase in the size of the nucleus from 3 - 5μ to 6 - 9μ .

After the pachytene stage an increase in the size of the oocyte is observed (Plate 1(b)) and the chromosomes become less conspicuous, shorten, thicken and become scattered throughout

PLATE 1

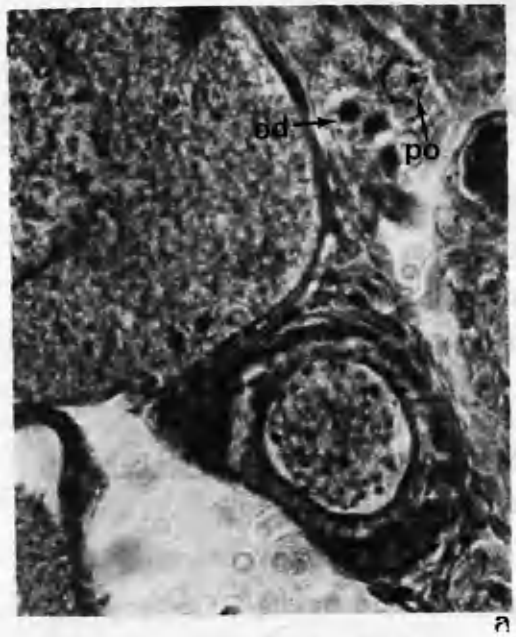
Oogenesis in *T.aurea*

a) T.S. of ovary of immature female showing primary oogonia (po) and oogonial division (od). Stained with Heindenhein's iron haematoxylin and Bouin fixation. X.620.

b) T.S. of ovary of immature female showing primary oocyte in the initial period of protoplasmic growth (arrow). Stained with Heindenhein's iron haematoxylin and Bouin fixation X.620.

c) T.S. of ovary of immature female showing protoplasmic oocyte in the leptotene or "lamp-brush" stage; and yolk nucleus (yn) Stained with Heidenhein's iron haematoxylin and Bouin fixation X.310.

d) T.S. of ovary of maturing female showing the ovarian cell-wall of three oocytes. zona radiata (zr); zona granulosa (zg) and connective-tissue zone (ctz). Stained with Azan and Bouin fixation. X.620.



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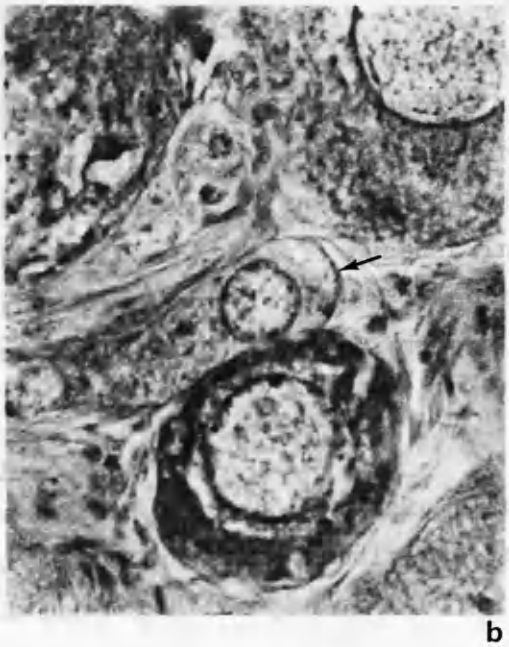

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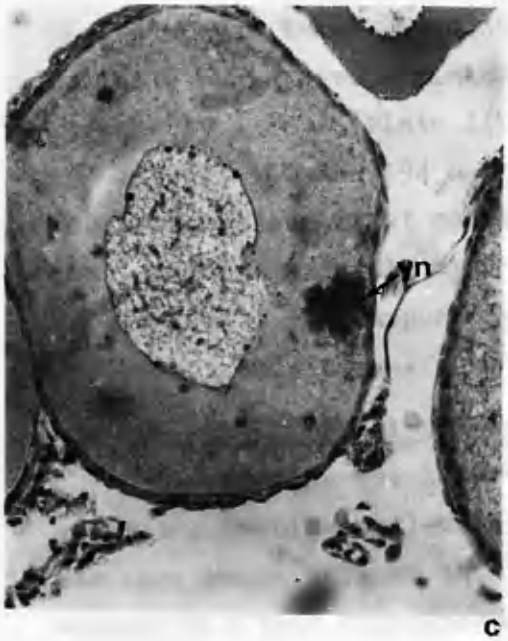

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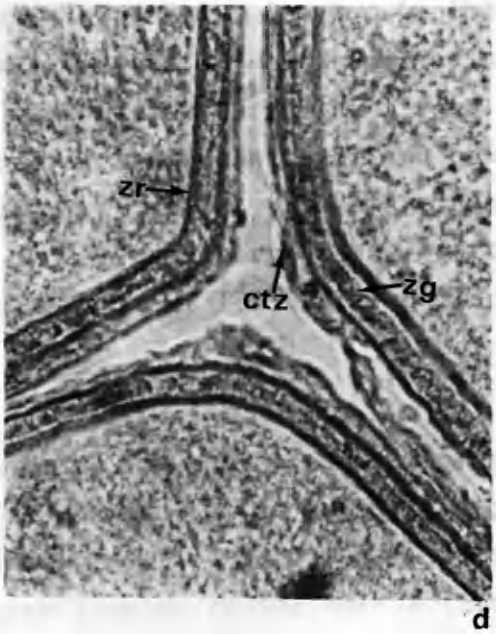


d

the granular karyoplasm. This marks the formation of the "lamp-brush", a characteristic of the diffusing or diplotene stage (Plate 1(c)). The nucleus of the smallest oocyte found measured 19 - 20 μ. There are a few nucleoli present at this stage and they occupy the peripheral parts of the nucleus. With a further increase in the size of the nucleus and the cytoplasm of the cell, coupled with an increase in the number of nucleoli, the oocyte enters the next stage of development.

Stage III - Protoplasmic oocytes.

For a clearer picture of the changes that take place in the protoplasmic oocytes, this stage has been subdivided into (i) juvinile phase and (ii) cellwall-formation phase.

(i) The Juvinile Phase

This phase embraces young oocytes, the cell-wall of which consist of connective tissue. A general increase in the size of the oocyte takes place during this period. Oocytes in the juvinile phase have a minimum cell size of 29 - 30 μ and the nuclei measure 19 - 20 μ (Plate 1(b)). The diameters of the larger cells are between 150 and 194 μ and that of the nuclei - 57 and 65 μ. The yolk nucleus first makes its appearance at this stage near the naucleus and gradually migrates to the periphery of the oocyte (Plate 1(c)). The appearance of a layer of follicular cells marks the end of this phase.

(ii) Cellwall-formation Phase

During the latter phase of the protoplasmic oocyte stage the ovarian cell-wall begins to form. The zona radiata makes its appearance between the follicular cells and the cytoplasm of the oocyte as a thin homogeneous layer lacking radial canals (Plate 1(d)). The follicular layer or the zona granulosa becomes more conspicuous with tall single-layer cells measuring 6 - 7 μ (Plate 1(d)). The latter is followed by a single layer of connective tissue, the

cells of which are stretched in a tangential direction (Plate 1(d)). By the end of this phase the size of the oocyte increases to 450 - 480 μ . The nuclei measure 95 - 110 μ. The yolk nucleus, now at the periphery of the oocyte begins to disintegrate.

From the primary oocyte stage to the present stage, increase in the volume of the oocyte is due to protoplasmic inclusion. After the chorion has been completely formed the oocyte enters the next stage.

Stage IV - Vitellogenesis.

For convenience, this stage has been subdivided into (i) peripheral vacuolization phase, and (ii) growth phase. A general characteristics of Stage IV oocytes is a considerable increase in the volume of the cells as a result of accumulation of vitellin and other trophic substances.

(i) Peripheral vacuolization phase

This phase is characterized by the appearance of a ring of vacuoles in the periphery of the oocyte (Plate 2(a)). The PAS test revealed that these vacuoles contain mucopolysaccharides. Yolk vesicles then make their appearance as minute granules throughout the oocyte. Simultaneously, small vacuoles are found scattered among the yolk vesicles (Plate 2(b)). The osmium tetraoxide test revealed the presence of fat droplets in these vacuoles.

(ii) Growth Phase

During the growth phase of the vitellogenetic oocyte a considerable increase in the size of the cell takes place. The yolk vesicles increase in size and so do the fat droplets. At the end of the growth phase the yolk is present as large globules and the vacuoles that contained fat become enlarged (Plate 2(c)). At the attainment of its maximum size, the oocyte measures 1800 - 2000 μ.

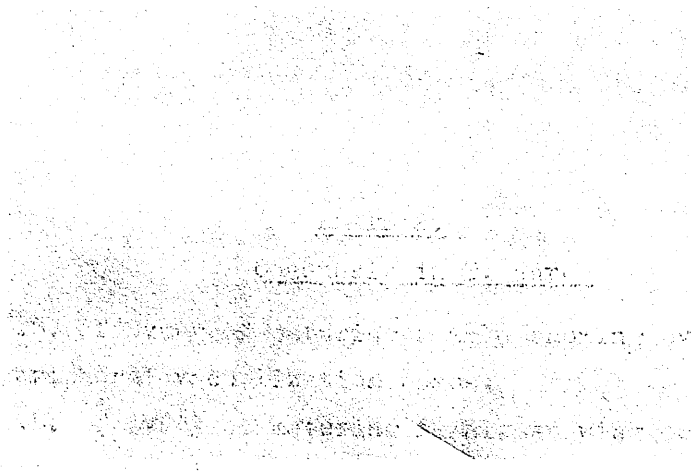
PLATE 2.

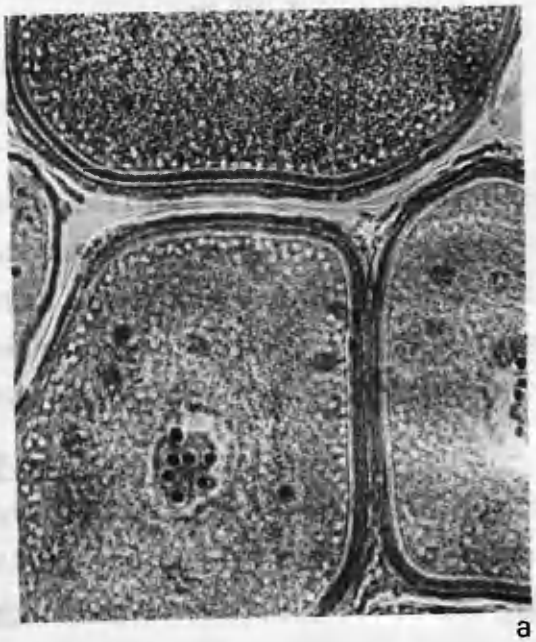
Oogenesis in T. aurea

a) T.S. of ovary of maturing female showing ovaries in the peripheral vacuolization phase.

b) T.S. of ovary of maturing female showing oocyte in early vitellogenetic phase.

c) T.S. of ovary of mature female showing oocyte in late vitellogenetic phase. All stained with Azan, and Bouin fixation and X.155.





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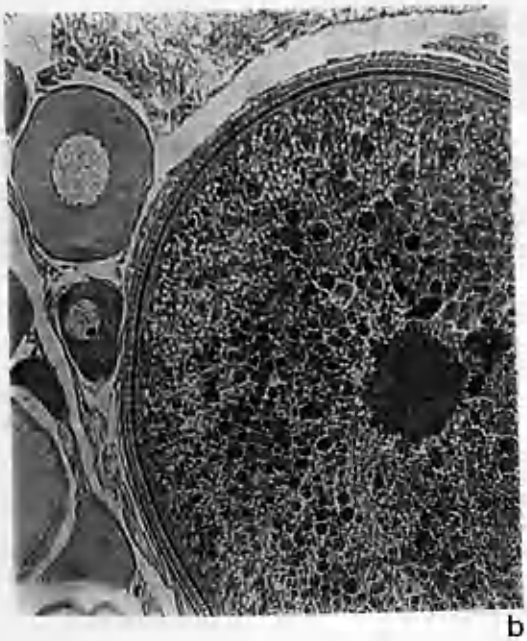

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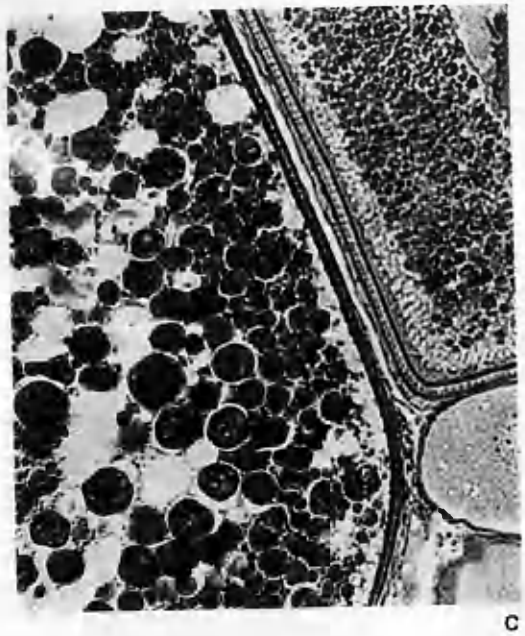

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Stage V - Maturation

The migration of the nucleus from the center to the periphery of the oocyte marks the beginning of the maturation stage. This is followed by preovulatory nuclear changes, production of the polar bodies and the release of the egg to the exterior. This marks the end of development. The latter phases have not been observed on microscopic slides, and it is presumed that they take place just before ovulation.

Stage VI - Post-spawning

This is the period after the egg has been extruded to the exterior, and spent follicles are present in the ovary. Since T.aurea is a polycyclic fish, the ovaries of a mature fish contain cells at all stages of development. Therefore in the ovary of a post-spawning fish, apart from the spent follicles, oocytes in Stages I to IV are also present.

A summary of the process of growth is presented in Table 4.

Table 4. Summary of the Process of Growth of Oocytes in T.aurea.

Growth Stages	Phase of development	Maximal size of oocyte (μ)	Relative increase in size of oocyte	Character of growth
I	Oogonia	15 - 20	1	Protoplasmic
II(a)	Primary oocyte	29 - 30	1.6	Protoplasmic
(b)	Juvinile	150 - 194	98	Protoplasmic
II - III	Cell-wall formation and peripheral vacuolization	450 - 480	268	Protoplasmic & Trophic
III	Early vitellogenetic	450 - 480	268	Trophic
IV	Late vitellogenetic	1800 -2000	1086	Trophic
V	Maturation	1800 -2000	1086	Pre-ovulatory changes.

SPERMATOGENESIS IN T. AUREA

Five stages have been distinguished during the development of the testis.

Stage I - Spermatogonia

Primary spermatogonia are the primary germ-cells found in the testicular tissue of T. aurea. They are the largest cells in the testis and are found either singly or in groups. Solitary germ-cells may be up to 16μ in diameter with a nucleus 10μ in diameter; germ-cells in groups are smaller, but both types display distinct chromatin structure of the nuclei and a large nucleolus (Plate 3(a)).

Through mitotic divisions several generations of germ-cells are formed. Stages in nuclear division may be seem occasionally in individual cells, but more often in grouped germ-cells (Plate 3(b)). After the last mitotic division, the daughter spermatogonial cells, following a short period of growth, transform into primary spermatocytes.

A synchronous division is observed in the cells which originate from the same spermatogonium. These cells are always found grouped together and are enclosed by a thin connective tissue forming a cyst. Usually, a cyst containing cells at one stage of development is found together with other cysts differing from each other in the developmental stages of their cells as a whole. They are found enveloped by a common fibrous connective tissue forming a larger cyst. The cells of the connective tissue of the larger cyst are frequently amoeboid in appearance. Occupying the interstices between the cysts are aggregatious of Leydig cells with a nuclear diameter of 4 - 6μ.

Although not spermatogenetic cells, the Sertoli cells with nuclear diameter ranging from 5 to 8μ are also observed as a characteristic component of the germinal epithelium. The nuclear

PLATE 3

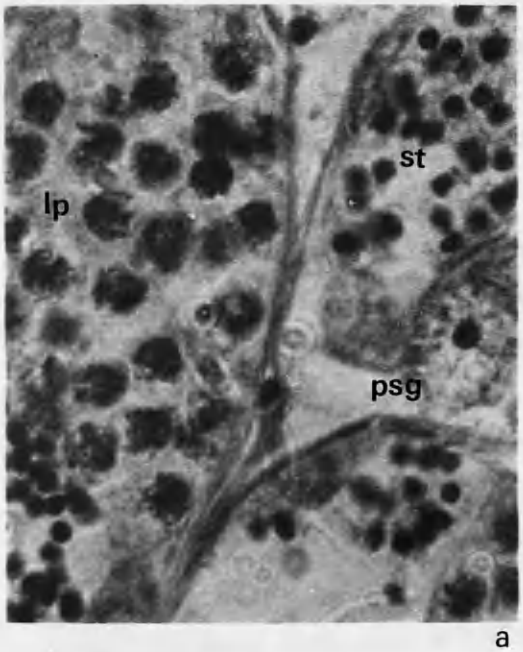
Spermatogenesis in T.aurea

a) T.S. of testis of maturing male showing (l) primary spermatogonia (psg); the bouquet or leptotene stage of meiotic prophase (lp), and spermatids (st).

b) T.S. of testis of maturing male: showing (i) the metaphase (m); and (ii) the leptotene (lp) phases of meiotic prophase.

c) T.S. of testis of maturing male; showing primary spermatocytes in meiotic prophase.

d) T.S. of testis of maturing male showing secondary spermatocytes (ss). All stained with Heindenhein's iron haematoxylin, Bouin fixation, and X.1550.



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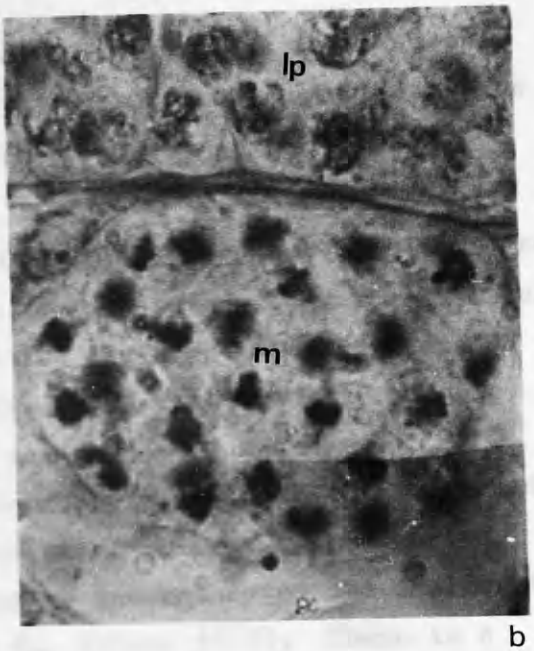

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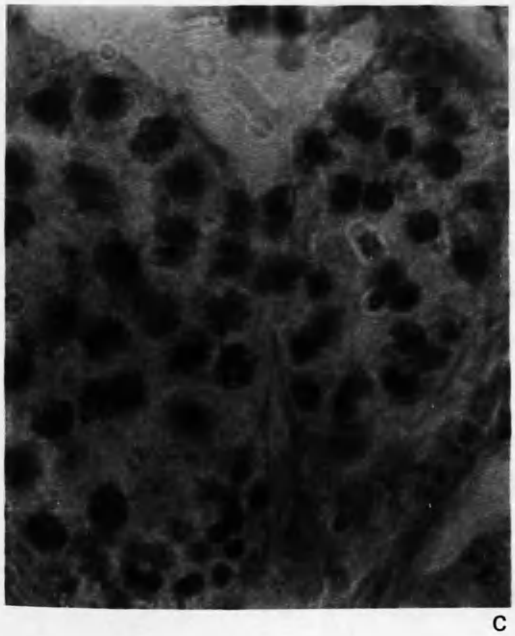

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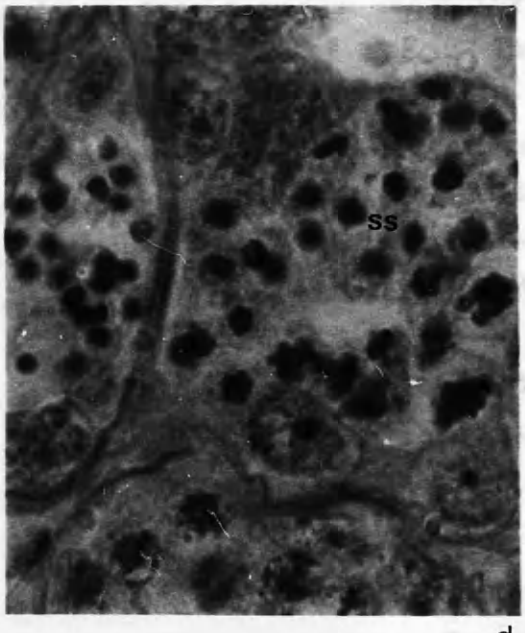


d

wall is well defined, with a prominent nucleus which is only slightly smaller than that of the primary spermatogonium. The cytoplasm of the cell is usually obscured.

Stages II to V - Maturation

Primary spermatocytes, after the short period of growth following the division of the last daughter spermatogonial cell, enter into the phase of reduction division. The reorganised chromosomes clearly exhibit the different stages of meiotic prophase (Plate 3(a)(b)(c)), metaphase and anaphase.

During Stage III secondary spermatocytes are formed as a result of reduction division of primary spermatocytes. The nuclear diameter of the secondary spermatocytes are smaller than the primary ones and measure 3μ (Plate 3(d)). There is a further maturation division which transforms the cells into spermatids.

Spermatids are slightly smaller than the secondary spermatocytes and they have a nuclear diameter of 2.5μ. The two spermatids produced as a result of the second division of maturation both contain a chromatin mass which becomes arranged in the inner margin of the nucleus, usually on one side of it and attaining among other irregular shapes, the shape of a half-moon (Plate 4(a)). These cells lack distinct cell boundaries and nuclear membranes.

During the last stage of maturation (spermiogenesis), the spermatids are gradually transformed into small round cells with a diameter of 1.6μ (Plate 4(b)).

Stage VI - Post-spawning

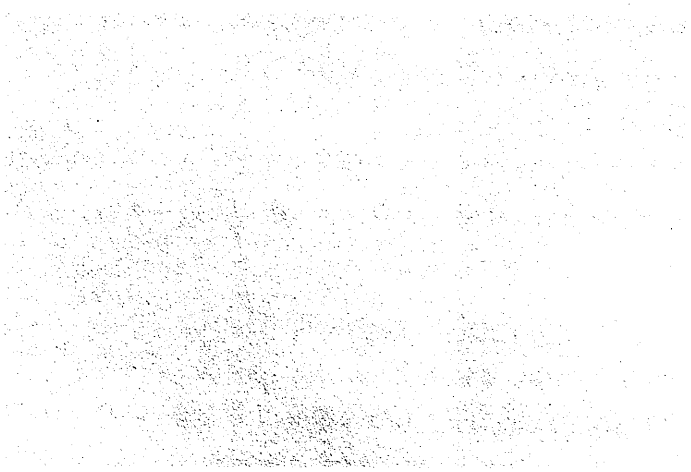
The testis of the post-spawning fish contains empty ducts and lobules once occupied by ripe spermatozoa. Sometimes, residual sperms are found in the empty lobules. These soon undergo

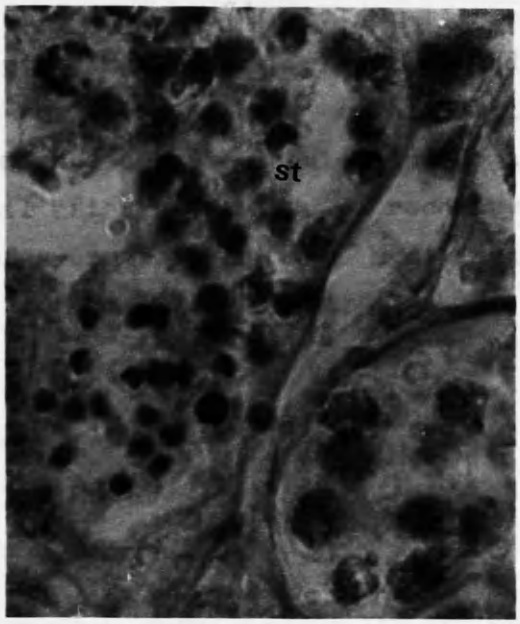
PLATE 4

Spermatogenesis in T. aurea

a) T.S. of testis of maturing male showing : (i) Spermatids (st); and (ii) the bouquet or leptotene stage (lp) of meiotic prophase.

b) T.S. of testis of mature male showing ripe spermatozoa. All stained with Heidenhein iron haematoxylin, Bouin fixation, and by X.1550.





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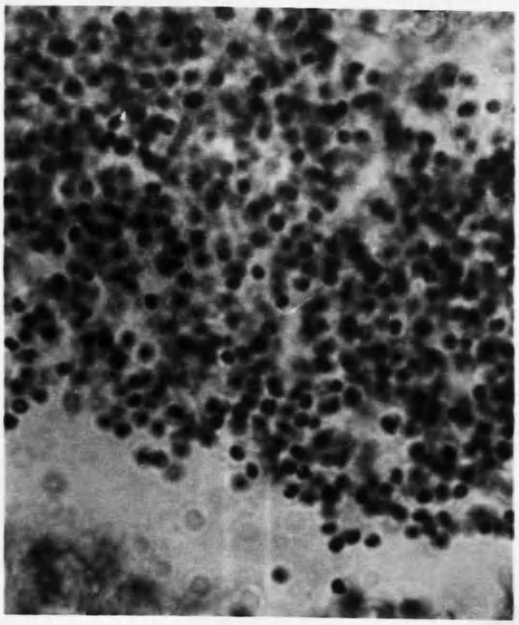


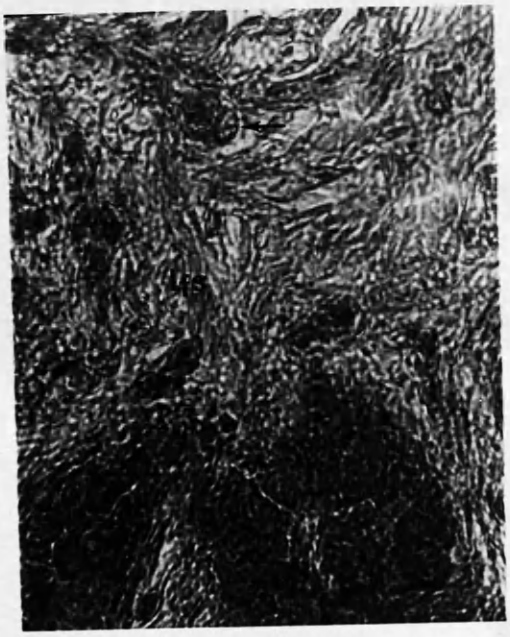
b

resorption. Apart from the empty lobules and sperm duct in the testis of a "spent" fish, ripe sperm are still common. The latter, however, is confined only to lobules and do not appear in the sperm duct until after a few days of recovery. Cells at all other stages of spermatogenesis are also found in the testis of a "spent" fish.

PLATE 5

Cytology of the pituitary of T.mossambica

Sagittal section of the pituitary showing the neurohypophysis (i) pituicytes (arrow) (ii) loose fibrous strands (lfs). Stained with Azan and Bouin fixation. X.620.



THE PITUITARY GLAND

General Morphology

The pituitary gland of T. mossambica is, as is typical in teleosts, an oval body situated ventral to the brain between the optic chiasma and the saccus vasculosus. It is attached to the brain by means of a slender stalk along which blood vessels enter the organ. The pituitary is divided into a nervous component - the neurohypophysis, and a glandular component - the adenohypophysis. The latter is made up of three distinct parts - the pro-adenohypophysis the meso-adenohypophysis and the meta - adenohypophysis. The components are demarkated by their distinct cell types.

The pituitary stalk enters the glandular part of the pituitary and fuses into its substance as the neurohypophysis, which is composed of loose fibrous strands with the nuclei of the neuroglia cells (pituicytes) scattered among their interstices. Quantities of colloid drops staining with azocarmine are also found there (Plate 5). The neurohypophysis is in contact with all the three parts of the adenohypophysis through sheets of cords of ramifying branches of neurohypophyseal tissue. The meta-adenohypophysis is the most extensively penetrated by the ramifying branches, followed by the meso-adenohypophysis, and then the pro-adenohypophysis.

The Cytology of the Pituitary Gland of T. mossambica

(i) The eta or Prolactin cells.

Two groups of fish (10 fish in each group) were maintained for studies of the eta or prolactin cells. The first group was kept in freshwater at a temperature of 28°C. The second group was kept in half seawater (salinity of 17.5 $o/_{oo}$) at the same temperature. After 15 days all the fish were sampled for histological studies.

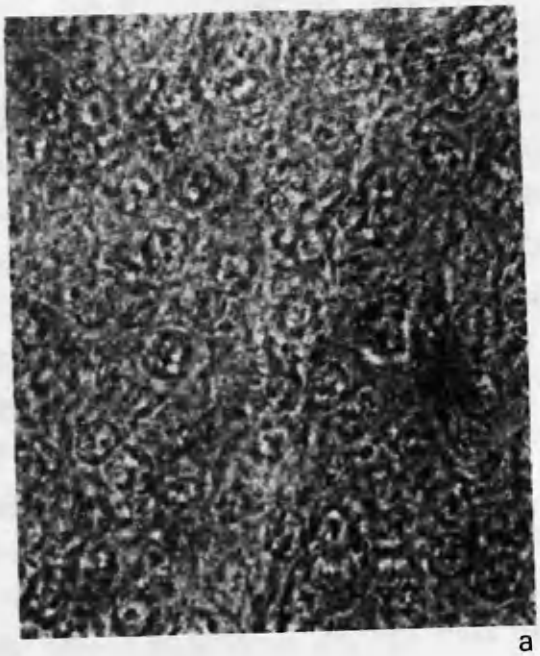
PLATE 6

Cytology of the pituitary of T.mossambica.

Sagittal sections of the pituitary: showing

a) Eta or prolactin cells in the pro-adenohypophysis of fish maintained in freshwater. Large, well-granulated, with large rounded nuclei and prominent nucleoli. X.1550.

b) Eta or prolactin cells in the pro-adenohypophysis of fish maintained for 15 days in half-seawater. Small, poorly granulated cells, with a small rim of cytoplasm around the nuclei X.620. All stained with Azan and Bouin fixation.



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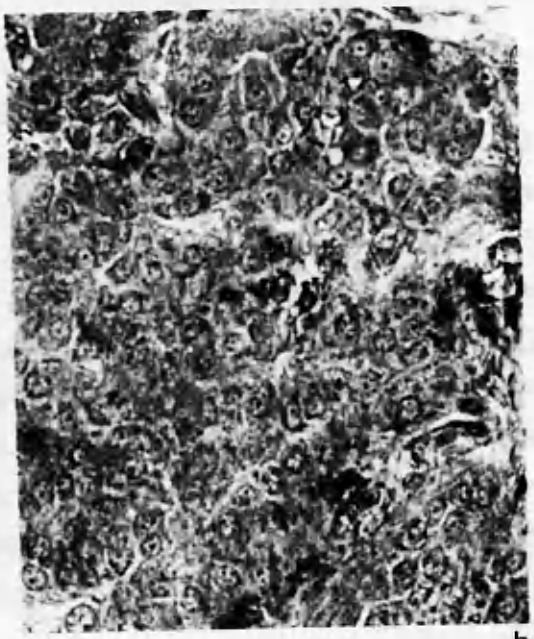


b

Results

Freshwater Fish

The pro-adenohypophysis of the pituitary of fish maintained in freshwater (the usual environment of the fish) for 15 days consisted almost entirely of eta cells. They are large, measure 10-11μ and bear a large spherical nuclei. The nucleoli which are prominent lie in the center of the nuclei (Plate 6(a)). The cytoplasm of the cells stain intensively with Azocarmine and less intensively with Orange G.

Seawater Fish

The pro-adenohypophysis of the pituitary of fish maintained in $\frac{1}{2}$-seawater differed from that maintained in fresh water. There was a reduction in the area occupied by the eta cells. The cells were in a shrunken condition. They were smaller in size (3-7μ) and appeared less active. The cytoplasm was thinly scattered as a narrow rim surrounding the nucleus. The nucleoplasm was not well defined (Plate 6(b)).

(ii) The α_1-or ACTH cells

Two sets of ten fish were kept under conditions similar to those outlined in the previous experiment and injected daily with 0.5 mg of Metopirone (methyl - 2 bis (3-pyridyl) - 1,2 - proparone - 1) in 0.05 ml. doses. Five days were allowed for the half-seawater fish to acclimatize before injection started. Ten control fish remained in freshwater and were injected with saline. After 12 days of treatment all the fish were sampled for histological examination of the pituitaries. The pituitaries of 10 fish maintained at 13 - 15^{o}C (from another experiment) were also examined for comparative analysis.

Results

Control Fish

Faintly-staining acidophilic cells, usually one layer thick occur in the pro-adenohypophysis at the border with the

PLATE 7

Cytology of the pituitary of T. mossambica

Sagittal sections of the pituitary, showing

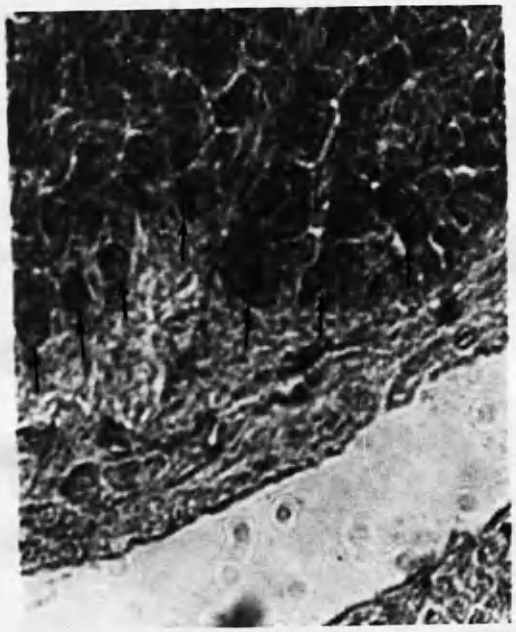
a) α_1 - or ACTH cells in the pro-adenohypophysis of normal fish maintained in freshwater. Small cells (arrows) bordering the neurohypophysis (nh).

b) α_1 - or ACTH cells in the pro-adenohypophysis of fish maintained in freshwater and injected for 12 days with Metopirone. Note hypertrophy in α_1 - cells (arrows) which are as conspicuous as the eta cells.

c) α_1 - or ACTH cells in the pro-adenohypophysis of fish maintained in half-seawater for 12 days with simultaneous Metropirone treatment. Hypertrophied cells (arrows) and more prominent than the hypertrophied eta cells.

d) α_1 - or ACTH cells in the pro-adenohypophysis of fish kept at 13° - 15°C for three months. Note increase in quantity of the cells. All stained with Azan, Bouin fixation, and X.620.

a



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neurohypophysis (Plate 7(a)). They differ from the eta cells previously described in the inconspicuousness of their cellular cytoplasm. However, they are sometimes difficult to differentiate from the eta cells lying immediately behind them, since the latter unlike the eta cells of the other parts of the pro-adenohypophysis also often stain faintly with Azan. The cytoplasm of the cells are not always distinct but the nucleus and its substance are distinguishable.

Freshwater Fish Treated with Metopirone

There was a hypertrophy of the single-layered (α_1-) cells as a result of metopirone treatment. The cytoplasm of the cells became conspicuous and the α_1-cells attained almost the same size as the eta cells and with the same staining affinity for acid dyes. For this reason, the two cell types become difficult to distinguish from each other (Plate 7(b)). No change was observed in the eta cells as a result of Metopirone treatment.

Half-seawater Fish Treated with Metopirone

The cell types of the pro-adenohypophysis of the pituitary are easily identified under this experimental condition. As a result of Metopirone treatment the α_1-cells bordering the neurohypophysis had become hypertrophied. The prominence of the cells was further increased due to the saline environment of the fish which had resulted in a reduction in size and activity of the adjacent eta cells (Plate 7(c)). Apart from a few which were irregularly shaped, most of the α_1-cells were polygonal. The largest cells found measured 7 - 10μ. They were granulated and showed obvious signs of secretive activity.

Low-temperature Fish

A considerable increase in the number of α_1-cells was observed in the pro-adenohypophysis of fish subjected to low temperatures (13-15°C) for three months. The cells which are

PLATE 8

Cytology of the pituitary of T.mossambica

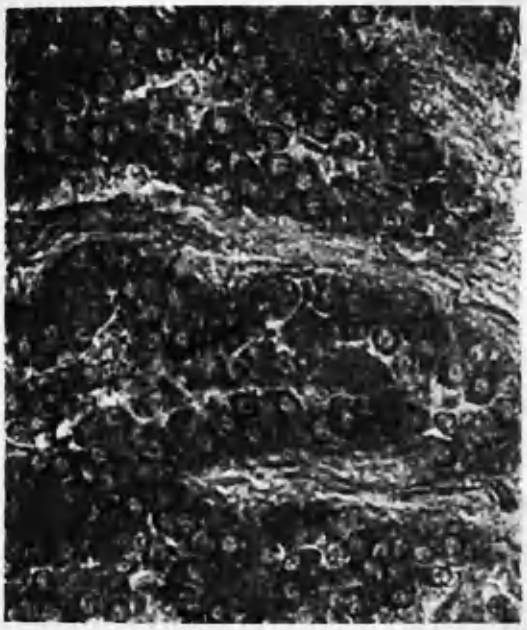
Sagittal sections of the pituitary; showing

a) α- or STH cells in the meso-adenohypophysis of normal fish. Well granulated with prominent nuclei and nucleoli, and distinct sinusoid pattern of distribution.

b) α - or STH cells in meso-adenohypophysis of fish kept at 13° - 15°C for three months. Poorly granulated with inconspicuous nuclei and nucleoli, and without the usual sinusoid pattern of distribution.

c) The basophil (B_1- and B_2-) cells in the meso-adenohypophysis of normal fish. B_1- cells oval and granulated; the B_2- cells conical and also granulated. All stained with Azan and Bouin fixation.

d) The PAS positive reaction in the B_1- and B_2- cells in the meso-adenohypophysis. Stained by Periodic Acid Schiff technique and Zenker-formed fixation. All X.620.



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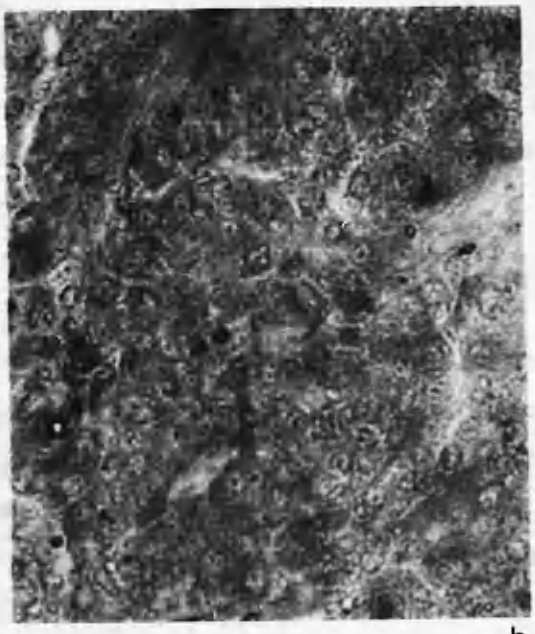

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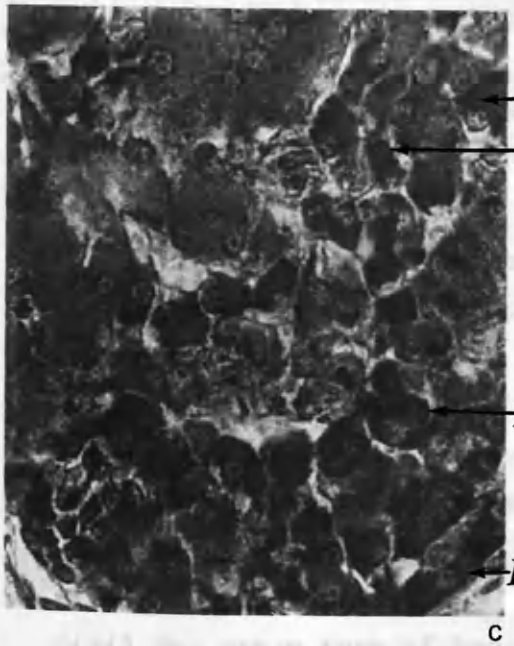

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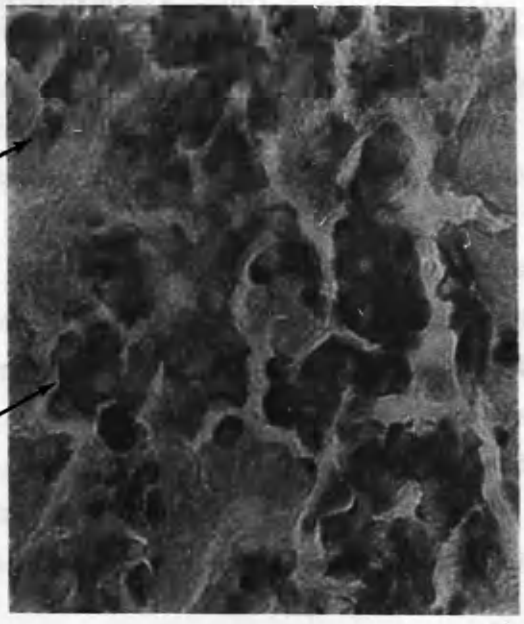


d

normally one-layered as compared with the numerous eta cells, had become multilayered and formed between 1/4 and 1/3 of the cell population in this region of the pituitary. The nuclei become prominent as well as the nucleoplasm (Plate 7(d)).

The meso-adenohypophysis

(i) The α- or STH cells

These correspond to the classic acidophilic cells the granules of which display strong affinity for acid dyes - azocarmine, Orange G, and acid fuchsin. They are usually one or two layered, situated dorsally at the border with the neurohypophysis, and are easily recognised by their undulating distribution and their intense coloration (Plate 9(a)). In the pituitary of fish maintained at 13 - 15°C for three months, the α-cells were inactive and often showed less cytoplasmic contents (Plate 8(b)).

(ii) The Basophil (B_1- and B_2) Cells

The greater part of the meso-adenohypophysis is composed of two types of basophil cells : B_1- cells and B_2- cells. These cells differ from each other basically in their morphological characteristics and to some extent, in distribution and staining intensities.

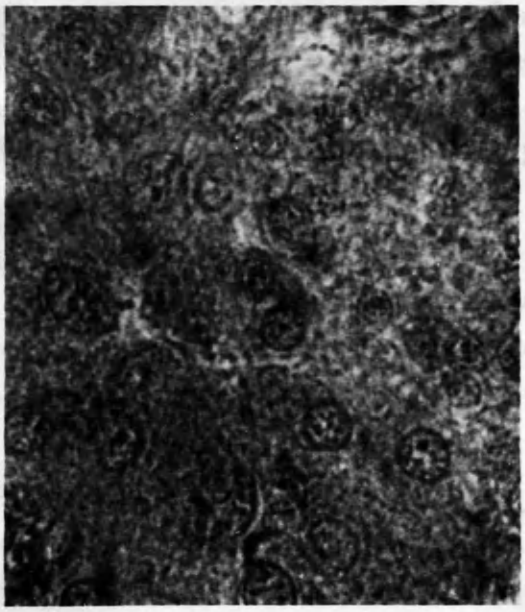
One type of basophil (B_1-) is of variable size and shape and ranges from a small roundish cell to one that is large and oval-shaped (Plate 8(c)). These large oval-shaped cells measure 20μ across the widest diameter. The normally eccentric nucleus is often round or oval. The cytoplasm of these cells is composed of granules which stain intensively with aniline blue and alcian blue combined with PAS. The PAS reaction reveals that these granules are rich in glycoproteids.

(iii) The other type of basophil (B_2-) cell is polygonal in shape and is less numerous than the B_1-cell. The cytoplasm of the cell stains slightly less intensively with basic dyes as compared with

PLATE 9

Cytology of the pituitary of T. mossambica

Saggital section of the pituitary showing the chromophobic cells of the meso-adenohypophysis. Stained with Azan and Bouin fixation. X.1550



the B_1- cells, and is also PAS positive (Plate 8(c)(d)).

There is no strict differential distribution according to cell shape of the two types of basophils in the meso-adenohypophysis. However, there is a tendency for the larger oval-shaped cells to be nearer the centre of the region while the polygonal cells lie towards the periphery.

(iv) The Chromophobe or Gamma (γ-) Cells

In the meso-adenohypophysis, the γ-cells occur in the peripheral parts. Very often they are found grouped together in the ventral side of the region close to the meta-adenohypophysis (Plate 9). The cytoplasm of the cells is colourless or weakly acidophilic. The γ- cells in contrast to the B_1- and B_2- cells, never show vacuoles.

The Meta-adenohypophysis

The acidophilic cells of the meta-adenohypophysis can be separated into two types on the basis of differences in (1) cell shape and size (2) staining intensity and distribution.

(i) The first cell type is large, oval-shaped and strongly acidophilic (Plate 10(a)(b)). It measures 13μ across the widest area. The nucleus with the general chromatin structure distributed within it, is round and seldom centrally located. The large nucleolus which often lies in the center is very conspicuous. These cells demonstrate an increased activity as evidenced by the presence of granules in the dense cytoplasm and are slightly PAS positive. (Plate 10(a)(b)). The Golgi region is evidenced in these cells as a faint area just above the nucleus. This cell type is usually distributed along the area bordering the ramifying branches of the neurohypophysis.

(ii) The second cell type of the meta-adenohypophysis is small, and to a lesser degree, acidophilic. They are differently shaped, ranging from irregular with somewhat oval appearance to polygonal

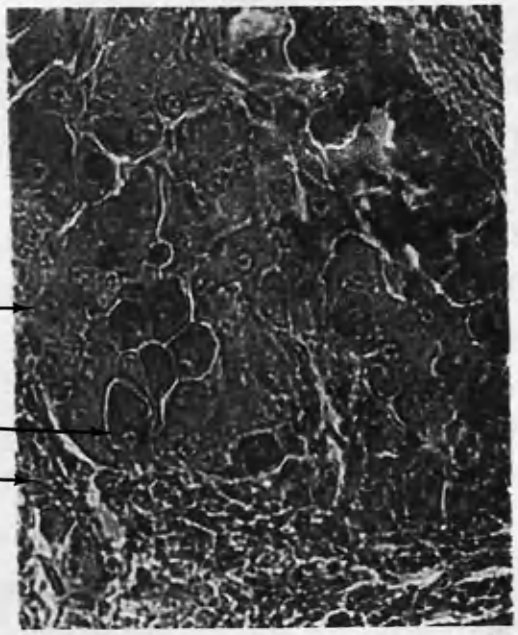
PLATE 10

Cytology of the pituitary of Tilapia

Sagittal sections of the meta-adenohypophsis, showing

a) two acidophilic cell types (a_1 and a_2) in T.mossambica. a_1-oval large and oval-shaped; a_2-small and conical. Neurohypophyseal branch filled with loose fibrous strands (lfs), stained with Azan and Bouin fixation.

b) two acidophilic cell types in (a_1 and a_2) in T.aurea. Neurohypophyseal branch filled with neuro-secretory material (nsm). Stained with Thionin - PAS - Naphtol Yellow, and Bouin - Hollande fixation. All X.620.



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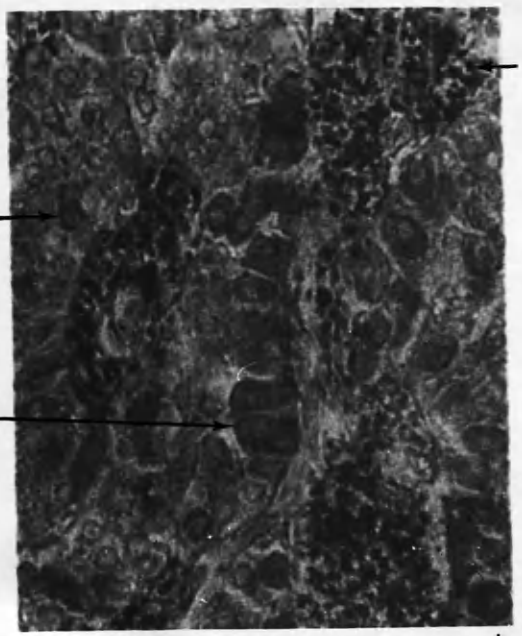

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and are situated in the central and ventral parts of the region, constituting the dominant cell type (Plate 10(a)(b)). Cells with amphoteric characters, staining bluish-pink by the azan method are sometimes found in this zone.

In summary, eight cell types have been distinguished from the adenohypophysis of T.mossambica: two from the pro-adenohypophysis - eta or prolactin cells (on the basis of response to environmental salinities) and α_1- or ACTH cells (on the basis of response to Metopirone treatment); four from the meso-adenohypophysis - the α- which are possibly STH cells (on the basis of tinctorial affinity and localization), the B_1- and B_2- cells which are possibly gonadotrophs (on the basis of tinctorial affinity and PAS test) and the γ-cells; and two cell types from the meta-adenohypophysis, the functions of which are not clearly established.

The Cytology of the Pituitary Gland of T.aurea

The Pro-adenohypophysis

Two types of acidophilic cells are distinguished; the eta cells, whose cytoplasm stain s intensively with azocarmin, Orange G, erythrosin, naphthol yellow and acid fuchsin. They form the dominant cell types in this region and are identical with the eta cells found in T. mossambica; and the α_1-cells which are single-layered and occupy the borders with the neurohypophysis are also identical, in their staining affinities and distribution, with the ACTH cells in T. mossambica

The Meso-adenohypophysis

The α-cells, unlike those in T. mossambica are usually single-layered but they occur as in the latter, in the same region of the meso-adenohypophysis and are also recognised by their strong acidophilic qualities and sinusoid appearance.

Two types of basophils (B_1- and B_2- cells) were also identified from this region in T. aurea. The PAS technique adopted in combination with Alcian blue and Orange G revealed that these two cell types stain differently. In the pituitary of castrates, and fish in the spawning condition vacuolation was observed in the oval-shaped (B_1-) cells (Plate 22(a)) Those cells in which vacuolation had not started, stained dark red with PAS. At this time the polygonal (B_2-) cells occupying the peripheral parts of this region even though they were PAS positive stained only light red (Plate 22(a)). It could therefore be postulated that the B_1- cells are responsible for the production of LH while the B_2- cells produce FSH.

The γ- cells in this region like those of T.mossambica are colourless or weakly acidophilic and never show vacuoles.

The Meta-adenohypophysis

The Thionin-PAS-Naphtol Yellow proved the most effective technique in demonstrating the presence of the large secretive cells in the meta-adenohypophysis. Using this technique, neurosecretory material was observed scattered within the ramifying branches of the neurohypophysis as green droplets. The large cells bordering the branches of the neurohypophysis were counterstained pinkish-red.

The second cell type which was small and non-secretary was identical with that found in T. mossambica.

A summary of the histochemical studies is given below :

Table 5. Summary of histochemistry of the Pituitary Gland of Tilapia

Group	Dye	Adenohypophysis			
		Acidophil Cells			Basophil Cells
		STH	LTH	ACTH	GTH (LH + FSH)
Basic	PAS				++
	Alcian Blue				++
	Aniline Blue				++
	Thionin				++
	Aldehyde fuchsin				++
Acid	Orange G	++	+	+	
	Naphtol Yellow	++	+		
	Azocarmine	++	++	+	
	Acid Fuchsin	++	++		
	Erythrosin	++	++	+	

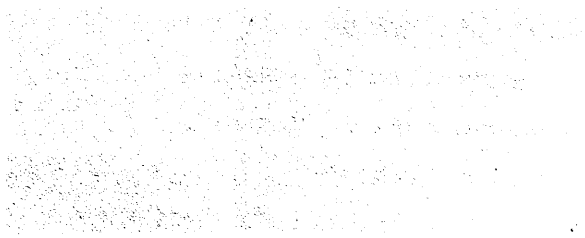


PLATE 11

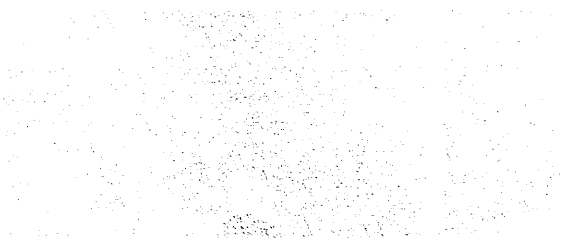
Cyclical changes in the ovary of T.mossambica

a) T.S. of ovary of female showing oocytes in the immature phase (Stage I)

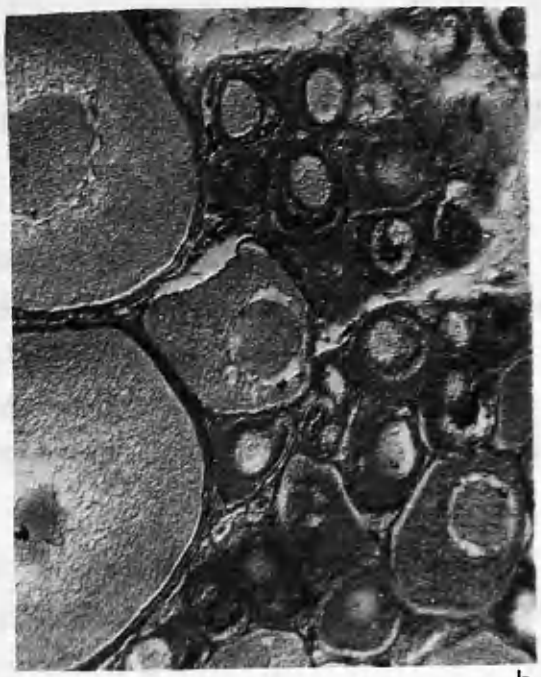
b) T.S. of ovary of female showing oocytes in the growth phase (Stage II)

c) T.S. of ovary of female showing oocyte in the vitellogenetic phase (Stage III)

d) T.S. of ovary of female showing oocyte in the vitellogenetic phase (Stage IV). All stained with Azan, Bouin fixation, and X.155.



a



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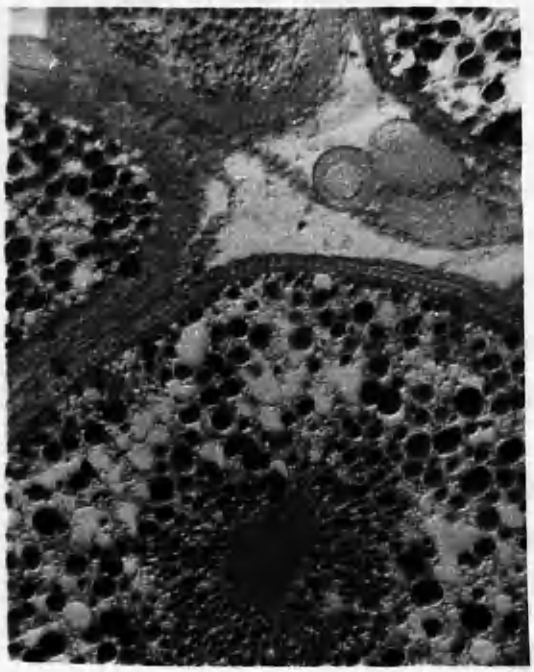

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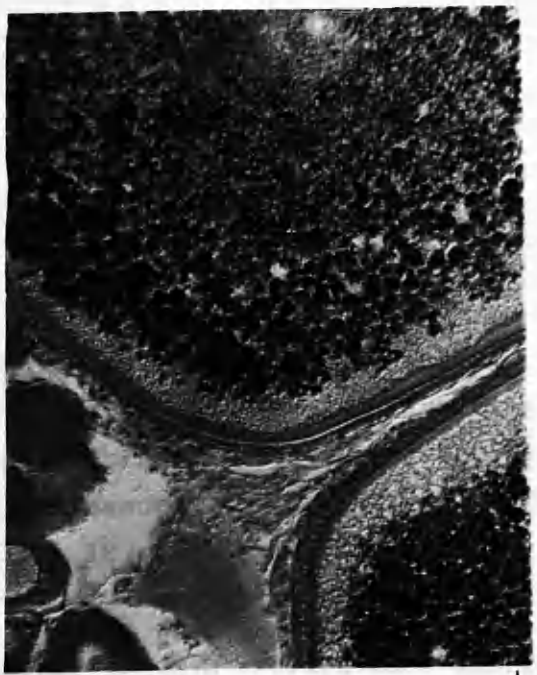


d

THE RELATIONSHIPS BETWEEN THE CORRELATIVE CYCLICAL CHANGES IN THE GONADS AND THE STRUCTURE OF THE PITUITARY GLAND OF T. MOSSAMBICA

The Ovary

The ovary is covered by a thin sheet of peritoneum beneath which lies the ovary wall - the tunica, a connective tissue structure 3 to 49 μ thick. The organ gets its blood supply from a long vessel running ventrally along its whole length. Branches of ovarian blood vessels arise from the main vessel, forming a network enclosing the ovary.

Cyclical changes

A clearer and better understanding of the cyclical changes in the ovaries of T. mossambica is obtained if the whole process is divided into the following phases :

1) The Immature Phase
2) The Growth Phase
3) The Prespawning Phase
4) The Spawning Phase
5) The Post-spawning Phase

See Table 6 for a summary of the use of the terms "phase" and "stage".

1) The Immature Phase

Juvinile fish which have not spawned before are in this phase of development. The ovaries contain oogonia with a nuclear diameter of 6 - 8 μ and primary oocytes (Plate 11(a)). The smallest of the primary cells measured had a diameter of 20 - 25 μ and a nuclear diameter of 10 - 12 μ.

2) The Growth Phase

This phase incorporates fish with ovaries in a relatively advanced stage. Increase in size of the oocyte is due to protoplasmic accumulation and it is otherwise called the period of protoplasmic

growth. The nuclear contents of protoplasmic oocytes become distinct; the nucleoli are grouped around the periphery and the general chromatin structure distributed in the centre. Large cells measuring 285 - 310μ with nuclear diameter of 62 - 74μ are found (Plate 11(b))

3) The Prespawning Phase

In this phase there is a rapid increase in the size of the oocytes due to accumulation of trophic substances, e.g. fat, yolk and polysaccharides. The smallest oocytes found in this phase had a cellular diameter of 520 - 600μ with nuclear diameter of 100 - 126μ. When the advanced oocytes of this phase attain their maximum size (890 - 1102μ and 126 - 133μ for the nucleus) the prespawning phase is complete (Plate 11 (c)(d))

4) The Spawning Phase

Polarization, followed by preovulatory nuclear changes occur in the animal pole of the oocyte which has completed the prespawning phase. These nuclear changes are followed by the release of the egg from its follicle (ovulation) via the oviduct to the exterior. This marks the last phase in the development.

5) The Post-spawning Phase

Immediately after the eggs are extruded, the ovaries contain spent oocytes as well as oocytes in the growth and even prespawning phase (Plate 12(a)). The ruptured follicle contracts, probably under the influence of elastic fibres in the connective tissue layer, which become strongly basophilic and hyperplasic only in the post-spawning phase (Plate 12(b)). Similarly, there is a large degree of hypertrophy of the follicular cells which become multilayered, and measure between 58 and 113μ (Plate 12(b)). In contrast the single-layered follicular cells in the prespawning phase measure 6 - 10μ. The hypertrophy and hyperplasia of the

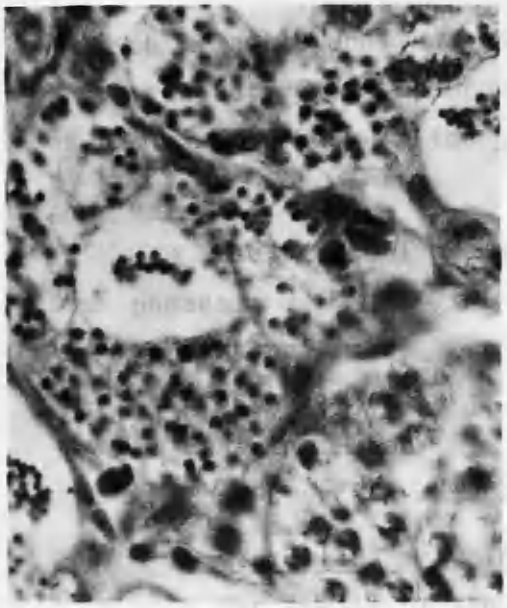
PLATE 13

Cyclical changes in the testis of T.mossambica

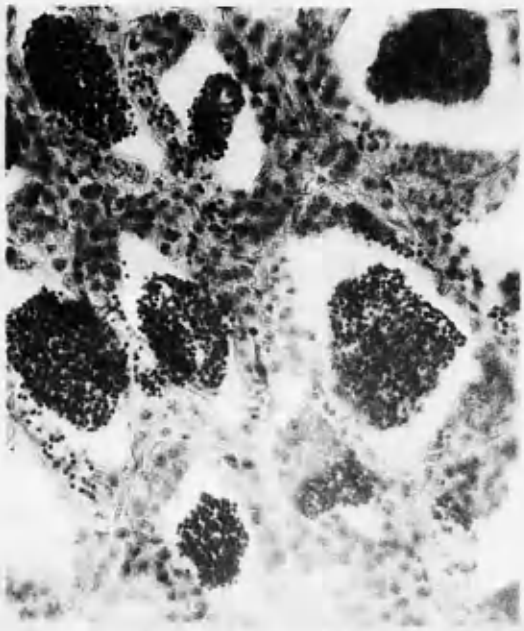
a) T.S. of testis of male in maturing phase (Stage III). Note the presence of only small quantity of spermatozoa. X.620.

b) T.S. of testis of male in prespawning phase (Stage IV). Note the presence of large quantities of spermatozoa. X.310

c) T.S. of testis of male in post-spawning phase (Stage VI). Note the presence of residual sperm (rs) and reserve sperm. X.620



a



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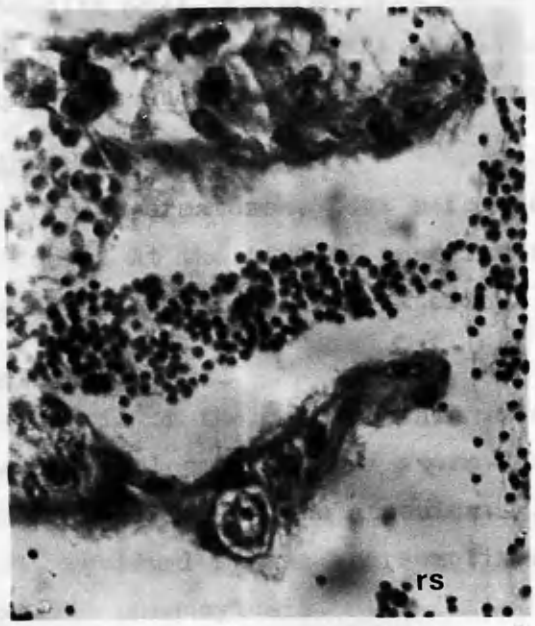


c

follicular cells and the elastic fibres in the connective tissue layer respectively, are probably connected with the formation of the atretic body or the corpus atreticum.

The Testis

The Cyclical changes in the testis are divided into four phases :

1) The Immature Phase
2) The Prespawning Phase
3) The Spawning Phase
4) The Post-spawning Phase.

1) The Immature (and maturing) Phase

The testis of the young, virgin fish contains spermatogonia, primary and secondary spermatocytes, and spermatids (Plate 13(a)

2) The Prespawning Phase

Intensive cell proliferation takes place in this phase and the testes are filled with cells at all stages of spermatogenesis including spermatids and numerous ripe spermatozoa (Plate 13(b)).

3) The Spawning Phase

The ripe spermatozoa, just before spawning, gather in the sperm duct. At the time of spawning they are released through the genital papilla to the exterior.

4) The Post-spawning Phase

The testis of the post-spawning fish contains cells at all stages of development. Ripe sperm are also present but they are usually enclosed in the ampoules. In the empty ampoules and sperm duct, residual sperms are sometimes present (Plate 13(c)). These soon undergo phagocytosis.

Cyclical Changes in the Histology of the Pituitary in connection with Maturation.

Three types of cells which undergo quantitative, qualitative (intensity of staining) and morphological changes during the reproductive cycle will be considered in this section. These cells will be called chromophobes, acidophils and basophils, according to the terminology commonly used in the literature. Chromophobes will be referred to as those cells which are either colourless or weakly acidophilic. The acidophils are those which take an intense red after Azan stain. Basophil denotes those cells whose cytoplasmic inclusions stain with aniline blue.

The meso-adenohypophysis seems to be the only region of the pituitary where profound changes, in connection with maturation, take place. For this reason quantitative data of the different cell types, in this region only, will be given, together with a description of the general changes occurring in all the parts of the organ as a whole.

1. The Immature Phase

a) The Pro-adenohypophysis

Chromophobe and acidophil are the cell types in this region of the pituitary. The latter are very conspicuous with distinct cell boundaries, while the chromophobes are small with indistinguishable cell boundaries. The cells are compact and occupy the whole of this zone.

b) The Meso-adenohypophysis

All the three types of cells in the pituitary are present in this region. Acidophils are the most numerous and make up 68% of the total cell population. Sixteen percent of the remaining cell types is made up of chromophobes while the basophils constitute only 12% (Plate 14(a)). The latter gave positive results to the Periodic Acid Schiff (PAS) test.

c) The Meta-adenohypophysis

Two cell types are found in this region - chromophobes and acidophils. Some of the latter are large with large nuclei, and others small, with conspicuous nuclei. In most cases, only the nuclei of the chromophobes are conspicuous at this time of the reproductive cycle.

2. The Growth Phase

a) The Pro-adenohypophysis

The chromophobe and acidophil cells both become conspicuous, especially the acidophils which occupy a greater portion of this region. There is no evidence of the appearance of any new cell type.

b) The Meso-adenohypophysis

Acidophils still dominate in this zone during the growth phase but their percentage is reduced from 68 to 60%, while that of the basophils increase greatly - from 12 to 33%. The chromophobes become reduced at this time - six percent, as compared with 16% during the immature phase (Plate 14(b); Fig 1 and Table 7).

c) The Meta-adenohypophysis

No marked difference is found in the cell types except for the appearance of certain bluish-pink cells. These are not basophils, but they rather have a slight affinity for basic dyes.

3. The Prespawning Phase

a) The Pro-adenohypophysis

No sign of secretive activity is found among the chromophobic and acidophilic cells which remain the only cell types, with the latter dominating. This zone, however, due to the expansion of the meso-adenohypophysis, looks comparatively smaller,

PLATE 14

Cyclical changes in the meso-adenohypophysis of T.mossambica.

a) Sagittal section of the pituitary of fish in the immature phase showing the basophil cells. Note the less basophilic tincotrial affinity of cells.

b) Sagittal section of the pituitary of fish in the growth phase, showing the basophil cells. Note the increased staining intensity of the cells.

c) Sagittal section of the pituitary of fish in the pre-spawning phase, showing the basophil cells. Note the intensified staining intensity and granulation of the cells. All stained with Azan, Bouin fixation and X.620.

a

b

c

FIGURE 1

Percentage of chromophils and chromophobes in the mese-adenohypophysis of T.mossambica at different phases of the reproductive cycle : I - immature phase; II - growth phase; III - prespawning phase; IV - spawning phase; V - post-spawning phase.

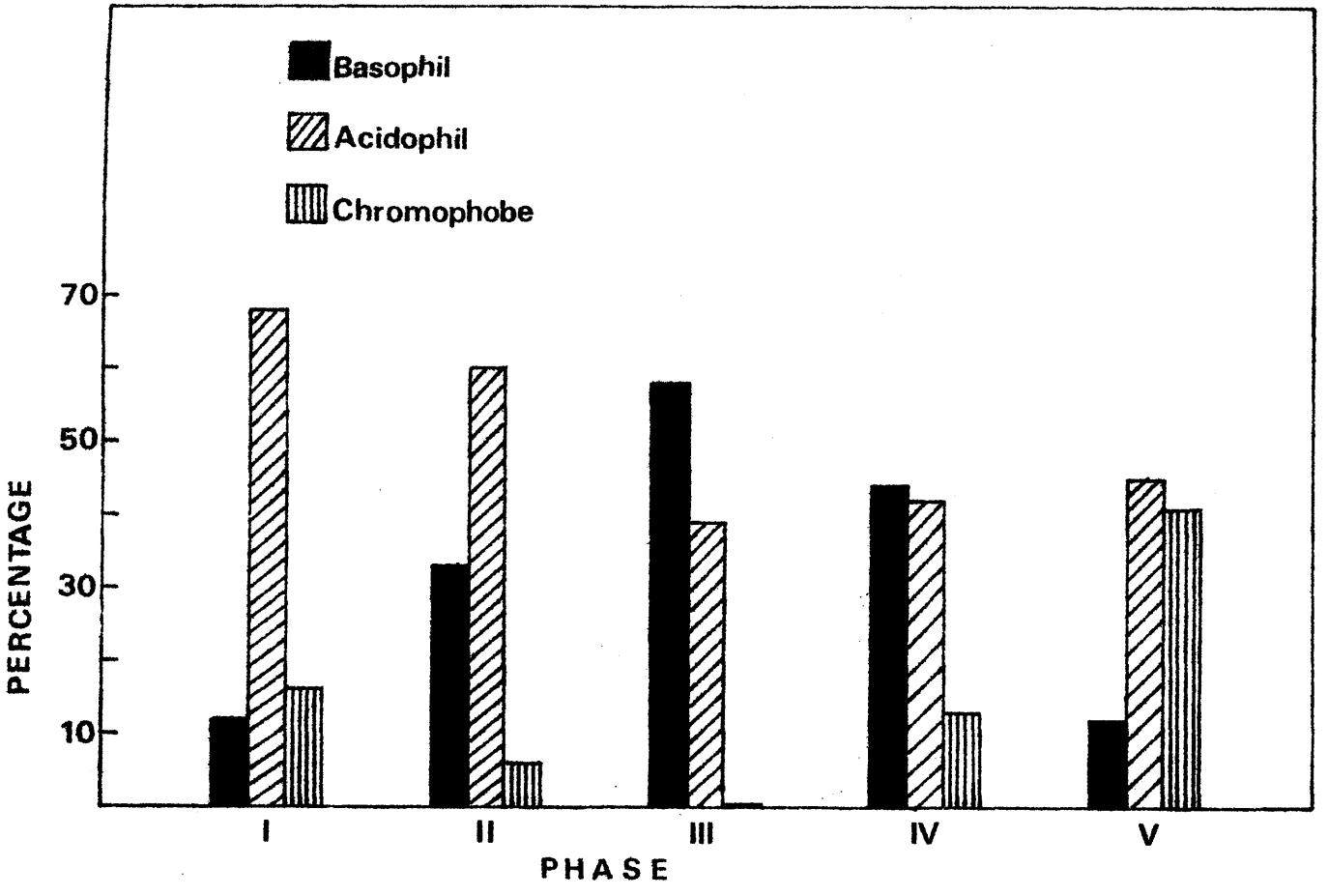

Basophil
Acidophil
Chromophobe
70
50
30
10
PERCENTAGE
I
II
III
IV
V
PHASE

Table 7. Quantitative data on Chromophils and Chromophobes in Meso-adenohypophysis of T.mossambica during the reproductive cycle.

Phase	Basophil		Acidophil		Chromophobe	
	No.	% ± SE	No.	% ± SE	No.	% ± SE
Immature	57	12 ± 2.55	265	68 ± 4.2 (6)*	65	16 ± 2.2
Growth	143	33 ± 2.3	267	60 ± 1.9 (9)	28	6 ± 1.0
Prespawning	492	58 ± 1.8	336	39 ± 1.8 (4)	9	0 ± 0.3
Spawning	250	44 ± 1.2	239	42 ± 0.7 (4)	77	13 ± 0.7
Post-spawning	51	12 ± 0.3	179	45 ± 0.2 (6)	163	41 ± 0.2

Note: Values for cell types are mean numbers and percentages of six saggital sections of pituitary per fish.

* Number of fish in parenthesis

forming only about one-fifth of the two zones (the pro- and meso-adenohypophysis) taken together.

b) The Meso-adenohypophysis

During the prespawning phase the meso-adenohypophysis becomes the largest component of the pituitary. There is a great increase and difference in the quantity and quality of the basophils, which are clearly PAS positive. They constitute about 58% of the total cell population. Acidophils form 39% while the chromophobes do not constitute any significant percentage of this zone. The staining intensity of the acidophils, and especially the basophils become increased and their nuclei clearly demonstrate intensified secretory activities. The basophils are large and granulated, and some have liquefied cytoplasmic contents. However, there is no sign of vacuolation (Plate 14(c); Fig. 1; and Table 7).

c) The Meta-adenohypophysis

The cell constituents of this region do not change except for an increase in the number of the bluish-pink cells. This zone is small as compared with the meso-adenohypophysis. Some of the acidophils, especially the large ones, bordering the branches of the neurohypophyseal tissue, exhibit secretive activities. These are granulated cells and much less in number than the smaller acidophils.

4. The Spawning Phase

a) The Pro-adenohypophysis

No significant change occurs except for a slight increase in size of the region. The cell types remain the same.

b) The meso-adenohypophysis

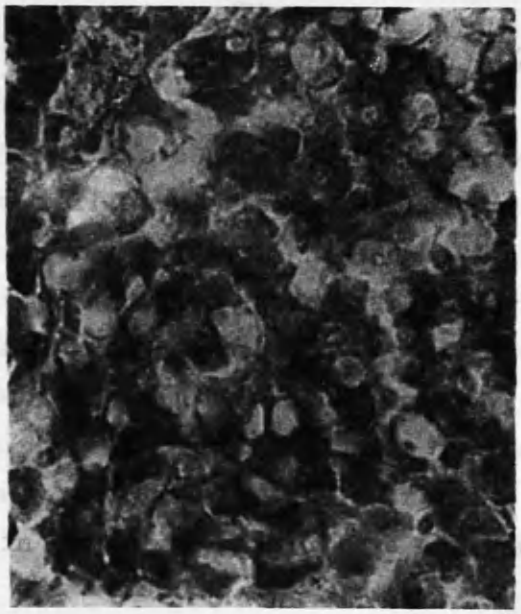
Most of the basophils are still granulated and are prominent by their large size. However, some degree of degranulation occurs as evidenced by the presence of vacuoles (Plate 15(a)). For this reason there is a slight reduction in the number

PLATE 15

Cyclical changes in the meso-adenohypophysis of T. mossambica

a) Sagittal section of the pituitary of fish in the pre-spawning phase, showing degranulation in some of the basophils. Stained with Masson's trichrome stain and Zenker-formol fixation.

b) Sagittal section of the pituitary of fish in the post-spawning phase. Note the presence of only a few basophils and numerous undifferentiated cells (chromophobes) and persisting vacuoles. Stained with Azan and Bouin fixation. All X.620.



a

b

of secretory basophils and they now form 44% of the total percentage of the cell types (Fig 1 & Table 7). Basophils with granulated cytoplasmic contents become common and due to the extent of granulation cell boundaries are difficult to distinguish. The cells are therefore distinguished by their nuclei which become well defined with distinct karyoplasm. During the latter part of the spawning phase many basophils are denuded of their cytoplasmic contents and they become partly or wholly vacuolated.

The percentage of acidophils in the meso-adenohypophysis of the pituitary increases slightly from 39% in the pre-spawning phase to 42% in the early part of the spawning phase (Fig 1 and Table 7).

The chromophobes which were hardly visible during the previous phase become conspicuous, forming 13% of the average percentage of the cell types in this region (Fig. 1 and Table 7).

c) The meta-adenohypophysis

Most of the bluish-pink cells still persist though there is a sign of a slight reduction in their staining affinity. The other acidophils remain small and non-granulated.

5. The Post-spawning Phase

a) The Pro-adenohypophysis

The most conspicuous change is found in the size of this region which now becomes almost proportional to the other two. No change in the morphology or number of cell types is observed.

b) The Meso-adenohypophysis

The whole picture of this zone is completely changed during this phase. The degree of degranulation is reduced and vacuoles are present, together with a substantial quantity of chromophobic cells (Plate 15(b)). The percentage of the deeply-

staining basophils drops sharply from 42 to 12% and so does the staining intensity (Fig 1; and Table 7).

A slight increase in the quantity of the acidophils is observed. The latter now becomes dominant and constitute 45% of the cell types of the pituitary. The chromophobes, exhibit, for the first time during the reproductive cycle, a great numerical increase and form 41% of the total cell population of the meso-adenohypophysis (Fig. 1 and Table 7).

c) The Meta-adenohypophysis

There is a depletion of the bluish-pink cells, and the secretive activity of the cells bordering the branches of the neurohypophyseal tissue is abolished. This region also increases and becomes almost equal to each of the other two.

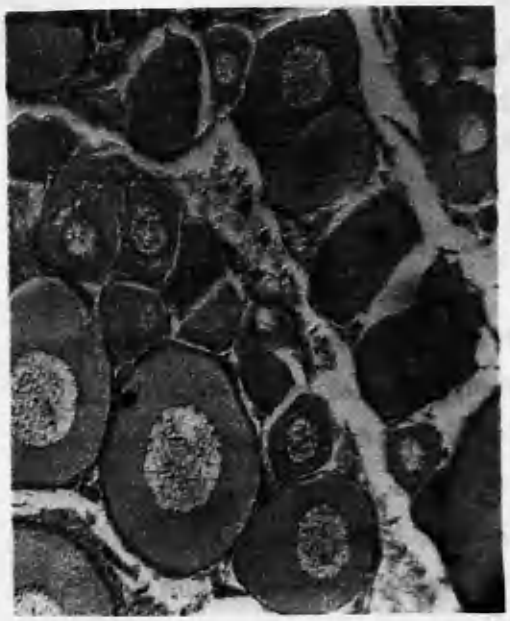
PLATE 16

Effects of low temperature and total darkness on the gonads and pituitary of T.mossambica

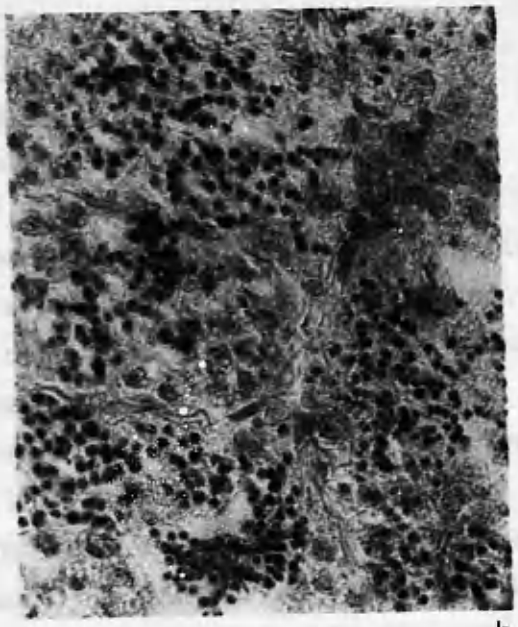
a) T.S. of ovary of control female at the beginning of the experiment, showing oocyte in the immature phase. Stained with Azan and Bouin-Hollande fixation. X.155.

b) T.S. of testis of control male at the beginning of the experiment, showing germ cells in the maturation phase. Stained with Heindenhain's iron haematoxylin and Bouin fixation. X.620.

c) Sagittal section of the pituitary of control fish at the beginning of the experiment; showing the basophils in the meso-adenohypophysis. Stained with Azan and Bouin-Hollande fixation. X.620.



a



b

c

THE EFFECTS OF LOW TEMPERATURE AND TOTAL DARKNESS ON THE REPRODUCTIVE SYSTEM OF T. MOSSAMBICA

Seventy males and females of T.mossambica weighing 4.0 - 5.4 gm and measuring 6.0 - 6.8 cm were acclimatized in the laboratory at 27° - 30°C. After a period of two weeks ten fish were sampled to determine the state of the gametogenetic activity. These fish formed the initial controls. Twenty of those remaining were kept in total darkness for three months at the same temperatures as the initial controls. Another twenty fish were subjected to a temperature of 13°- 15°C under the same light regime (~~normal~~ natural daylight) as the controls, while the remaining fish continued under favourable conditions and served as the final controls. Each group was fed with 2 gm of Tetramin per day. The little amount of food given was to ensure that not much of it was left to pollute the water, especially in the total darkness group where tempering of the experiment was reduced to a minimum.

Results

a) Initial control group

The Ovaries.

Histological examination of slides of the ovaries revealed oogonia and numerous young oocytes in the initial period of protoplasmic growth. Only a few oocytes had entered into the advanced phase of protoplasmic growth (Plate 16(a)). Two of the six females examined had ovaries in the third stage of maturation - presence of trophic substances in the oocytes. However, these vitellogenetic oocytes were few as compared with the numerous protoplasmic ones. Data on the size of fish, diameter of the large oocytes and the stages of maturation of the ovaries are given on Tables 8 and 9 and Appendices i and ii.

The Testes

In the male control fish spermatogenesis was in progress. Spermatogonia, spermatocytes and spermatids were common in the testes. Spermiogenesis, however, was not completed and so spermatozoa

Table 8. Effects of Low Temperature and Total Darkness on Growth and Oogenesis T. mossambica

Group	Mean wt of fish ± SE (gm)	Mean length of fish ± SE (cm)	Mean size of advanced oocytes ± SE	Mean Stage of Maturation
Initial control	(6)* 4.7± 0.7	6.4±0.4	461±87	II - III
Low Temperature	(5) 1.7±0.3	4.9±0.3	398±29	II
Total Darkness	(6) 2.0±0.2	5.1±0.2	256±28	II
Final control	(6) 7.5±0.6	7.9±0.4	983±32	IV

* Number of fish in parenthesis. Fish with atretic oocytes in low-temperature group not included in analysis.

NOTE: Values for oocyte sizes represent the mean of mean measurements per ovary present over an area of six cross sections.

Mean weight of final control group statistically significant from (1) low-temperature group at $p \leq 0.001$; (2) total darkness group at $p \leq 0.001$

Mean length of final control group statistically significant from (1) low-temperature group at $p \leq 0.001$; (2) total darkness group at $p \leq 0.001$

Table 9. The effects of low-temperature and total darkness on growth and spermatogenesis in T. mossambica

Group	No. of Fish	Mean Wt. (gm)	Mean length (cm)	Spermatogenetic condition of testes ± SE				
				SG	PS	SS	ST	S
Initial control	4	2.3	5.3	44±6	30±2	18±3	50±8	-
Low temperature	4	1.5	4.8	94±8	1±0	-	-	-
Total darkness	4	1.7	5.0	107±8	-	-	-	-
Final control	4	8.7	8.6	5±2	17±8	9±4	18±8	58±8

NOTE: Values for spermatogenetic types are average of each germinal stage in five cross-sectioned lobules.

Mean weight of final control group statistically significant from; (1) low-temperature group at $p \leq 0.001$; (2) total darkness group at $p \leq 0.001$

Mean length of final control group statistically significant from; (1) low-temperature group at $p \leq 0.001$; (2) total darkness group at $p \leq 0.001$.

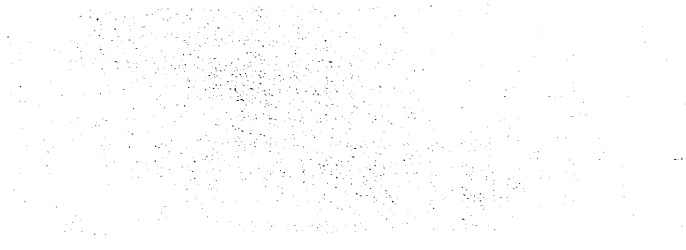
PLATE 17

Effects of low temperature and total darkness on the gonads and pituitary of T. mossambica

a) T.S. of ovary of final control female: showing mature, vitellogenetic oocytes. Stained with Azan X.155.

b) T.S. of testis of final control male: showing ampoules filled with ripe spermatozoa and cells at all stages of development. Stained with Heindenhein's iron haematoxylin X.310.

c) Sagittal section of the pituitary of final control fish, showing numerous granulated basophils in the meso-adenchypophysis. Stained with Azan. X.620. All fixed in Bouin's fixative.



were absent from the testis (Plate 16(b); Fig 2 and Table 9). Appendix iii gives the data on the sizes of the fish.

The Pituitary

Chromophobe and numerous acidophil cells were found in the pituitaries of both males and females. In spite of the lower stage of maturation of the fish, their pituitaries revealed some degree of basophilia (Plate 16(c) and Table 10).

b) Final Control Group

In three months the control fish grew quite rapidly. There was an average increase of 6.4 gm per male and 2.8 gm per female (Tables 8 and 9) Secondary sexual characters became evident in the males, aggressive behaviour was noticed and spawning occurred at the end of the second month.

The Ovaries.

The ovaries were large and revealed many oocytes in the period of vitellogenesis, when they were examined histologically. Oocytes as large as 1102 μ were measured. The mean size of advanced oocyte was 983 μ (Table 8). At the same time cells at all stages of oogenesis were observed (Plate 17(a)).

The Testes

Microscopic examination of the testes revealed lobules containing ripe spermatozoa as well as cells at all stages of maturation (Plate 17(b) and Fig.2).

The Pituitary

The meso-adenohypophysis of the pituitary contained a high percentage of basophils as compared with the total cell population in this region, and with the basophils of the same region in the pituitary of the initial controls. They were also numerous and granulated (Plate 17(c) and Table 10).

Low temperature Group

In three months, the fish subjected to low temperatures

FIGURE 2

Spermatogenetic condition of *T.mossambica* maintained at low temperatures and total darkness. I - Initial control group; II - Low temperature group; III - Total darkness group; IV - Final control group. SG - Spermatogenia; PS - Primary spermatocytes; SS - Secondary spermatocytes; ST - Spermatids; S - Spermatozoa.

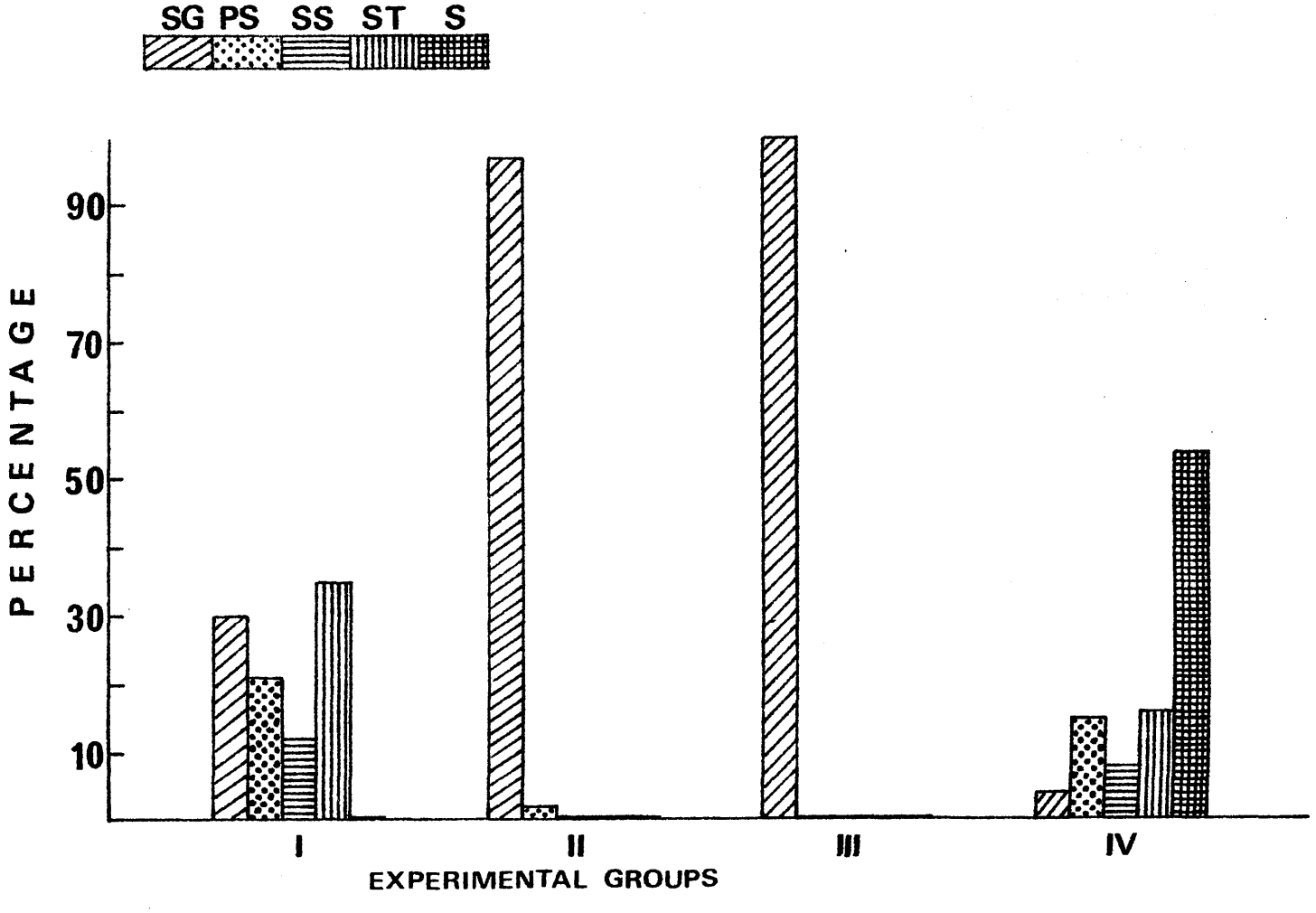

SG PS SS ST S
PERCENTAGE
90
70
50
30
10
I
II
III
IV
EXPERIMENTAL GROUPS

Table 10. Effects on low temperature and total darkness on percentage basophil counts in the pituitary

Treatment	No. of Fish	Mean No. of basophils	Mean percentage of basophils
Initial controls	7	48±18.30	12±5.41
Final controls	4	492±77.99	58±3.11
Low temperature	15	42±17.59	9±3.39
Total darkness	15	0	0

NOTE: Cell counts expressed as average percentage of total cells in meso-adenohypophysis. Values represent average percentages of basophils in 4-6 saggittal sections per pituitary, with total area of meso-adenohypophysis in view.

PLATE 18

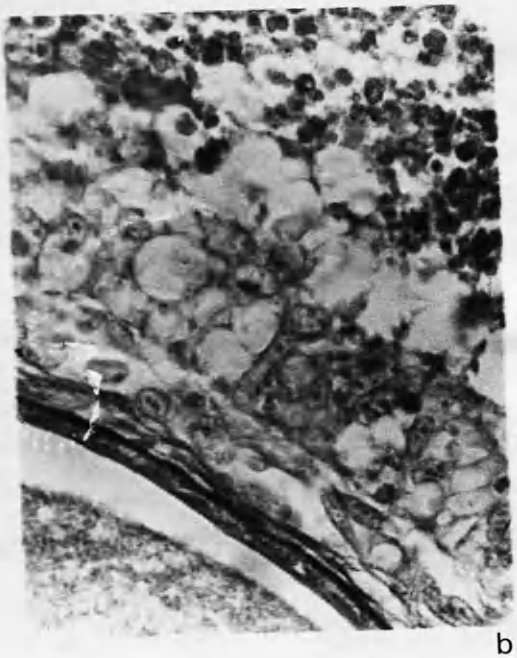
Effects of low temperature on oogenesis in *T. mossambica*

Transverse sections of ovaries of fish maintained at 13°- 15°C. for three months; showing

a) abolition of oogenesis with formation of circumnuclear ring (cnr) and accumulation of organelles (o) near the nucleus, of the protoplasmic oocytes. Stained with Heindenhein's iron haematoxylin. X.310.

b - d) atretic stages in vitellogenetic oocytes. Stained with Azan. X.620. All fixed in Bouin's fixative.

a



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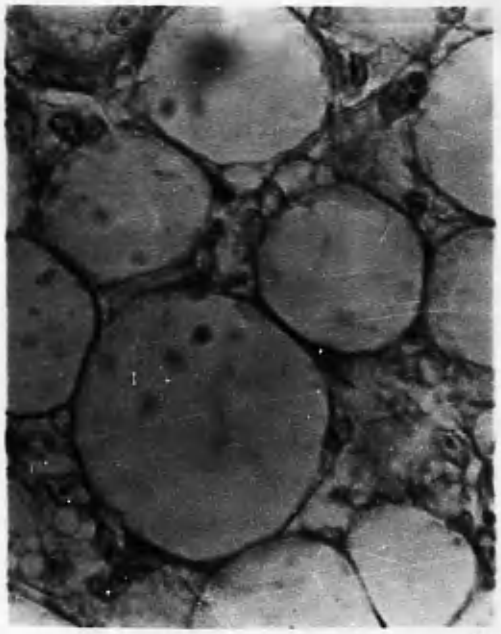

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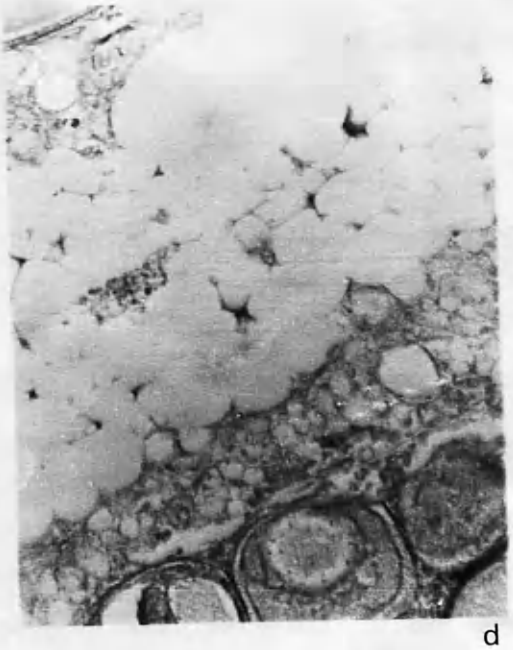


d

lost weight. There was an average decrease of 7.2 gm per male and 5.8 gm per female, as compared with an average weight gain of 6.4 gm in the male and 2.8 gm in the female control fish (see Tables 8 and 9 and Appendices i - iii). The fish remained small, passive and fed rarely.

Changes in the Ovaries.

Histological examination of slides of the ovaries showed oogonia and numerous young protoplasmic oocytes most of which were in the "resting" phase. Very few of the cells, in relation to the many resting ones, were in an advanced stage of protoplasmic growth. However, none of these advanced cells showed any sign of transformation into the period of vitellogenesis. In the cytoplasm of the "resting" oocytes a dark zone, probably consisting of organoids was commonly found around the nucleus or near it (Plate 18(a)).

Atresia of yolky oocytes.

Some of the ovaries of the initial control fish, when examined, revealed yolky oocytes. The oocytes of such fish when subjected to low temperatures for three months showed various stages of atresia. The onset of atresia became evident when the follicular cells become hypertrophied, detached and henceforth behaved as independent functional entities. The zona radiata ruptured and this facilitated invasion of the yolk deposits in the oocyte by the hypertrophied follicular cells (Plate 18(b)). As the yolk was gradually removed, concomitantly, the fat droplets come together, initially, as small aggregates (Plate 18(c)). As more yolk was removed, especially from the center of the oocyte, the aggregates of fat became bigger and bigger until they eventually coalesced (Plate 18(d)). Meanwhile, the disintegrated yolk granules invaded by the follicular cells were forced to the periphery of the oocyte where they degenerated. The disappearance of the atretic oocyte from the ovary was probably by resorption.

Changes in the Testes.

The testes, when examined under the microscope revealed a total inhibition of spermatogenesis. Almost all the cells were in the primary and secondary spermatogonial stages. The majority of them were in the resting phase (Plate 19(a)). Only an extremely few cells could be seen to be in the early stages of meiotic division (Fig.2 and Table9). These were also passive cells and showed no tendency for further development. Mitotic divisions were not found. There were clusters of pycnotic nuclei indicating that some of the cysts were becoming necrotic.

Changes in the Pituitary

The percentage of basophils in the meso-adenohypophysis of fish subjected to low temperatures was very low. Nine percent of basophil cells were estimated in this region as compared with 12% and 58% in the same region of the pituitary of the initial and final controls respectively (Plate 19(b) and Table 10).

d) Total darkness Group

Three months of confinement of T.mossambica in total darkness resulted in retardation of growth. There was an average decrease of 0.5 gm per body weight in male and 1.3 gm per female as compared with the average weight gain of 6.4 gm in the male and 2.8 gm in female control fish (Tables 8 and 9 and Appendices i - iii). In spite of the favourable temperature, the fish remained small and were inactive at the end of the experiment. The peritoneum was thin (fat free) indicating a low degree of feeding.

The Ovaries.

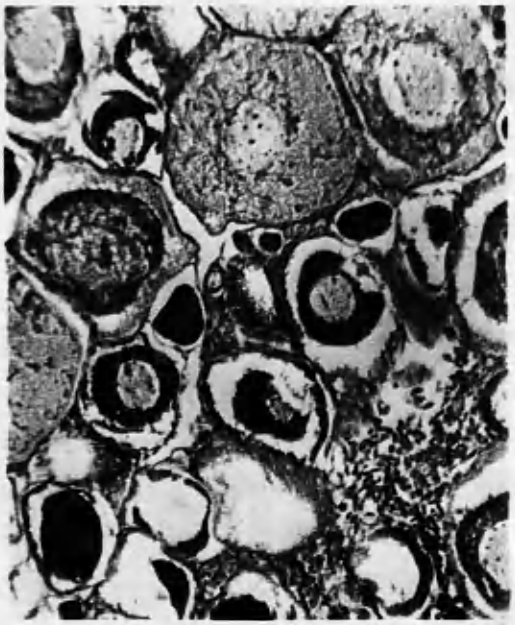
Oogonia in the resting stage and numerous primary oocytes were common in the ovaries when they were examined under the microscope. As in the previous findings, there were a few cells in an advance stage of protoplasmic growth but they were also inactive (Plate 20(a) & Table 8). The dark zone around the nucleus, or circum-nuclear ring and the accumulation of organelles near the nucleus were also found. Fish with ovaries in the third stage of maturation were not found.

PLATE 20

Effects of total darkness on the ovary and pituitary of *T. mossambica.*

a) T.S. of ovary of female kept in total darkness for three months: abolition of oogenesis. Stained with Heindenhain's iron haematoxylin. X.155.

b) Sagittal section of pituitary of fish kept in total darkness for three months: absence of basophil cells in the meso-adenohypophysis. Stained with Azan. X.620. All fixed in Bouin's fixative.



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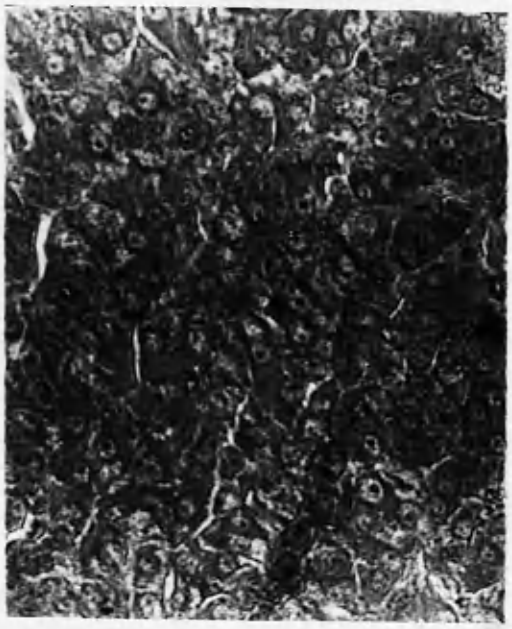

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The Testis

The whole of the testis was dominated by primary and secondary spermatogonia . No other germinal tissue was found in the testis. Apart from spermatogonia, connective tissue cells, Sertoli cells, and interstitial cells of Leydig were also present.

The Pituitary

The pituitary of fish kept in total darkness for three months showed no affinity for basic dyes. No basophil cells were found in the meso-adenohypophysis and the cells in the pituitary were all PAS negative. They were mainly acidophils and a few chromophobes (Plate 20(b) and Table 10).

The results of the present investigations suggest that temperatures between 13° and 15°C have an inhibitory effect on the development of the pituitary and the gonads of T.mossambica. Total darkness also has effects on the reproductive system, similar to those of low temperatures.

THE EFFECTS OF GONADECTOMY IN TILAPIA

1) Effects on the Secondary Sexual Characters and Behaviour

In T.aurea, gonadectomy resulted in the disappearance of the secondary sexual characters. The red coloration of the borders of the fins and the greenish-blue coloration of the upper half of the body in the males were abolished. These males came to resemble the females or riverted to the condition found in the immature fish of both sexes. Castration also resulted in the decrease in the length of the genital papilla in fish of both sexes (see Table 13)

In T.mossambica the removal of the gonads had similar effects on the secondary sexual characters as has been reported for T.aurea - the overall disappearance of the black coloration of the body, the bright red coloration of the borders of the fins and the brilliantly shining operculum.

The behaviour of the fish was equally affected as a result of castration in both species of Tilapia. In a tank containing males and females gonadectomized T.mossambica, spawning behaviour ceased soon after the operation. The gravel at the bottom of the tank was left undisturbed, instead of the usual, constant, nest-building activities, coupled with aggressive behaviour. The normal activity of the fish, however, apart from spawning behaviour, did not seem to be affected by the operation. They fed more readily than during the pre-operative period.

In a tank with an all male gonadectomized T.mossambica population one male displayed the secondary sexual characters, indulged in nest building and began to court a totally castrated male which did not show the secondary sex characters. At autopsy, a regeneration of the testis was observed in the male which showed the sex characters. The interesting point here is that, the courting of a castrated male by a male with regenerated testis confirms the inference that total gonadectomy in male Tilapia renders it a feminine morphological characteristics.

2) Effects on Growth in T. mossambica

Twenty male and female T.mossambica were gonadectomized and divided into two groups of 10 fish (5 ♂ and 5 ♀) in each group. Five males and five females were sham operated and maintained in another 76 x 32 x 30 cm tank as the control group. Each group was fed with 10 gm of Tubifex three times a day for 50 days. The fish were tagged to facilitate the identification of fish individually. The effect of the operation on growth is reported separately for males and females.

Results.

It is evident from Tables 11 and 12 that castration enhanced somatic growth in T.mossambica. In the two groups of castrated males there was a mean weight gain of 16.3 gm and 5.9 gm respectively, as compared with only 1.7 gm in the sham-operated control males (Table 11). In the gonadectomized females, the first group gained an average weight of 15.0 gm, the second group 9.2 gm while the controls gained an average weight of only 2.0 gm (Table 12).

3) Effects on the Pituitary in T. aurea

Twenty four adult T. aurea (12 ♂ and 12 ♀) were totally gonadectomized. Four fish (2 ♂ and 2 ♀) were sampled on the 5th, 10th, 20th, 30th, 40th and 50th day after the operation for histological studies of the pituitary. All the testes removed from the males were in Stage IV, and the ovaries were in Stages III and IV. Equal number of controls were maintained and sampled at the same time as the gonadectomized fish.

Results.

The effects of castration were seen only in the meso-adenohypophysis of the pituitary. For this reason, only this zone will be considered in describing the cytological changes in the pituitary.

Controls.

The pituitary glands of the controls sampled on the day of the operation revealed features characteristics of adult, maturing

Table 11. Effect of Gonadectomy on Growth in male T. mossambica

Group	Before operation		50 days after operation		Mean wt gain ± SE (gm)	Mean increase in length ± SE (cm)
	Mean wt. of fish (gm)	Mean length of fish (cm)	Mean wt. of fish (gm)	Mean length of fish (cm)		
1	18.2	10.6	34.5	12.9	(5)* 16.3±0.7	2.3±0.3
2	12.9	9.5	18.8	10.6	(5) 5.9±1.0	1.1±0.2
3**	9.9	8.8	11.6	9.2	(5) 1.7±0.5	0.4±0.1

* Number of fish in parenthesis.

** Control group

Mean weight gain in Group 1 significantly different from controls at $p < 0.001$

Mean increase in length in Group 1 significantly different from controls at $p < 0.001$

Mean weight gain in Group 2 significantly different from controls at $p < 0.01$

Mean increase in length in Group 2 significantly different from controls at $p < 0.05$

(See appendix iv for individual weight increases)

Table 12. Effect of Gonadectomy on Growth in female T.mossambica

Group	Before operation		50 days after operation		Mean wt gain ± SE (gm)	Mean increase in length ± SE (cm)
	Mean wt. of fish (gm)	Mean length of fish (cm)	Mean wt of fish (gm)	Mean length of fish (cm)		
1	12.4	9.6	27.4	11.9	(5)* 15.0±0.6	2.4±0.1
2	13.0	9.5	22.2	11.2	(5) 9.2±1.1	1.8±0.3
3**	7.3	7.8	9.3	8.7	(5) 2.0±0.2	0.9±0

* Number of fish in parenthesis

** Control group.

Mean weight gain in Group 1 significantly different from Controls at $p < 0.001$

Mean increase in length in Group 1 significantly different from Controls at $p \leq 0.001$

Mean weight gain in Group 2 significantly different from Controls at $p < 0.001$

Mean increase in length in Group 2 significantly different from Controls at $p < 0.01$

(See Appendix V for individual weight increases).

fish - the presence of only a few chromophobes, and numerous acidophils and basophils (Plate 21(a). With subsequent samplings, an increase in the quantity of secretive basophils was observed until the 50th day, when degranulation was evidenced in a few basophils (Plate 22(c)). Degranulation of the cells confirmed the spawning condition of the fish, as evidenced by the presence of ripe oocytes and spermatozoa in the gonads.

Gonadectomized Fish

On the fifth day after gonadectomy there was a conspicuous change in the meso-adenohypophysis of the pituitary. Unlike those of the controls, a few basophil cells were found with degranulated cytoplasmic contents (Plate 21(b)). Chromophobe cells, even though they were not so numerous, were encountered more frequently than in the controls. A second effect of gonadectomy concerned the acidophil cells, which in the castrates demonstrated a stronger affinity for acid dyes.

Further evidence of degranulation was observed in the basophils of the pituitary of fish ten days after castration. By the 20th day, a comparatively large number of vacuoles were present in the meso-adenohypophysis (Plate 21(c)). Most of them revealed the morphologically typical "castration" or signet ring cells - a large vacuole, pressing the nucleus against the cell wall (Plate 21(d)) A considerable reduction in the number of PAS positive cells was also observed. Concomitantly, the strongly-acidophilic cells in the pituitary of the castrates, unlike those in the controls, revealed hyperplasic and hypertrophic characteristics and were densely granulated (Plate 22(d)).

The pituitary of the gonadectomized fish sampled on the 30th and 40th days after operation showed a similar, but a more pronounced effect (Plate 22(a)). Most of the PAS positive cells were in an advanced stage of degranulation. The hypertrophied acidophils seemed to increase in quantity, and chromophobes were common in the meso-adenohypophysis.

PLATE 22

Effects of gonadectomy on the cytology of the meso-adenohypophysis of T. aurea.

a) Sagittal section of the pituitary of a 30 day gonadectomized fish, showing advanced stage of degranulation of basophils. Almost all B_1-cells and part of B_2- cells are degranulated. B_1-cells stain deep red and B_2-cells - light red. Stained with Alcian Blue-PAS-Orange G.

b) Sagittal section of the pituitary of a 50 day gonadectomized fish, showing (i) complete depletion of basophils, (ii) disappearance of vacuoles, and (iii) the appearance of numerous chromophobic cells. Stained with Azan.

c) Sagittal section of the pituitary of control fish sampled on the 50th day of experiment, showing the presence of secretive basophils in meso-adenohypophysis. Few basophils are degranulated (Charactertistics of spawning phase). Stained with Azan.

d) Sagittal section of the pituitary of 20 day gonadectomized fish, showing hypertrophy and secretory activity in the STH cells. Stained with Cleveland-Wolfe. All fixed in Bouin-Hollande, and X.620.

By the 50th day after the operation, there was no further evidence of degranulation. Instead, chromophobe cells, the cytoplasm of which had slight affinity for acid dyes, or no tinctorial affinity at all, were found in the area once occupied by degranulated basophils (Plate 22(b)). If basophils were present at this stage, they were extremely few and in isolation, and showed a weak affinity for aniline blue.

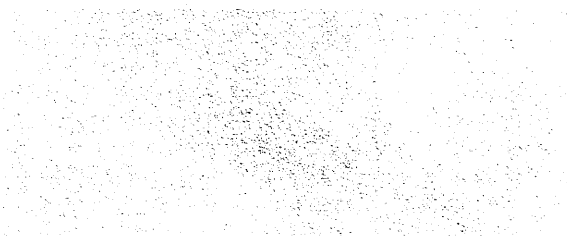
In T.mossambica, identical observations were made in the meso-adenohypophysis of the pituitary. Increasing degranulation occurred in the basophils till the 30th day after castration. From the 30th day, the vacuoles gradually disappeared. By the 46th day the basophils became almost completely depleted and gave way to the appearance of chromophobe cells (Plate 23(a)). The complete depletion of the basophils was confirmed by an almost PAS negative result when the latter test was made (Plate 23(b)).

PLATE 23

Effects of gonadectomy on the cytology of the meso-adenohypophhsis in T. mossambica

a) Sagittal section of the pituitary of a 46 day gonadectomized fish; showing (i) an almost total depletion of basophil cells, and (ii) the appearance of chromophobic cells in the region once occupied by the basophils. Stained with Azan.

b) Sagittal section of the pituitary of a 46 day gonadectomized fish, showing a PAS negative reaction in the meso-adenohypophysis. Stained with Periodic Acid and Schiff's reagent. All fixed in Bouin's fixative, and X.620.



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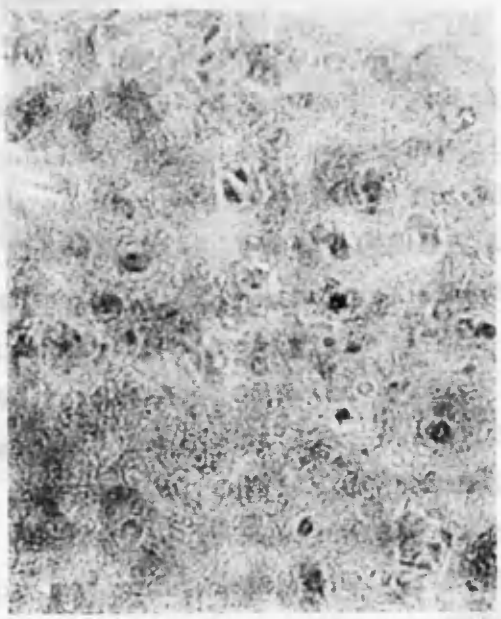

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THE EFFECT OF REPLACEMENT THERAPY ON THE SECONDARY SEXUAL CHARACTERS AND BEHAVIOUR IN GONADECTOMIZED T. AUREA

Thirty six mature T. aurea were gonadectomized and divided into three groups of 12 fish (6 ♂ and 6 ♀) in each group. Forty days after the operation they were subjected to steroid hormone treatments. The lengths of the genital papilla in fish of both sexes were measured at the time of the operation, on the 40th day (just before injections began) and also at the end of the replacement therapy. The hormones tested were : Testosterone propionate (TP) and Oestradiol Benzoate (OB). The individual dosage of TP was 0.62 mg in 0.1 ml volume, and that of OB was 0.30 mg. in 0.1 ml. The third group of gonadectomized fish served as the controls and were treated with the same volume of Arachis oil. Injections were given six times per week for 30 days. The effects on the males are presented separately from those of the females.

Results

Gonadectomy resulted in a reduction in the size of the genital papilla, while the administration of TP caused a rapid growth of the papilla during the 30 days of treatment (Table 13). There was an average gain of 1.8 m.m. in length of the genital papilla as a result of TP administration. During the same period the genital papilla of the control fish receiving oil-treatments revealed a decrease of 0.12 m.m. (Table 13). Oestradiol benzoate treatment also resulted in a mean increase of 0.55 m.m. in the genital papilla of the males.

In the female gonadectomized fish, TP stimulated the development of the genital papilla, as evidenced by an increase of 0.56 mm in length of the papilla. Using OB, a similar stimulatory effect was realized. The OB treated females gained an average length of 0.80 mm while the genital papilla of the

control females, receiving injections of oil decreased by 0.07mm. over the same period of 30 days (Table 13).

Apart from its effect on the genital papilla in both males and female gonadectomized fish, Testosterone propionate also reinstated the breeding dress in the males which is usually fully developed only at the height of the breeding period - the red coloration of the borders of the fins and the greenish-blue coloration of the upper half of the body. However, in the OB treated males, no such coloration appeared. On the other hand, administration of TP into gonadectomized female fish, resulted in some degree of masculinization - the appearance of red coloration on the borders of the fins.

The behaviour of the gonadectomized fish was also affected as a result of replacement therapy. The castrated males, after the reinstatement of breeding coloration as a result of TP treatment built nests, demonstrated aggressive behaviour and courted the females. OB treatment caused a heightened awareness in fish of both sexes, especially in the females.

Table 13. Effects of Gonadectomy, and of Steroid administration on the Genital papilla in male and female T.aurea

Sex	Treatment	Mean wt. of fish (gm)	Mean length of fish (cm)	Mean length of Genital Papilla ± SE (mm)			Mean increase/loss in length of papilla after treatment ± SE (m.m)
				Before operation	40 days after operation	30 days after treatment	
♂	TP	(6)* 85.5	18.6	3.14±0.296	2.79±0.278	4.59±0.455	1.80±0.350
♂	OB	(6) 99.7	18.3	2.90±0.181	2.63±0.180	3.18±0.233	0.56±0.153
♂	AO**	(6) 84.2	17.6	2.73±0.089	2.56±0.073	2.44±0.050	-0.12±0.037
♀	TP	(6) 53.4	15.0	1.96±0.210	1.73±0.201	2.25±0.290	0.52±0.180
♀	OB	(6) 42.5	13.9	1.93±0.129	1.70±0.091	2.50±0.345	0.80±0.282
♀	AO	(6) 38.7	13.5	1.67±0.179	1.48±0.166	1.41±0.175	-0.07±0.023

* Number of fish in parenthesis

** Arachis oil (treatment for controls).

p values calculated by Student's t test in males between :TP treated and AO treated at $p < 0.001$; OB treated and AO treated at $p < 0.02$

p values calculated by Students t test in females between : TP treated and AO treated at $p < 0.05$; OB treated and AO treated $p < 0.02$.

Results of individuals sizes of the genital papilla before operation and after Methallibure hormone treatment are given in Appendices VI and VII

METHALLIBURE TREATMENT

1) Effects of Methallibure on the Secondary Sexual Characters and Behaviour

T.mossambica : Before treatment started, most of the males (both experimentals and controls) displayed distinct secondary sexual characters - a black coloration of the body, coupled with a sharply contrasting pale and shining opercular, and a red coloration of the borders of the fins, especially, those of the dorsal and caudal fins. Spawning behaviour - nest building and territorial establishments, were also observed.

Five days of Methallibure treatment had no effect on the secondary sexual characters in the males. Spawning behaviour continued, but at a somewhat reduced rythm. From the fifth to the eighth day of treatment, the black coloration of the body began to fade and the borders of the fins which were previously red, began to look light-green in colour. By the eighth day nest-building had completely stopped and the fish had abolished their spawning behaviour. By the tenth day, there was no sign of the secondary sexual characters, and the treated males, morphologically, were indistinguishable from both the treated and control females and also from the immature fish of both sexes. Feeding activities, however, were not disturbed by this treatment.

In the control tank, secondary sexual characters persisted in the males and spawning behaviour intensified.

T. aurea: The secondary sexual characters in T.aurea are less conspicuous than those in T. mossambica, and comprise of red coloration of the borders of the fins and a greenish-blue coloration of the upper half of the body. It is therefore difficult, usually, to differentiate a male in an out-of-spawning coloration, from a female, when observing the fish in a tank.

As a result of Methallibure treatment, territorial

establishments and aggressive behaviour, which were so characteristic of the fish in spawning conditions, were abolished. The secondary sexual characters also disappeared and the colours of the males riverted to those of females and immature fish of both sexes. A closer examination of the fish revealed a considerable reduction in the size of the genital papilla in the males. The genital pore in the female was hardly discernible. For this reason, a separate experiment was set up to determine the effect of Methallibure on the size of the genital papilla in males.

The Effect of Methallibure on the Genital Papilla in male T.aurea.

Two groups of males (10 in each group) were treated with Methallibure for six weeks. The third group, was treated with Tween 80 (the suspension medium) and served as the controls. The length of the genital papilla of each fish was measured before and at the end of the experiment. All the fish were tagged to facilitate individual identification of each fish.

Results.

(a) Description of the Genital Papilla

The genital tube is a flaccid papilla situated just caudal to the anus. In the male fish, the papilla are conical with only one aperture near the tip which serves as a combined ureter and genital opening. In the female, the genital is broader and has two apertures - the oviduct, which opens as a transverse slit across the papilla; and the ureter, which has a small opening into the oviduct.

b) The Effect of treatment

From Table 14 it is evident that Methallibure caused a reduction in the length of the genital papilla. The ten males of the first group lost an average of 1.08 mm in length of the papilla, while those of the second group lost 1.05 mm. In the control fish, there was a gain of ~~0.12~~ 0.75 mm. in length of the genital papilla during the same period (Table 14).

Table 14. The Effect of Methallibure on the Length of the Genital papilla in T. aurea.

Experiment Number	Mean wt of fish (gm)	Mean length of fish (cm)	Mean length of genital papilla ± SE (mm)		Length of Genital Papilla ± SE (mm)
			Before treatment	After treatment	
1	(10)* 84.9	16.6	2.48±0.306	1.49±0.261	-1.08±0.146
2	(10) 71.5	15.8	2.25±0.145	1.20±0.103	-1.05±0.055
3(Control)	(10 75.3	16.5	2.48±0.266	3.23±0.319	+0.75±0.117

* Number of fish in parenthesis.

Detailed results on lengths of genital papilla before and after treatment are given in Appendices VIII - X

The Effects of Methallibure on Growth and Survival in T.mossambica

Three groups of fish were treated for 35 days with Methallibure. They were maintained in 76 x 32 x 30 cm tanks and each tank contained 34 fish (17 males and 17 females). The fourth group which served as the control was treated with Tween 80 (the suspension medium). All the fish were fed with Tubifex and commercially dried food (Tetramin). The feeding procedure was as follows : 5 gm. of Tetramin in each tank in the morning, 15 gm of Tubifex in the afternoon and 5 gm. of Tetramin again in the evening. The fish were fed every day except Sundays.

Results

(a) Effects on Survival

During the whole period of treatment there was no mortality in any of the experimental or control tanks. There was, therefore, a survival rate of 100%.

(b) Effects on Growth

The treatment of fish with Methallibure did not seem to affect the feeding habits of the fish, as all the experimental groups as well as the controls fed readily. The treated fish in all the tanks grew more rapidly than the controls. There was an average increase of 1.69 gm. per fish in the controls during the 35 experimental days whereas the Methallibure treated fish gained an average weight of 3.07 gm., 3.31 gm and 3.27 gm per fish in the first, second and third groups respectively (Table 15).

Table 15. Effect of Methallibure on Growth in T.mossambica

Group	Before treatment: Mean wt of fish ± SE (gm)	Before treatment: Mean length of fish (cm)	After treatment: Mean wt of fish ± SE (gm)	After treatment: Mean length of fish (cm)	Mean wt increase (gm)	Mean length increase (cm)
1	(34)[1] 1.78±0.16	5.9	(30)[2] 4.85±0.16	6.8	3.07	0.9
2	(34) 2.07±0.18	6.2	(30) 5.38±0.31	6.9	3.31	0.7
3	(34) 3.06±0.32	6.9	(30) 6.33±0.43	7.5	3.27	0.6
4[3]	(34) 2.53±0.17	6.7	(30) 4.22±0.16	6.9	1.69	0.2

1. Number of fish in parenthesis.

2. " " " " (Four fish from each group sampled for hostological examination before treatment ended.

3. Control group.

p values calculated by Student's t test between groups: 1 and 4 $p < 0.05$; 2 and 4 $p < 0.01$; 3 and 4 $p < 0.001$

Effects of Methallibure on the Gonads and Pituitary of T.mossambica

One hundred and twenty maturing T.mossambica weighing between 4 and 12 gm. and measuring 6-12 cm. were maintained in four tanks and treated with Methallibure as described earlier. The control fish received the suspension medium.

Samples of 12 fish (6 males and 6 females) were taken. The first after 10 days, and subsequently at 5 day intervals.

Results

1) The Ovaries

Eight cross-sections from the central portion of each ovary were selected and examined for oocytes containing intact yolk or atretic oocytes as evidence of ovarian maturation or regression respectively.

a) Controls. Initial Sampling

The ovaries of six females sampled at the beginning of treatment revealed oogonia, protoplasmic oocytes and oocytes in the period of vitellogenesis (Stage III).

Ten Days of Treatment (Controls)

The oocytes of the control fish, treated with the suspension medium only, developed rapidly. Large oocytes, approaching the last phase of development (Stage IV) were observed in the ovaries (Plate 24(a)

b) Treated Fish

The ovaries of Tilapia treated for ten days with Methallibure were in a state of regression, when the organs were examined microscopically. Oocytes at various stages of atresia were found (Plate 24(b)). An increase in the size of the tunica was also observed (6-13 μ, as compared with 3-9 μ in controls). All the slides of the ovaries studied revealed numerous protoplasmic oocytes (Plate 24(b).

PLATE 24

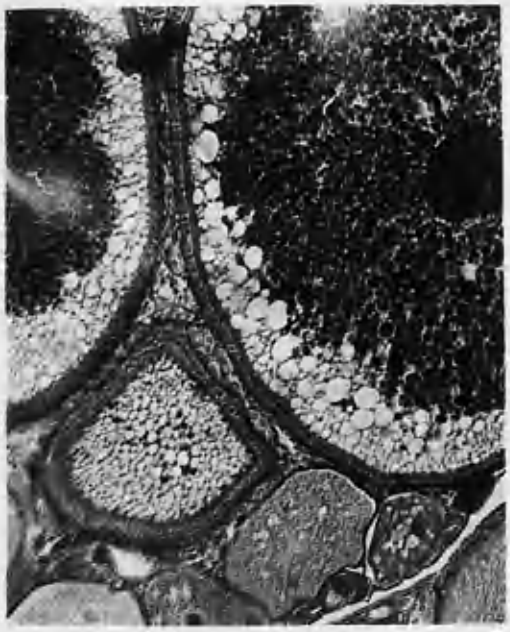
Effects of Methallibure on the ovary of T.mossambica

a) T.S. of ovary of maturing control female. Stained with Azan.

b) T.S. of ovary of adult female treated for 10 days with Methallibure; invasion of oocyte-yolk by phagocytosing follicular cells. Stained with Heindenhein's iron haematoxylin.

c) T.S. of ovary of adult female treated for 20 days with Methallibure: further evidence of atresia. Stained with Heidenhein's iron haematoxylin.

d) T.S. of ovary of adult female treated for 25 days with Methallibure : reconstitution phase of ovary - resorption of all atretic vitellogenetic oocytes leaving protoplasmic ones. Stained with Azan. All X.155, and Bouin fixation.



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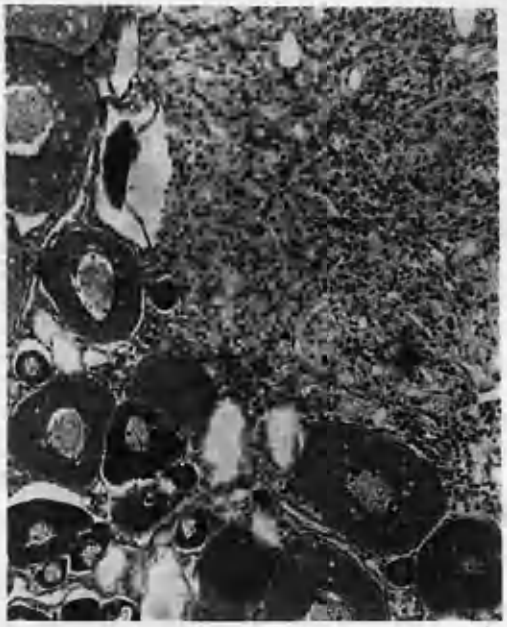

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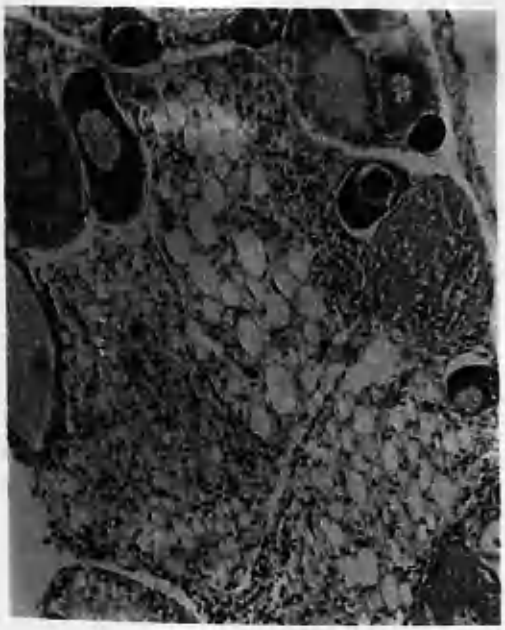

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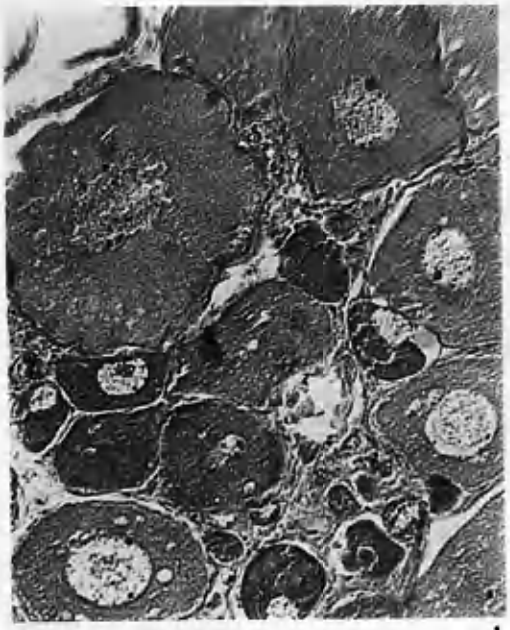

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15-20 Days of Treatment

a) Controls

Two females had spawned and were carrying the eggs in their mouths. The rest had ovaries in Stage IV of maturation (as proved by microscopic examination, and the escape of eggs to the exterior when the peritoneum was pressed).

b) Treated Fish

Numerous atretic oocytes invaded by follicular cells were found. The number of the latter in each oocyte was so great that the whole structure resembled a multinucleated mass of protoplasm - a syncytium (Plate 24(b)(c)). In five of the six ovaries studied all the oocytes beyond the protoplasmic stage were in the advanced stage of atresia described. Oocytes in the early stages of atresia were found only in one female.

There was a further increase in the size of the tunica which now measured 83 - 249 μ as compared with 30-49 μ in the controls. In the ovaries of the treated fish oogonia and protoplasmic oocytes were also present but their number seemed to be reduced.

25 Days of Treatment

Only a little evidence of atresia existed in the ovaries of fish treated with methallibure for 25 days. A few remnants of atretic oocytes persisted and soon underwent resorption. The condition of the ovary, in contrast with that of the previous observation, resembled that of young, immature females (Plate 24(d)) It contained oogonia and numerous young protoplasmic oocytes. The average diameter of the latter was 200-215 μ with a maximum of 270-285 and a mean nuclear diameter of 65-67 μ with a 95-100 μ as the maximum.

In the ovary, the spaces once filled by vitellogenetic oocytes, and then by atretic ones, disappeared, partly by the contraction of the ovary and partly by the growth of the protoplasmic oocytes.

PLATE 25

Effects of Methallibure on the testis of T.mossambica

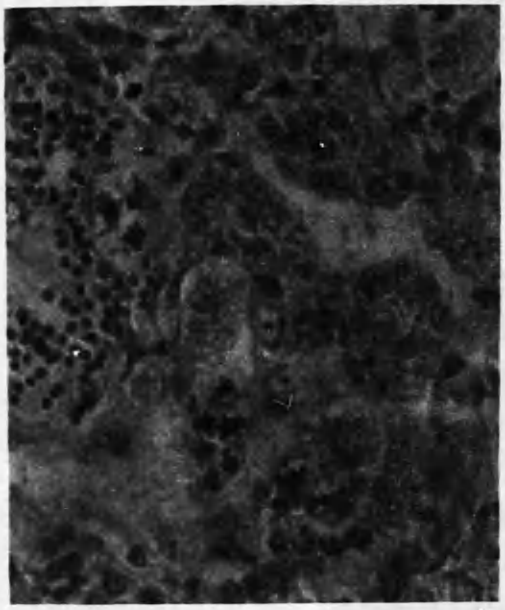
a) T.S. of testis of maturing control male.

b) T.S. of testis of adult male treated for 10 days with Methallibure: necrotic changes in intermediate germ cells.

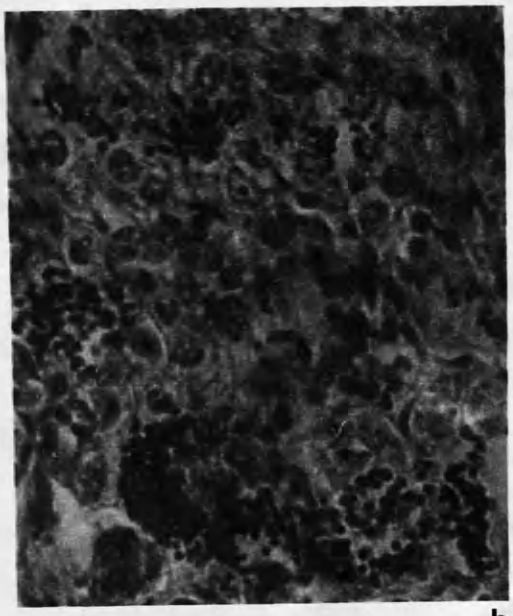
c) T.S. of testis of adult male treated for 20 days with Methallibure; further necrotic changes in intermediate germ cells - presence only of spermatogonia and spermatozoa.

d) T.S. of testis of adult male treated for 30 days with Methallibure: presence of large number of spermatogonia in the latent period and a few traces of spermatozoa.

All stained with Heindenhein's iron haematoxylin, Bouin fixation, and X.620.



a



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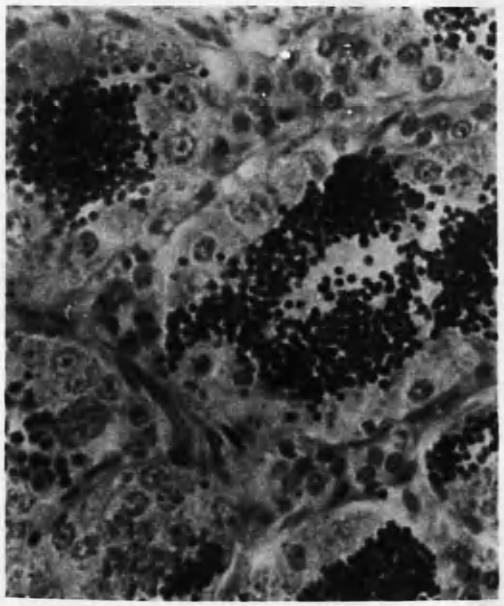
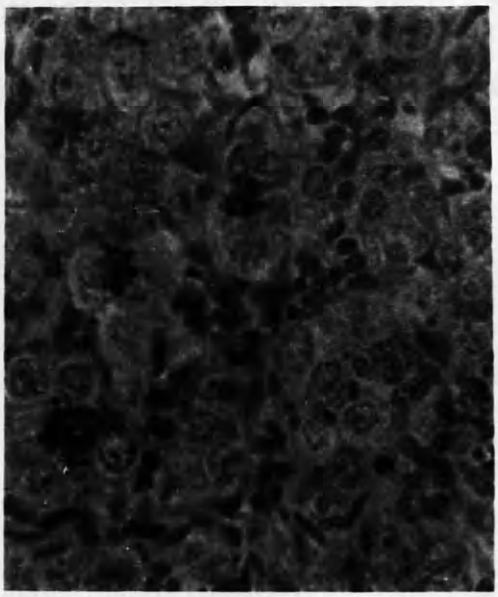


c



d

From the 25th to the last day of treatment (the 35th day) reconstitution of the ovary continued and the tunica during this period, decreased to 40-50μ. As long as treatment continued, vitellogenetic oocytes were no longer formed.

2) The Testis

Six cross-sections of the testis of each fish were selected and the degree of spermatogenetic activity was estimated by counting the total number of germinal cysts of each spermatogenetic stage found in six randomly selected areas.

a) Controls. Initial Sampling

Histological examination of the testes of six males sampled at the beginning of the experiment revealed spermatogonia, primary and secondary spermatocytes, spermatids and a few ripe sperm - Stage III (Fig. 3 and Table 16).

Ten Days of Treatment (Controls)

Spermiogenesis was in progress and as a result, the testis of the control fish contained numerous lobules packed with spermatozoa (Stage IV). Cells at all stages of development were also in evidence (Plate 26(b) Fig. 3 and Table 16).

b) Treated Fish

Ten days of Methallibure treatment resulted in a remarkable state of regression of the testis. As compared with the controls, there was considerable increase in the quantity of spermatogonial cells, followed by a great reduction in the number of the primary spermatocytes. Secondary spermatocytes and spermatids were completely absent from the testis. Spermatozoa, however, were present and their quantity did not differ very much from that of the 10 day controls (Plate 25(b), Fig 3, Table 16).

15-20 Days of Treatment (Controls)

Spawning had occurred and empty lobules were present in the testis, together with germinal cells at all stages of development (Fig.3)

FIGURE 3

Effects of Methallibure on Spermatogenesis in T.mossambica. Upper series: controls. Lower series: Methallibure -treated. I - 10 days of treatment; II - 15 - 20 days of treatment; III - 25 - 30 days of treatment; IV - 35 days of treatment. SG - Spermatogonia; PS - Primary spermatocytes; SS - Secondary spermatocytes; ST - Spermatid; S - Spermatozoa.

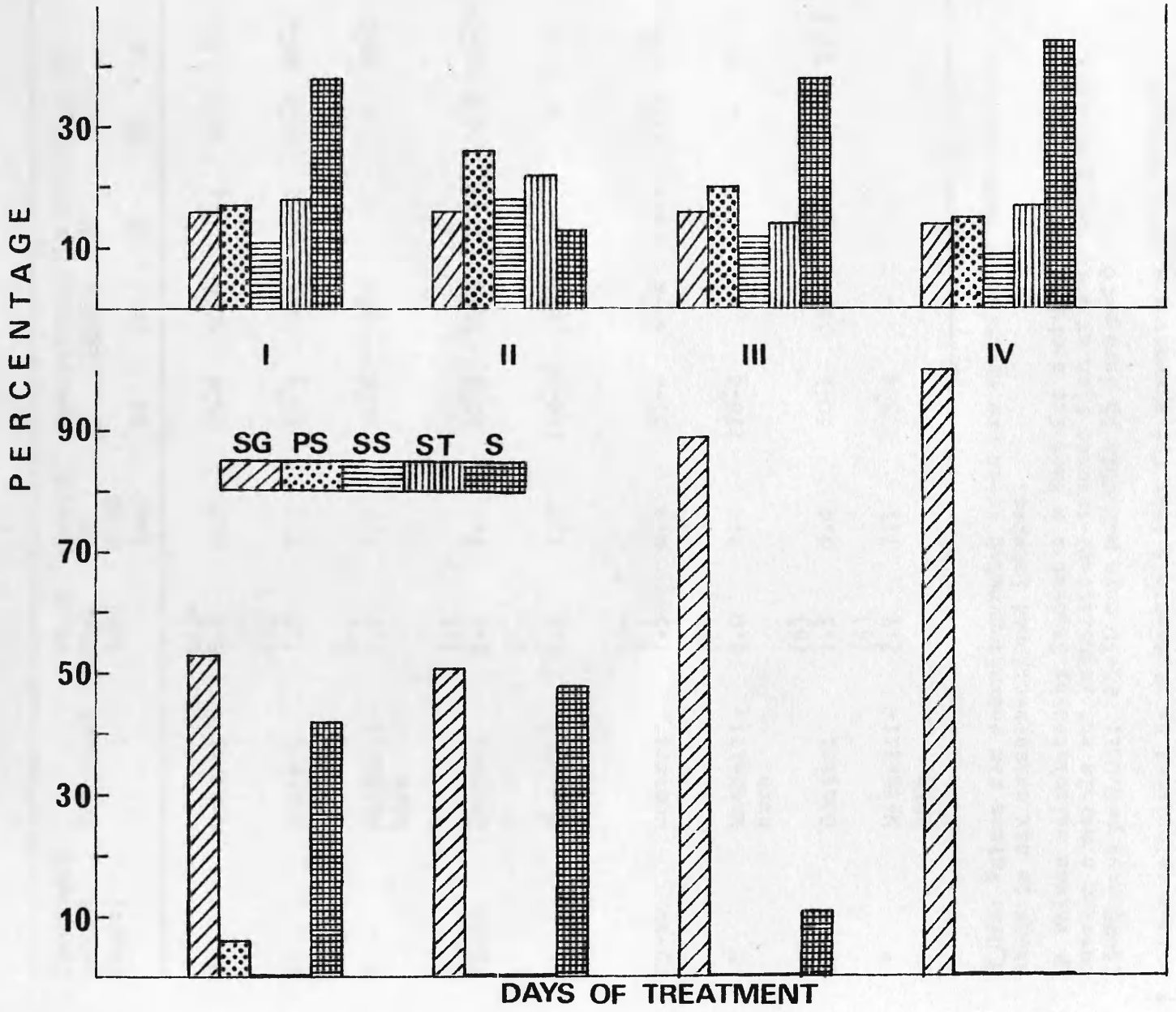

30
10
I
II
III
IV
90
70
50
30
10
SG PS SS ST S
PERCENTAGE
DAYS OF TREATMENT

Table 16 Effect of Methallibure on Spermatogenesis of T.mossambica

Treatment period (days)	Treatment	Wt.of fish (gm)	Length of fish (cm)	Spermatogenetic condition of testes ± SE				
				SG	PS	SS	ST	S
0	Control	(6)* 8.1	8.0	23±8	32±6	26±4	53±9	7±4
10	Control	(6) 7.7	7.5	33±3	36±5	23±2	38±5	80±4
"	Methalli-bure	(6) 6.9	7.5	81±6	9±2	-	-	64±2
15-20	Control	(6) 5.6	7.0	22±3	35±4	24±3	34±3	20±3
"	Methalli-bure	(6) 5.4	7.0	124±12	2±1	-	-	117±1
25-30	Control	(6) 7.5	8.3	37±2	46±2	28±1	33±1	90±1
"	Methalli-bure	(6) 8.0	8.0	178±4	-	-	-	22±9
35	Control	(6) 7.3	5.4	30±1	32±2	20±2	35±3	94±4
"	Methalli-bure	(6) 7.7	7.1	205±4	-	-	-	-

NOTE: Values for spermatogenetic types are averages of each germinal stage in six cross-sectioned lobules.

p values calculated by Student's t test for spermatogonial cells between controls and Methallibure-treated fish at : 10 days $p < 0.001$; 15-20 days $p < 0.001$; 25-30 days $p < 0.001$; 35 days $p < 0.001$.

p values calculated by Student's t test for spermatozoa cells between controls and Methallibure-treated fish at: 10 days $p < 0.01$; 15-20 days $p < 0.001$; 25-30 days $p < 0.001$.

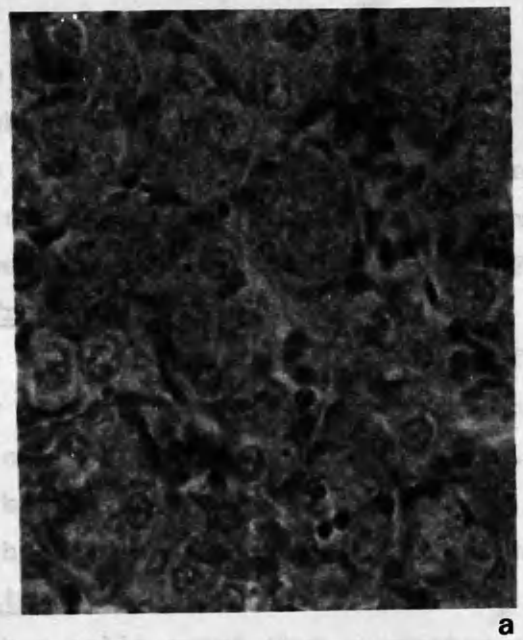
* Number of fish in parenthesis.

PLATE 26

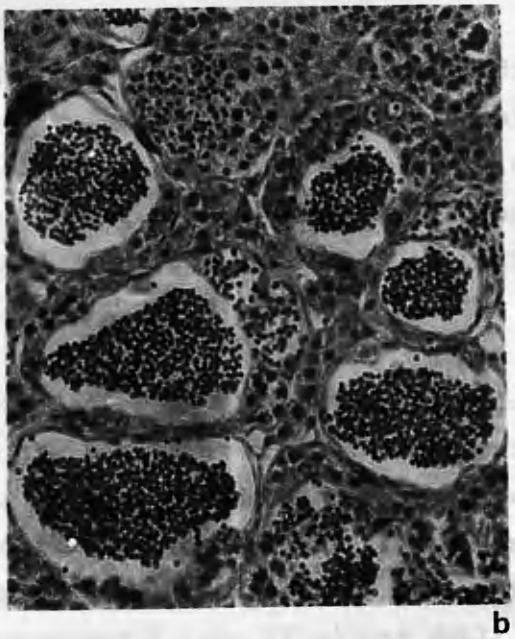
Effects of Methallibure on the testis of T.mossambica

a) T.S. of testis of adult male treated for 35 days with Methallibure: testis filled with numerous spermatogonia; all other germinal cell types degenerated. X.620.

b) T.S. of testis of mature control male: testis filled with germinal cells at all stages of spermatogenesis. X.310. All stained with Heindenhein's iron haematoxylin and Bouin fixation.



a



b

Treated Fish

There was a further reduction in the number of primary spermatocytes which were now extremely rare on microscope slides. Increase in the number of the spermatogonial cells was observed, and a further increase in the quantity of spermatozoa. The intermediate cells were still absent (Plate 25(c); Fig 3 and Table 16).

25-30 Days of Treatment

There was a complete disappearance of all the intermediate cells - primary and secondary spermatocytes and spermatids. The testis was basically filled with spermatogonia. Spermatozoa were extremely few, but still present (Plate 25(d); Fig 3 and Table 16). Empty lobules which were once filled with sperm were also present.

35 Days of Treatment

No other cells apart from spermatogonia dominated the testes of five of the six males examined. Even in the last fish, there were only a few traces of sperm in the testis (Plate 26a, Fig. 3 and Table 16).

During the treatment, as the intermediate and finally the final germinal cells disappeared, the intestitial cells of Leydig, the Sertoli cell, and the connective-tissue cells became very conspicuous. Comparison of the sizes of the intestitial cells in the controls and treated fish revealed no differences.

The Pituitary

a) Controls

The pituitaries of the control fish developed from the growth phase, at the beginning of the experiment, to the pre-spawning and post-spawning phases, by the end of the experiment. This development was marked by the accumulation and subsequent depletion of strongly-staining secretive granules in the basophil cells of the meso-adenohypophysis (Plate 27(a) and Table 17).

b) Treated Fish

Qualitative, rather than quantitative changes were observed in the meso-adenohypophysis of all the pituitaries treated

with Methallibure.

From the tenth to the 35th day of treatment, histological examination of all the slides studied revealed a gradual diminishment of the blue-staining intensity of the basophil cells (Plate 27(b). There was a direct relationship between the period of treatment and the staining intensity of the basophils. The latter increased from (++) to a strong (+++) in the controls and reduced from (+) on the tenth day of treatment to (-) on the 35th day of treatment (Table 17). Eventually chromophobes dominated in the meso-adenohypophysis.

The results of the present investigation suggest that Methallibure causes marked regression in the testis, completely blocks mitosis in the spermatogonia, and prevents their transformation into spermatocytes. Spermatocytes and spermatids already present at the time of treatment undergo depletion, but ripe sperm, however, persist for some time. In the females, Methallibure causes atresia of all the yolky oocytes and the ovaries contain only oogonia and protoplasmic oocytes which do not transform into the vitellogenetic stage so long as treatment continues. Methallibure appears to block the pituitary-gonadotrophic activity by preventing the release of the gonadotrophic hormones. Methallibure does not disturb somatic growth as all the treated fish grew well

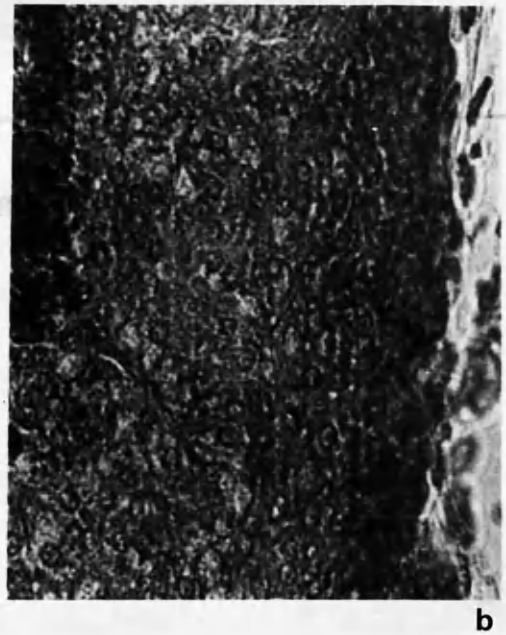
PLATE 27

Effects of Methallibure on the pituitary of T.mossambica

a) Sagittal section of pituitary of control mature fish: presence of granulated basophils. X.1550.

b) Sagittal section of pituitary of adult fish treated for 35 days with Methallibure: basophil cells absent from meso-adenohypophysis, presence of chromophobic cells with inconspicuous nuclei and nucleoli in region formerly occupied by secretive basophils. X.620. All stained with Azan and Bouin fixation.

a



b

Table 17. The effect of Methallibure treatment on the staining intensity of the basophils of the meso-adenohypophysis (+++ means maximal intensity; - means minimal intensity)

Treatment period	Staining intensity	No. of fish Studied
Initial Controls	++	12
10 days		
Control Fish	+++	12
Treated fish	+	12
15-20 Days		
Control Fish	++ *	8
Treated Fish	+-	12
25-35 Days		
Control Fish	+++	12
Treated Fish	-	12

* Post-spawning

5) The Effects of Different Modes of Methallibure Treatment on the Gonads in Relation to the Gonadosomatic Index

Experiment No. 1

Three groups of sexually mature female T.aurea, weighing between 58 and 100 gm. were maintained in three 400 - litre tanks at a density of 30 fish per tank.

The first group received an "external" treatment with Methallibure, while the second group received an "oral" treatment. The third group which served as the controls was treated with the suspension medium.

Four fish from each group were sampled at 10 day intervals, and the gonads, before fixing, were accurately weighed. The experiment lasted for a minimum period of 40 days.

Results

(i) External treatment

From Table 18 it is evident that as a result of external Methallibure treatment, there was a steady increase in the GSI of the fish. The peak GSI was reached only on the 30th day of treatment. From the 30th to the 40th day, there was a sharp decline in the GSI to 0.4 (Fig 4 and Table 18).

(ii) Oral treatment

The peak GSI in the orally-treated fish was reached within ten days, after which there was a somewhat steady decline. Below a GSI of 0.4 there was very little or almost no further decrease (Fig.4 and Table 18).

(iii) Controls

There was a constant increase in the GSI of the controls which reached its peak on the 30th,-40th day (Fig.4 and Table 18) Oogenesis was also in an advanced stage (Plate 29(a)).

(b) Histology of the Ovaries in Relation to the GSI

Histological slides of the ovaries of the first two groups of fish revealed that the peak GSI in both cases was reached when

FIGURE 4

Effects of different modes of Methallibure treatment on the Gonadosomatic Index in female T. aurea.

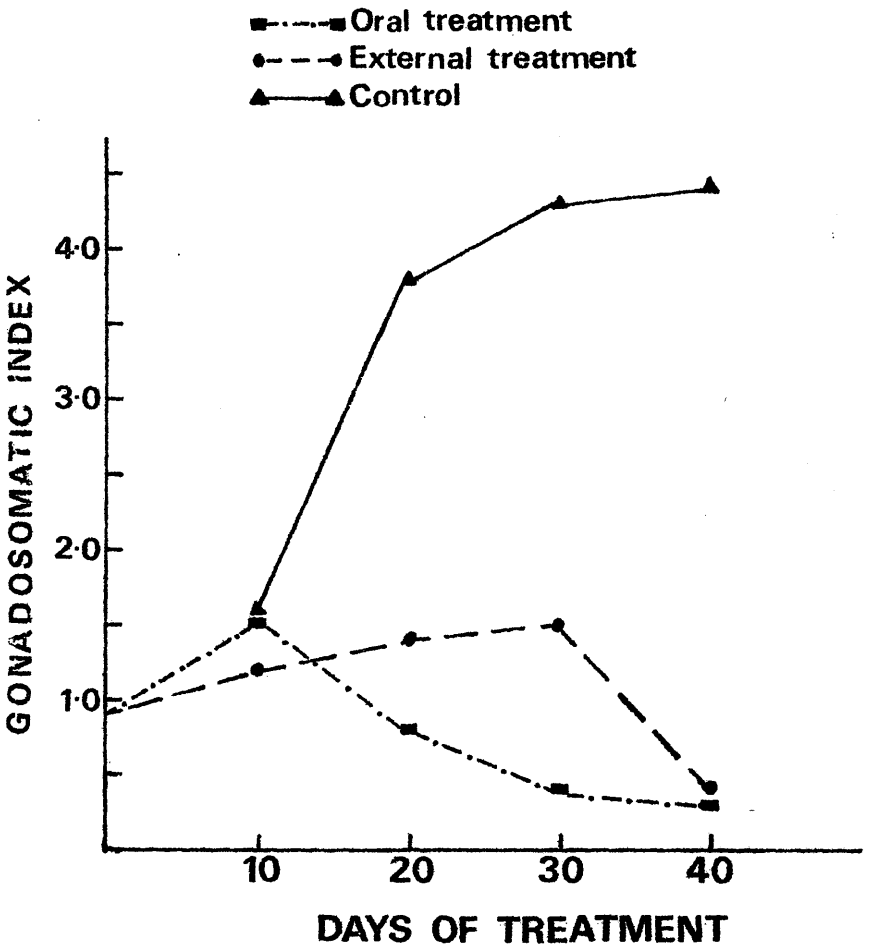

Oral treatment
External treatment
Control
GONADOSOMATIC INDEX
4·0
3·0
2·0
1·0
10
20
30
40
DAYS OF TREATMENT

Table 18. The Effects of Methallibure on the Gonadosomatic Index (GSI) in T.aurea

Experiment	Treatment	No. of fish per sample	Mean GSI ± SE				
			0 Day	10 days	20 days	30 days	40 days
1	External	4	-	1.294±0.244	1.443±0.252	1.531±0.181	0.417±0.061
	Oral	4	-	1.515±0.358	0.849±0.134	0.426±0.021	0.330±0.01
	Control	4	0.928±0.269	1.648±0.071	3.881±0.17	4.339±0.375	4.445±0.253
2a	External	4	-	1.572±0.333	0.781±0.027	0.53 ±0.035	-
	Oral	4	-	0.605±0.078	0.371±0.03	0.303±0.02	-
	Control	4	0.678±0.158	1.214±0.139	2.454±0.08	5.225±0.136	-
2b	External	4	-	0.371±0.103	0.133±0.02	0.115±0.022	
	Oral	4	-	0.211±0.037	0.105±0.026	0.06 ±0.015	
	Control	4	0.401±0.013	0.401±0.04	0.814±0.016	3.038±0.45	

Experiment No. 1: p values calculated by Student's t test for GSI between (a) externally-treated fish and controls, at 10 days $p<0.01$; 20 days $p<0.01$; 30 days $p<0.001$; 40 days $p<0.001$; (b) Orally-treated fish and controls, at: 10 days - NS; 20 days $p<0.002$; 30 days $p<0.001$; 40 days $p<0.001$

Experiment No. 2a: p values calculated by Student's t test for GSI between (a) externally-treated fish and controls, at: 10 days NS; 20 days $p<0.001$; 30 days $p<0.001$; (b) orally treated fish at : 10 days $p<0.01$; 20 days $p<0.001$; 30 days p 0.001

Experiment No. 2b: p values calculated by Student's t test for GSI between (a) externally-treated fish and controls, at 10 days - NS; 20 days $p<0.001$; 30 days $p<0.001$; (b) orally-treated fish at: 10 days $p<0.01$; 20 days $p<0.001$; 30 days $p<0.001$.

Details of length gravimetric analysis and GSI of individual fish are given on Appendices XI - XIII

hypertrophy and hyperplasia of the oocytes and their component cells were intensified. Atresia in the yolky oocytes started when the zona radiata lost its original contours and simultaneously the follicular cells became hypertrophied and multilayered (Plate 28(a)) Hydration of the atretic oocytes takes place, probably at this stage, as evidenced by the presence of enlarged oocytes with friable inclusions. The hypertrophied follicular cells then invaded the yolk in the oocyte (Plate 28(b)). In some sections blood cells were also found, at this stage, in the atretic oocyte. They were probably playing the same role as the follicular cells in egesting the yolk.

When the GSI of the externally and orally-treated fish showed a decline (Fig.4) the ovaries, when examined histologically revealed oocytes in advanced stages of atresia, during which little or no yolk was left in the oocyte (Plate 28(c)).

After the atretic oocytes had been resorbed from the ovary, the latter contained oogonia and protoplasmic oocytes only (Plate 28(d)). This accounted for the very low and almost non-fluctuating GSI of 0.4 and 0.3 in the 40 days externally and orally-treated fish respectively (Fig.4 and Table 18).

From Fig.4 and Table 18, it could be seen that oral treatment of the fish affected the fish more quickly than external treatment.

Some of the ovaries of the control fish when examined, revealed the presence of spent follicles as well as cells in different stages of oogenesis, indicating that some of the fish had spawned. The rest of the control females had ovaries with oogonia, protoplasmic and vitellogenetic oocytes as well as ripe oocytes.

Experiment No. 2.

Three groups of maturing male and female T.aurea, weighing between eight and 24 gm. were maintained in three 200-litre tanks at a density of 30 fish per tank. The first group was treated externally, the second orally, and the third group received Tween 80

PLATE 28

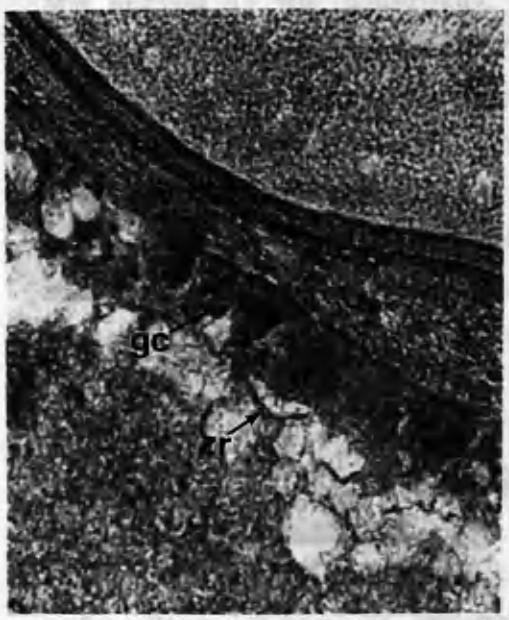
Atretic changes in gonads of Methallibure-treated T.aurea

a) T.S. of ovary of adult treated female in the early atretic stage : (i) distortion of the zona radiata (zr), and (ii) hypertrophy of the granulosa cells (gc). Stained with Azan X.620.

b) T.S. of ovary of adult treated female in subsequent atretic stage: invasion of oocyte-yolk by granulosa cells. Stained with Heindenhein's iron haematoxylin. X.620.

c) T.S. of ovary of adult treated female in latter atretic stage: ejection of most of the yolk and the presence of parenchymatous cells derived mainly from the granulosa. Stained with Azan X.620.

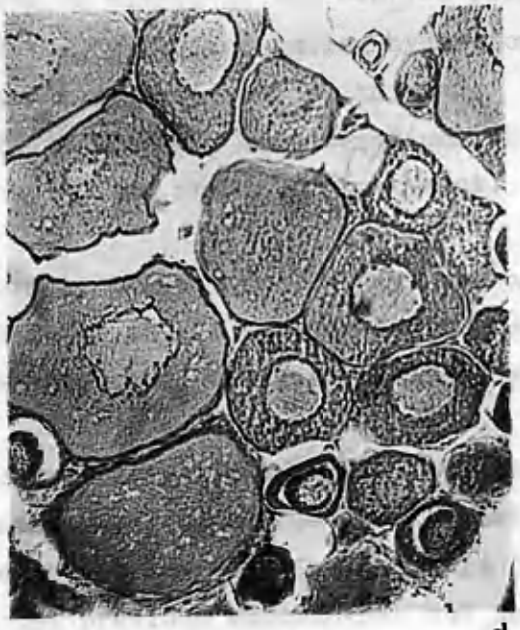
d) T.S. of ovary of adult treated female in the reconstitutional phase: presence of oogonia and protoplasmic oocytes in ovary. Stained with Azan. X.155. All fixed in Bouin's fixative.



a

b

c



d

and served as the controls.

The conditions of the present experiment, modes of treatment, dosage and sampling intervals were all the same as in the previous experiment. The only differences were in (1) the sizes of the fish and (2) the sex ratios - the previous experiment involved a monosex population of females while the present one involved a mixed population of males and females. The results of the present experiment, however, are presented separately for females and same for males.

Results.

(a) The GSI in Relation to the Histology of the Ovaries.

(i) External treatment

Fig. 5 and Table 18 show that the peak GSI was reached only after 10 days of external Methallibure treatment. This was followed by a rather sharp decline in the GSI. After a GSI of 0.7 there was a very slow decline to 0.5.

Microscopic examination of slides of the ovaries revealed hyperplasia and hypertrophy of the follicular cells, hydration of the ovaries with hypertrophied follicular cells, and an invasion of the oocyte-yolk. These findings were identical with those observed in the first group of the previous experiment.

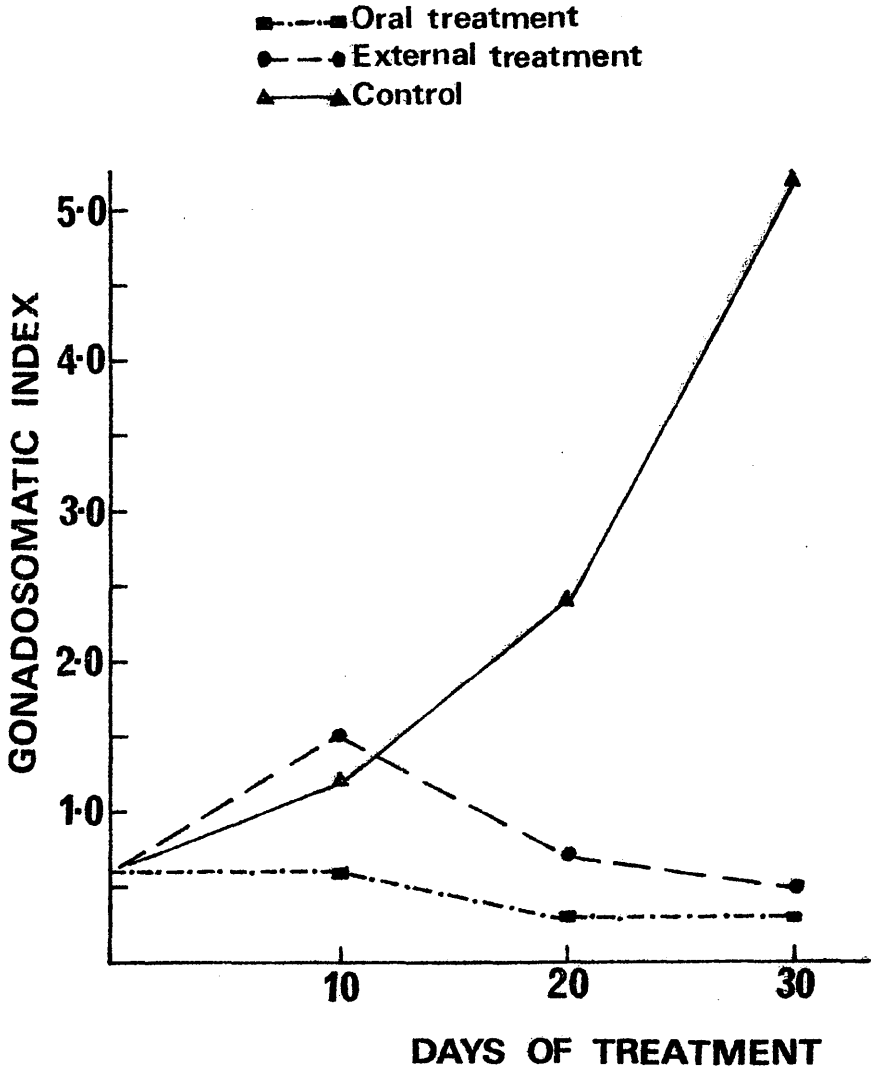
(ii) Oral treatment

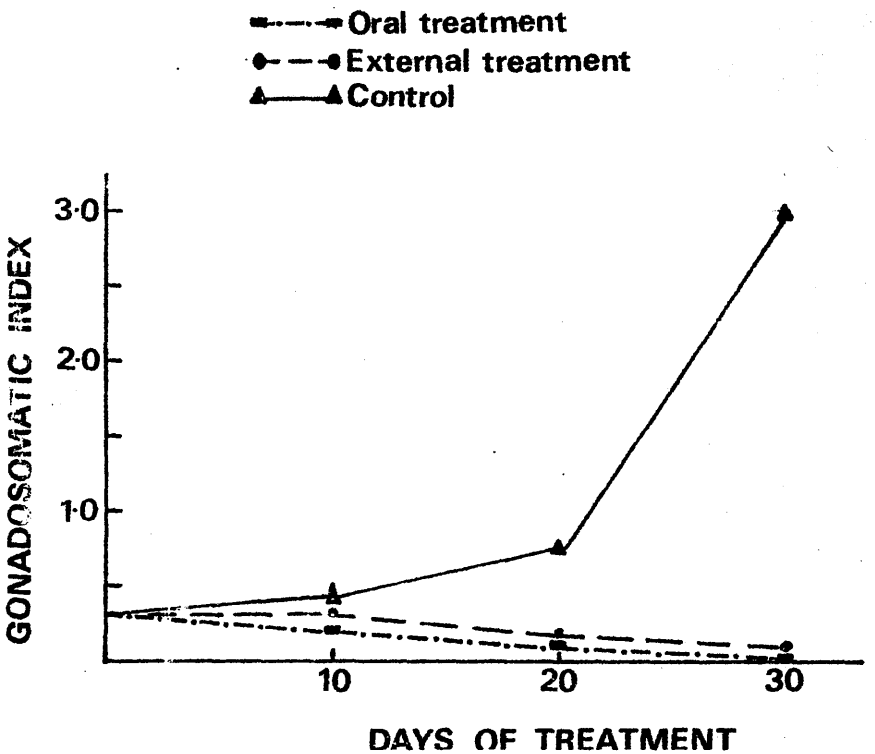
The GSI of the fish orally treated for 10 days was on the declining phase (Fig. 5 and Table 18). A further decline was observed on the 20th day of treatment and after it remained stable at 0.3

The ovary of the 10 days orally-treated fish revealed oocytes in advanced stages of atresia, i.e. complete or almost complete depletion of yolk - hence the low GSI of 0.6. This phase took a longer time to reach in the previous experiment, but the histology of the ovaries were the same. From the 20th to the 30th day only oogonia and protoplasmic oocytes were found in the

FIGURE 5

Effects of different modes of Methallibure treatment on the Gonadosomatic Index in female T. aurea


Oral treatment
External treatment
Control
GONADOSOMATIC INDEX
5·0
4·0
3·0
2·0
1·0
10
20
30
DAYS OF TREATMENT


Oral treatment
External treatment
Control
GONADOSOMATIC INDEX
3·0
2·0
1·0
10
20
30
DAYS OF TREATMENT

ovary, thus justifying the very low and stable GSI of 0.3. Histologicall and gravimetrically, the results were identical with those of the previous experiment.

(iii) Controls

A high GSI of 6.2 was reached in the control females on the 30th day. There were ripe, vitellogenetic and protoplasmic oocytes, as well as oogonia in the ovaries, when they were examined.

The results of this experiment confirmed those of the previous one that oral methallibure treatment acts quicker than external. It seems also that the response is even more rapid in small fish with yolky eggs than in large fish with yolky eggs, dose for dose.

(b) The GSI in Relation to the Histology of the Testes

(i) External treatment

The decline of the GSI in the males after 10 days of external treatment, as shown in Fig.6 and Table 18 was accompanied by a degeneration of primary and secondary spermatocytes, and spermatids in the testes (Plate 29(b)). From the 20th to the 30th day of treatment the GSI remained low, at 0.13 and 0.12 respectively and the cell types in the testes remained the same - spermatogonia and spermatozoa.

(ii) Oral treatment

Oral Methallibure treatment resulted in a consistent decrease in the GSI. On the 30th day, a very low GSI of 0.06 was reached (Fig. 6 Table 18). The testes of these fish, when examined under the microscope revealed numerous spermatogonia and spermatozoa. The histological picture of the testis was similar to that of the externally-treated fish except for the presence of rather more spermatozoa.

(iii) Controls

There was a high GSI of 3.0 in the control males (Fig 6 and Table 18). These fish were already sexually mature as evidenced by the presence of large quantities of ripe sperm and cells at all

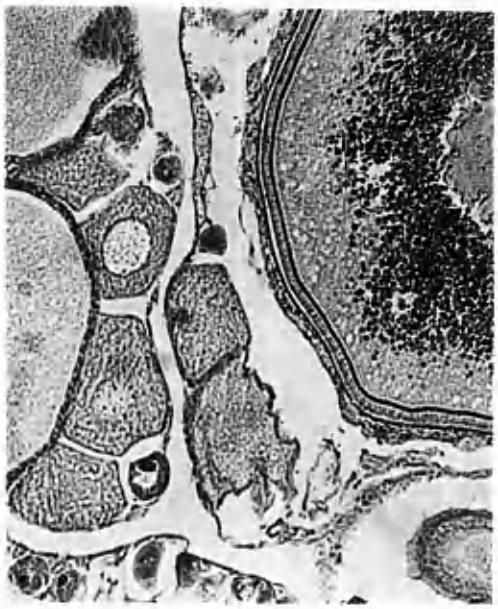
PLATE 29

Atretic stages in gonads of Methallibure-treated *T. aurea*

a) T.S. of ovary of normal female (control for Plate 28) : no evidence of atresia, fish with maturing oocytes. Stained with Azan X.155.

b) T.S. of testis of fish orally treated for 10 days with Methallibure: absence of intermediate cells (compare with Fig.C)

c) T.S. of testis of control fish: presence of cells at all stages of development. Figs b and c stained with Heindenhein's iron haematoxylin, and X.620. All fixed in Bouin's fixative.



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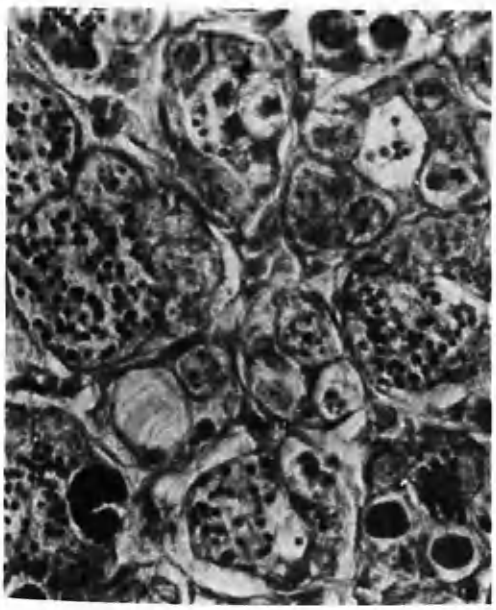

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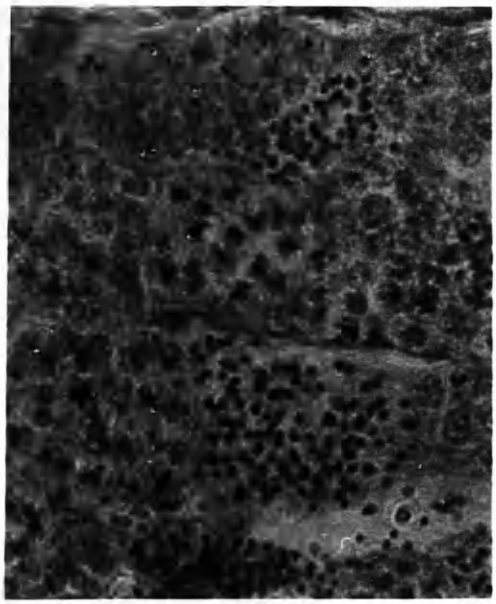


c

stages of spermatogenesis in the testis (Plate 29(c).

The results of these experiments suggest that Methallibure administered externally or orally, acts on the fish by reducing the GSI. This reduction in the GSI is accompanied by regressive changes in the gonads. The initial increase in the GSI of the females correspond with definite stages of atresia in the ovary. Oral treatment in both sexes, acts quicker on the gonads than external treatment.

Mortality

During the whole period of the experiment, only one fish died from the externally-treated group, and two from the orally-treated. Although there was no mortality in the controls, the very low mortality recorded in the experimental groups makes it unlikely that the cause of death was due to the Methallibure treatment.

The Secondary Effect of Methallibure on Growth of T.aurea in ponds

The secondary effect of Methallibure on growth is described as the difference in weight of experimental fish, previously treated with Methallibure, over controls during the period of recovery in ponds.

Experiment No. 1

Twenty five Methallibure-treated females (13 externally and 12 orally-treated) with an average weight of 92.1 gm. and measuring 13.9 - 17.7 cm. were transferred into a pond in early Summer when the temperature ranged between 26 and 31^{o}C, for recuperating after 40 days of treatment in tanks. The same number of control females with an average weight of 95.9 gm and measuring 14.1 - 18.9 cm. were transferred into another pond. Ten, untreated, ripe males were introduced into each of the ponds to facilitate spawning behaviour and spawning. Seining of the fish was planned at two weeks intervals to check for the presence of eggs in the mouths of the females and to record any weight differences.

Results

The fish were examined, for the first time, two weeks after being transferred into the ponds. At that time, spawning had been observed among the control fish as evidenced by the presence of fingerlings in the ponds. When the fish were caught some of them were found with eggs in their mouths.

These controls, upon weighing, revealed an average gain in weight of 15.1 grams per fish (Table 19).

Two weeks recovery period in the pond did not result in spawning in any of the fish previously treated with Methallibure. However, some of the females examined released yolky oocytes when the sides of the peritoneum containing the paired ovary was pressed.

These previously-treated females had gained an average weight of 45.4 grams per fish, as compared with 15.1 grams in the controls, representing an increase of 30.3 gm. per fish over the controls (Table 19).

Five days later, five females were found dead in the pond with the methallibure-treated fish. On the next day, nine dead fish were counted from the same pond, but this time, including two untreated controls. The experiment was therefore terminated. Unsuccessful attempts were made to determine the cause of death.

Experiment No. 2.

Twenty four Methallibure-treated fish (12 ♂ and 12 ♀) with an average weight of 63.8 gm in the females and 75.3 gm in the males, and measuring 13.7 - 18.4 cm and 14.9 - 18.5 cm respectively were transferred into a pond in Autumn for recovery after three months of treatment in tanks. Equal number of males and females, from the control tank were transferred into another pond.

Due to bad weather, periodic seining was impracticable. However, checks for spawnings (which was a relatively easier operation) in both the control and experimental ponds continued

every week using a mosquito net. At the end of the seventh week, the fish in both ponds were caught, checked for spawning and weighed. The temperature of the pond, during the experimental period fell from 26 - 25°C in September October to 21°C in November.

Results.

Spawning occurred in the control pond soon after the fish were transferred into it. Large numbers of fingerlings were found swimming in groups in the pond. Some females still carried incubating eggs.

The control females, during the period of seven weeks in the ponds gained an average weight of 27.2 gm per fish and measured between 16.0 and 20.5 c. while the previously Methallibure treated females gained an average of 52.2 gm in weight per fish representing an increase of 25 gm. over the controls, and measured 16.5 - 21.7 cm (Table 19). One previously treated female, however, was found with eggs in the early cleavage stage, in her mouth, indicating that spawning had just occurred.

The treated males, on the contrary, had gained less weight per fish as compared with the control males - 80.7 gm : 92.8 gm, representing an average loss of 12.1 gm over the controls (Table 19). The lengths of the experimental fish was 18.5 - 20.6 cm and that of the controls :- 20.0 - 22.5 cm.

The results of the second experiment confirms those of the first, that treatment of female T.aurea with Methallibure enhances growth of the fish in the ponds after the treatment has ceased. Spawning is also abolished during the period of treatment and the onset of maturation is delayed for at least seven weeks.

The fish were maintained in the same ponds as those of the previous experiment. No mortality was recorded during the second experiment, suggesting that the cause of death of some of the previously treated fish, was not due to the treatment.

Table 19. Secondary Effect of Methallibure on Growth in *T. aurea* in ponds

Experiment No.	Treatment	Sex	Gravimetric analysis of fish Before transferred into ponds			Gravimetric analysis of fish On termination of experiment				
			Total wt of fish (kg)	Mean wt of fish (gm)	No. of fish stocked	Mean wt of fish (gm)	Recovery Period (wks)	Mean wt increase (gm)	Wt.per fish over controls (gm)	Date
1.	External & Oral		2.282	91.2	25	(10)* 137.5	2	45.4	+ 30.3	6/6/69-20/6/69
	Control		2.398	95.9	25	(10) 111.0	2	15.1	-	"
2a	External		0.766	63.8	12	(9) 116.0	7	52.2	+ 25	25/9/69-12/11/6
	Control		1.097	91.4	12	(8) 118.7	7	27.3	-	"
2b	External		0.904	75.3	12	(12) 156.0	7	80.7	- 12.1	"
	Control		1.214	101.2	12	(11) 194.0	7	92.8	-	"

* Number in parenthesis denotes number of fish caught

The Effects of Unilateral Ovariectomy and of Methallibure treatment of Unilaterally-ovariectomized Fish on the remaining Ovary in T.aurea

Twenty female T. aurea were unilaterally ovariectomized and sampled at five day intervals after the operation (4 fish per sample). Ten other females were unilaterally ovariectomized, treated daily with Methallibure and sampled also at five day intervals (2 fish per sample). Methallibure treatment started on the same day, soon after the operation. Another ten fish were sham operated, also sampled at five day intervals (2 fish per sample) and served as the controls for the unilaterally ovariectomized fish, while the latter, in turn served as the controls for the hemicastrated methallibure-treated fish.

The increase in weight of the remaining ovary over that of the contralateral counterpart removed at the time of the operation roughly indicates the compensatory hypertrophy. The weight of one-half of the combined weight of both ovaries of sham-operated controls minus the weight of the ovary removed at operation from the hemicastrated group, may falsely indicate the periodical ovarian enlargement from the operation date to the sampling day. Further, the percentage weight gain or loss over the initial controls are indicated. For the sham-operated controls, the percentages are calculated upon the initial weight of the ovaries removed at the time of the operation from the hemicastrated fish.

Results

Histological examination showed that the ovary removed at the time of the operation contained oogonia and protoplasmic oocytes. The largest oocyte found had a diameter of 265 - 283 μ and a nuclear diameter of 90 - 100 μ (Plate 30(a)) and the ovary measured 1665 - 1850 across its widest area.

Only five days after the operation, there was a compensatory hypertrophy of 63% of the remaining ovary over that of the ovary

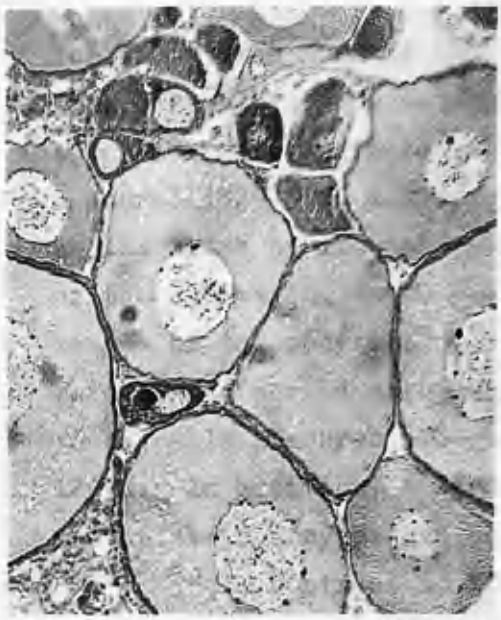
PLATE 30

Effects of unilateral ovariectomy on the remaining ovary in T. aurea : (i) Compensatory hypertrophy.

a) T.S. of ovary removed at the time of operation; showing protoplasmic oocytes. Stained with Heindenhein's iron haematoxylin.

b) T.S. of ovary of sham operated control female five days after operation; showing larger, but still protoplasmic oocytes. Stained with Heindenhein's iron haematoxylin.

c) T.S. of ovary of remaining contralateral counterpart five days after operation: showing vitellogenetic and numerous protoplasmic oocytes. Stained with Azan. All fixed in Bouin's fixative, and X.155.



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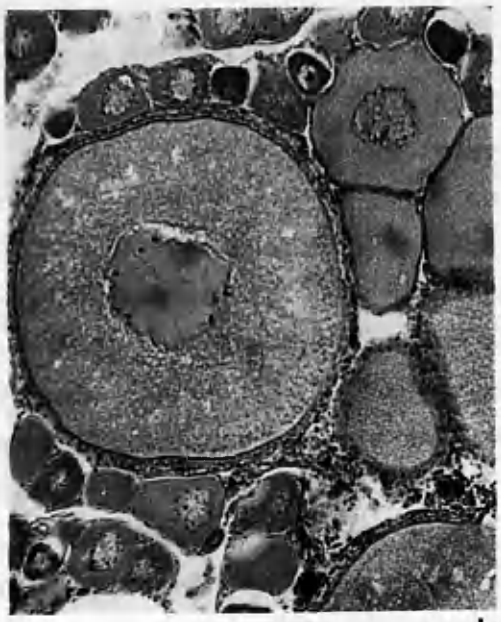

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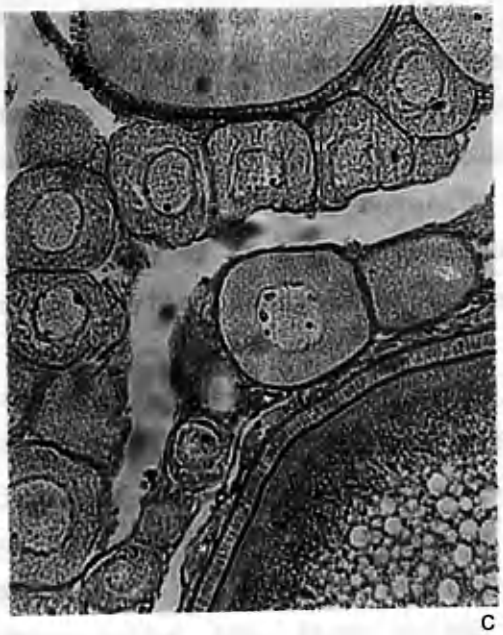


c

removed at the time of the operation (Fig. 7 and Table 20). This compensatory hypertrophy was accompanied, first of all, by a considerable increase in the volume of the remaining ovary which measured 3325-3350 μ along the width almost exactly twice as much as the width of its contralateral counterpart removed at the time of the operation. There was a difference also in the developmental stages of the oocytes. A few oocytes were found which had already entered the period of vitellogenesis (Plate 30(c). The largest of these cells measured 616-650 μ and the nuclear diamter was 100 - 120 μ. In addition, a large number of smaller oogonial and protoplasmic oocytes had appeared in the ovary (Plate 30[c]).

In contrast to the sham-operated controls, mitotic divisions in the oogonia of the ovary of the partially castrated fish were very common, indicating the recruitment of new oocytes from oogonial stocks (Plate 31(a)). Some of the protoplasmic oocytes also revealed nuclear polymorphism.

In the hemicastrated, methallibure-treated fish, five days post operation resulted in a compensatory hypertrophy of 80% of the remaining ovary (Fig. 7 and Table 20). Histological picture of the slides of the ovary revealed conditions similar to that found in the ovary of five days hemicastrated fish - presence of vitellogenetic oocytes, numerous protoplasmic oocytes and oogonia.

In the sham-operated controls, an increase of 7% was realized in the one-half of the combined weight of both ovaries. Microscopic slides of the ovaries did not reveal any peculiarities which could be associated with compensatory hypertrophy, although oocytes measuring 300 - 333 μ with a nuclear diameter of 98 - 102 μ were found. These cells, like those in the ovary removed at the time of the operation, were in the period of protoplasmic growth (Plate 30(b)). Increase in the size of the oocytes and the 7%

PLATE 31

Effects of unilateral ovariectomy on the remaining ovary in T.aurea (ii) functional mechanisms of remaining hypertrophy.

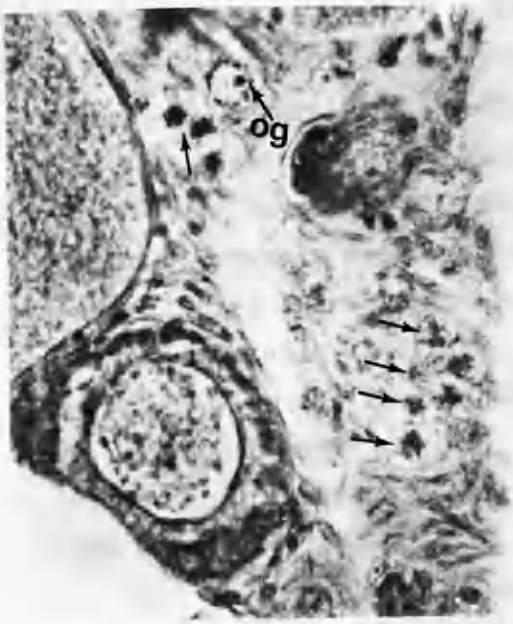
T.S. of remaining ovary five days after operation: showing

a) oogonia (og) and substantial number of dividing oogonia (arrows). Stained with Heidenhein's iron haematoxylin.X.620.

b) nuclear polymorphism in protoplasmic oocyte. Stained with Azan. X.310.

c) mitotic division in granulosa cell (arrow). Stained with Heidenhein's iron haematoxylin. X.620.

d) the hypertrophied ovarian cell-wall: zona radiata (zr) with minute radial canals; cells of the zona granulosa (zg) double-layered; and cells of the connective tissue zone (ctz) or theca also double-layered, forming theca interna and theca externa. Stained with Azan. All Bouin fixation. X.1550



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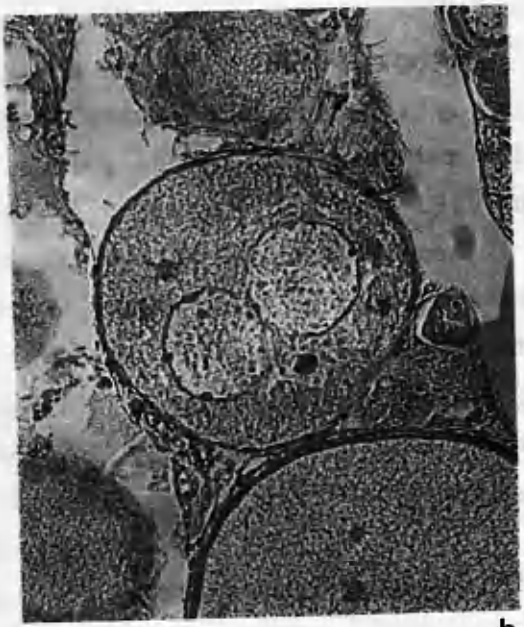

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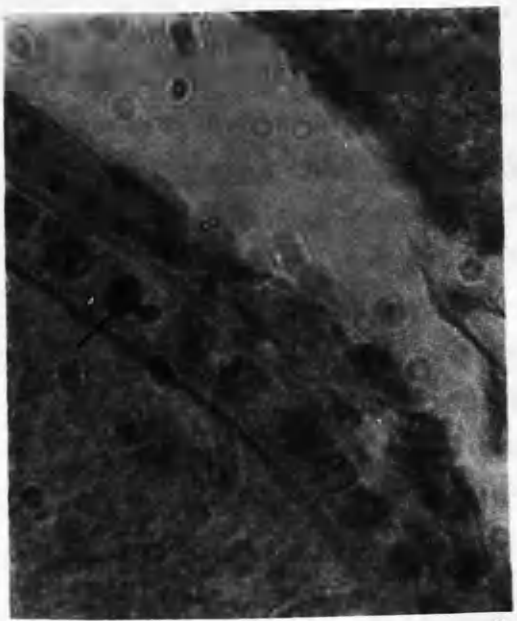

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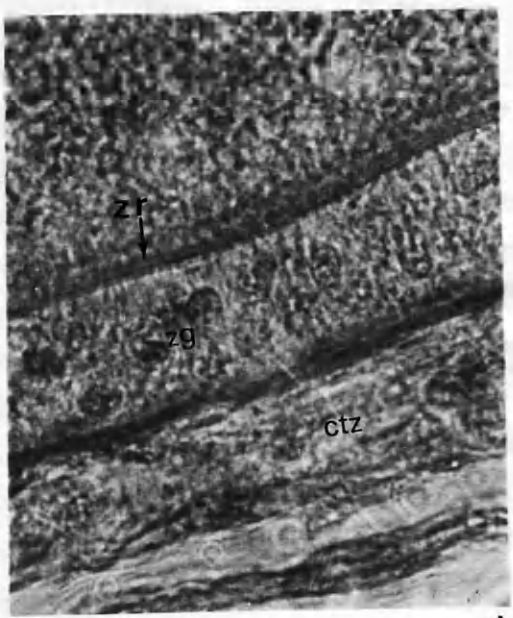


d

increase in weight of the ovary, as shown in Table 20 , could therefore be merely the periodical ovarian enlargement.

Subsequent samplings at 10, 15 and 20 days after unilateral ovariectomy revealed compensatory hypertrophy of 146%, 194% and 270% respectively over the initial ovarian weights of the contralateral counterparts removed at the time of the operation (Fig. 7 and Table 20). Finally, on the 25th day after the operation, which was also the last sampling day, a compensatory hypertrophy of 566% was observed (Fig. 7 and Table 20). Histological examination of the ovaries revealed large vitellogenetic oocytes measuring 1450 - 1500μ with a nuclear diameter of 135 - 144μ.

The ovarian cell wall of vitellogenetic oocytes of the hemicastrated fish, unlike those of the sham-operated controls, usually revealed a hypertrophied zona radiata with minute radial canals and a double layer of hypertrophied granulosa cells (Plate 31(d)). Mitotic divisions in the granulosa cells are fairly common, indicating their hyperactivity (Plate 31(c)). The connective tissue cells (the theca) was also double-layered - theca interna and theca externa (Plate 31(d)).

In the remaining ovary of the unilaterally ovariectomized Methallibure-treated fish, there was a compensatory hypertrophy of 150%, ten days after the operation (Fig. 7 and Table 20). The histology of the ovary, however, did not show the compensatory hypertrophy described previously, in the remaining ovary of the hemicastrated fish. The ovarian hypertrophy, in this case, was in connection with atresia. Beginning with day 15 after the operation, methallibure inhibited the compensatory hypertrophy of the remaining ovary, as evidenced by a loss in weight of 55% over the initial weight of the ovary removed at the time of the operation. This loss in weight continued to the 25th day after operation (the last sampling day) when a decrease of 94% by weight

FIGURE 7

Effects of unilateral ovariectomy and of Methallibure treatment of unilaterally ovariectomized T. aurea on the remaining ovary.

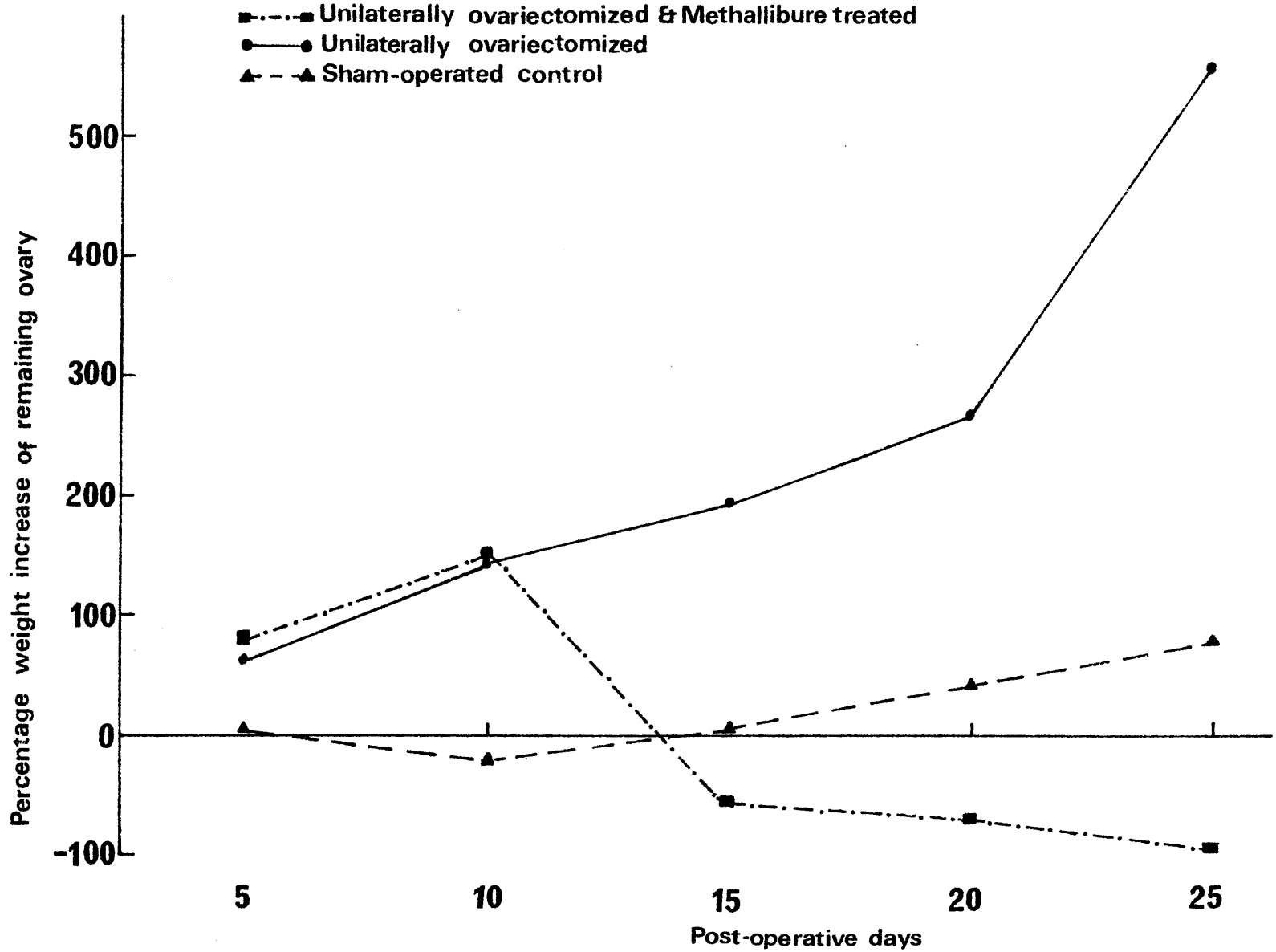

Unilaterally ovariectomized & Methallibure treated
Unilaterally ovariectomized
Sham-operated control
Percentage weight increase of remaining ovary
500
400
300
200
100
0
-100
5
10
15
20
25
Post-operative days

Table 20. Effects of Unilateral ovariectomy, and of methallibure treatment on the remaining ovary in T. aurea.

Period (days)	Mean wt of fish (gm)	Mean length of fish (cm)	Mean initial wt.of ovary ± SE (gm)	Mean final wt. of ovary* ± SE (gm)	Mean wt.gain/loss by remaining ovary ± SE (gm)	% wt.gain/loss over initial ovarian wt ± SE
-	44.8[a]	14.2 (4)**	0.734±0.055	1.181±0.016	0.447±0.058	63±11
5	31.6[b]	11.9 (2)	0.414±0.093	0.568±0.125	0.154±0.030	80±0
	68.6[c]	15.4 (2)	-	0.788±0.089	0.054	7-
	41.7	13.8 (4)	0.620±0.015	1.528±0.277	0.908±0.157	146±25
10	60.5	15.4 (2)	0.644±0.706	1.852±1.999	0.708±0.586	150±73
	52.3	15.0 (2)	-	0.501±0.036	-0.119-	-19
	52.6	14.9 (4)	0.642±0.118	1.765±0.025	1.123±0.114	194±50
15	51.5	13.7 (2)	0.738±0.273	0.359±0.111	-0.379±0.161	-51±3
	49.0	14.7 (2)	-	0.668±0.061	-0.026	4-
	56.7	16.1 (4)	0.478±0.155	2.199±0.254	1.721±0.135	270±31
20	54.5	15.1 (2)	0.794±0.014	0.247±0.114	-0.547±0.131	-69±15
	54.8	15.4 (2)	-	0.678±0.035	0.200	41
	58.5	15.4 (4)	0.432±0.041	2.877±0.113	2.446±0.105	580±68
25	48.2	13.8 (2)	0.758±0.194	0.045±0.028	-0.711±0.164	-94±3
	62.8	16.0 (2)	-	0.780±0.022	0.348	80

a - Unilaterally ovariectomized fish
b - Unilaterally ovariectomized and methallibure treated fish
c - Sham-operated controls.
* Mean final weight of ovary of controls represents the mean of the weight of one half of the combined weight of both ovaries, minus the mean initial weight of the ovary of the unilaterally-gonadectomized fish.

** Number of fish in parenthesis

p values calculated by Student's t test between unilaterally ovariectomized fish and unilaterally-ovariectomized, methallibure treated fish on : day 5 at p - NS; day 10 p - NS; day 15 $p < 0.1$(NS); day 20 $p < 0.01$; day 25 $p < 0.01$
Gonadal weights of individual fish are given in Appendices XIV-XVI

was recorded (Fig 7 and Table 20). When the ovaries were examined histologically, oocytes in advanced stages of atresia, typical of advanced atretic oocytes resulting from Methallibure treatment of normal fish were evidenced (see Plates 24 and 28).

The sham-operated controls revealed a weight loss of 19% ten days after the operation. However, a 4% increase which gradually increased to 80% was realized from the 15th to the 25th day after the operation (Fig. 7 and Table 20). Vitellogenetic oocytes measuring 580 - 600 μ with a nuclear diameter of 118 - 125 μ were found when the ovary was examined histologically. The weight gain in the ovary coupled with the presence of vitellogenetic oocytes in the ovary of the sham-operated fish is possibly, the normal periodical ovarian enlargement and development.

The Effects of Exogenous Gonadotrophins on the Gonads and Pituitary of Methallibure-treated T. mossambica

Four different gonadotrophic extracts were tested. They were all prepared commercially from mammalian sources. The four preparations were as follows :

(i) Follicle Stimulating Hormone or FSH (Armour)

(ii) Luteinizing Hormone or LH (Armour)

(iii) Human Chorionic Gonadotrophin or HCG (Organon)

Iiv) Pregnant Mare Serum or PMS (Organon)

The hormones were injected intraperitoneally on alternate days,. The individual doses of FSH and LH were 0.25 mg per fish. Those of HCG and PMS were 12.5 I.U and 10.0 I.U. respectively. When FSH and LH were used in combination, the two hormones were mixed in equal volumes before the equivalent of 0.25 mg was extracted for injection.

The fish used for these investigations were those used for the study of the effects of methallibure on growth and survival (see page 104.), and had therefore been treated for 35 days with methallibure. Injections started on the day after the previous experiment terminated.

Histological examination of the gonads and pituitary during the period of treatment revealed regressive changes similar to those described earlier as a result of methallibure treatment (see pp.105-9). The ovaries, therefore, on the commencement of the replacement therapy were in Stage II - presence of oogonia and protoplasmic oocytes. The testes were in Stage I - presence of spermatogonia. The meso-adenohypophysis of the pituitary did not show any basophilia.

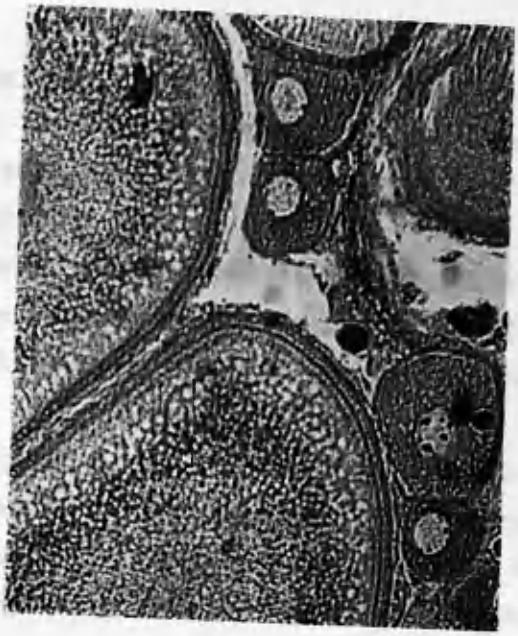
Seventy of the Methallibure treated fish were subjected to various hormonal treatments (see Tables 22 and 24) while the remaining 20 fish served as controls. The latter received injections of 0.1 ml. of saline, also on alternate days until the end of the experiment.

PLATE 32

Effects of exogenous gonadotrophins on maturation in Methallibure-treated T.mossambica

a) T.S. of ovary of Methallibure-treated female, injected for 30 alternate days with FSH: presence of oocytes in early vitellogenetic stage.

b) T.S. of ovary of Methallibure-treated female, injected for 30 alternate days with saline (control): oocytes still in the protoplasmic stage. All stained with Azan, Bouin fixative, and X.155



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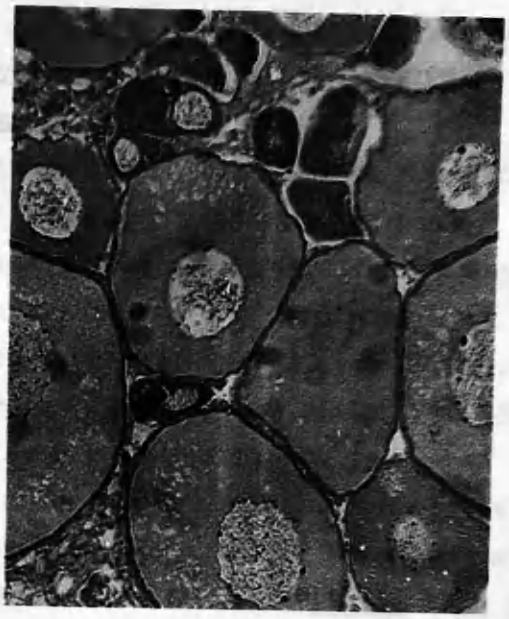


b

Results

Effects on Secondary Sex Characters and Behaviour.

On the fourth day of treatment, i.e. after two injections secondary sexual characters appeared on the males receiving injections of HCG, PMS and a combination of FSH and LH. These characters were less noticeable in the LH-treated fish until about the sixth-seventh day of treatment. Meanwhile, the HCG, PMS and FSH + LH-treated fish had built nests and were defending their territories.

Two of the LH-treated fish developed full secondary sexual characters on the sixth day of treatment and began to build nests. By this time, only one male from the FSH-injected group showed the secondary sex characters and spawning behaviour. From the seventh to the last day of treatment (30th day), the rest of the males receiving FSH and LH singly, showed varying degrees of the secondary sex characters which were never as intense as in those receiving HCG, PMS and a combination of FSH and LH.

The Pituitary Gonadotrophins, FSH and LH

(a) Effects on the Ovaries.

It is evident from Table 21 that FSH alone produced varying stimulatory effects on the gonadal tissues. Three of the eight females injected with this hormone for 30 days had ovaries in Stage IV. The ovaries of the remaining fish were less stimulated by FSH however, they had ovaries at an advanced stage as compared with the controls (Table 21 and Plate 32). Gravimetric data on the ovaries also revealed slightly increased ovarian weights in FSH-treated fish over Saline-treated controls (Table 22).

Luteinizing hormone was even less stimulating to the ovaries of methallibure-treated Tilapia than FSH as only two females matured out of eight (Table 21). There was, however, a greater increase in ovarian weights as compared with those of FSH and saline-treated fish (Table 22).

Table 21. Histological condition of ovaries of Methallibure-treated T.mossambica treated with Follicle Stimulating Hormone (FSH) Luteinizing Hormone (LH), Human Chorionic Gonadotrophin (HCG) and Pregnant Mare Serum (PMS)

Treatment	No. of Fish	Fish in different stages of stimulation						% of fish with ripe ovaries
		Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	
FSH	8	-	-	5	3	-	-	37
LH	8	-	-	6	2	-	-	25
FSH & LH	5	-	-	-	5	-	-	100
HCG	7	-	-	-	5	-	2	100*
PMS	7	-	-	2	5	-	-	71
Controls	10	-	10	-	-	-	-	0

* Including two spawned fish

Table 22. Effect of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Human Chorionic Gonadotrophin (HCG) and Pregnant Mare Serum (PMS) on the Ovaries of Methallibure treated T.mossambica

Group No.	Mean body weight (gm)	Treatment for 30 alternate days FSH (mg)	LH (mg)	HCG (I.U)	PMS (I.U)	Mean GSI* ± SE
1	7.2	0.25	0	0	0	3.563 ± 0.902 (8)
2	9.7	0	0.25	0	0	3.866 ± 0.613 (8)
3	8.0	0.125	0.125	0	0	4.554 ± 1.037 (5)
4	8.4	0	0	12.5	0	5.100 ± 1.127 (7)
5	7.9	0	0	0	10.0	4.930 ± 0.893 (7)
6	4.9	0	0	0	0**	0.286 ± 0.049 (10)

* GSI = $\frac{\text{Wt of testis}}{\text{Wt.of fish}}$ X 100. Figures in parenthesis indicate number of fish

** Control group

p values calculated by Student's t test for GSI between groups: 1 and 6 $p < 0.001$; 2 and 6 $p < 0.001$; 3 and 6 $p < 0.001$; 4 and 6 $p < 0.001$; 5 and 6 $p < 0.001$.

The combination of FSH and LH gave some of the best results of the whole of the replacement therapy experiments by consistently stimulating all the ovaries to full maturity (Table 21). The GSI were equally increased (Table 22).

(b) Effects on the Testes.

There was very little stimulation of the testes as a result of FSH treatment. Only one of the eight treated fish reached Stage IV (Table 23). The gonads of five others were only slightly stimulated or not stimulated at all, as evidenced by the presence of control fish in the same stage of maturation (Stage III). The testes of the remaining two fish were probably not stimulated at all, and were in Stage II. On the average, the FSH-treated fish gained more testicular weights than the controls, as judged by the high GSI (Table 24).

Luteinizing hormone did not stimulate the testes to any great extent. Two of the eight LH-treated fish had ripe sperm when their testes were examined histologically. All the remaining fish were in Stage III (Table 23). There was an increased mean GSI over FSH and saline-treated fish (Table 24).

When used in combination, FSH and LH were very efficient in stimulating the testes of all the treated fish to maturity (Table 23). Large quantities of ripe sperm were found in the sperm duct and numerous ampoules were packed with spermatozoa (Plate 33(a)). The GSI of the males in this group was the highest recorded in the experiment (Table 24).

The testes of the saline-treated controls were not stimulated (Plate 33(b)).

The Chorionic Gonadotrophins, HCG and PMS

(a) Effects on the Ovaries.

The data in Table 21 show that HCG was the most effective of all the hormones tested, in stimulating the ovaries. One female

Table 23. Histological condition of testes of methallibure-treated T.mossambica treated with Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Human Chorionic Gonadotrophin (HCG) and Pregnant Mare Serum (PMS)

Treatment	No.of Fish	Fish in different stages of stimulation						% of fish with tipe testes
		Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	
FSH	8	-	2	5	1	-	-	12
LH	8	-	-	6	2	-	-	25
FSH + LH	5	-	-	-	5	-	-	100
HCG	7	-	-	-	5	-	2	100*
PMS	7	-	-	1	6	-	-	85
Controls	10	-	4	6	-	-	-	0

* Including two fish with post-spawning testes.

Table 24. Effect of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Human Chorionic Gonadotrophin (HCG) and Pregnant Mare Serum (PMS) on the Testes of Methallibure treated T.mossambica

Group No.	Mean body weight (gm)	Treatment for 30 alternate days FSH (mg)	LH (mg)	HCG (I.U)	PMS (I.U)	Mean GSI*±SE
1	7.6	0.25	0	0	0	$0.222^{(8)}_{\pm}0.923$
2	10.0	0.	0.25	0	0	$0.321^{(8)}_{\pm}0.064$
3	9.1	0.125	0.125	0	0	$0.511^{(5)}_{\pm}0.050$
4	11.2	0	0	12.5	0	$0.449^{(7)}_{\pm}0.057$
5	9.6	0	0	0	10.0	$0.493^{(7)}_{\pm}0.096$
6	6.1	0	0	0	0**	$0.102^{(10)}_{\pm}0.039$

* GSI = $\frac{\text{Wt. of testis}}{\text{Wt. of fish}}$ x 100. Figures in parenthesis indicate number of fish.

** Control group.

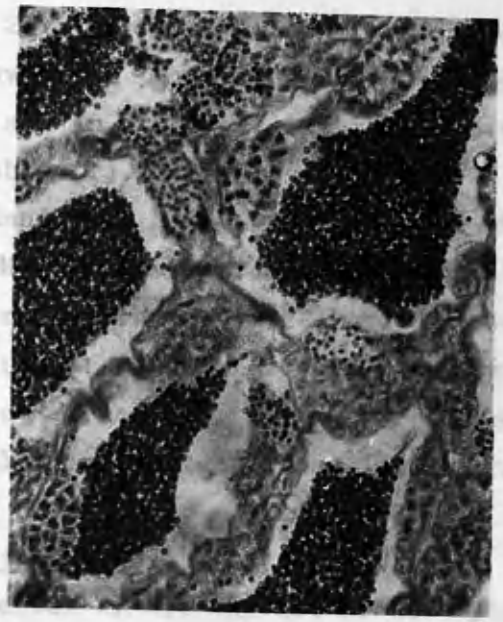
p values calculated by Student's t test for GSI between groups: 1 and 6 NS ; 2 and 6 $p < 0.01$; 3 and 6 $p < 0.001$; 4 and 6 $p < 0.001$; 5 and 6 $p < 0.001$

PLATE 33

Effects of exogenous gonadotrophins on maturation in Methallibure-treated T.mossambica

a) T.S. of testis of Methallibure-treated male, injected for 30 alternate days with a combination of FSH and LH: mature testis packed with ripe spermatozoa. X.310.

b) T.S. of testis of Methallibure-treated male, injected for 30 alternate days with saline (control): maturing testis still without spermatozoa X.620. All stained with Heidenhein's iron haematoxylin and Bouin fixation.



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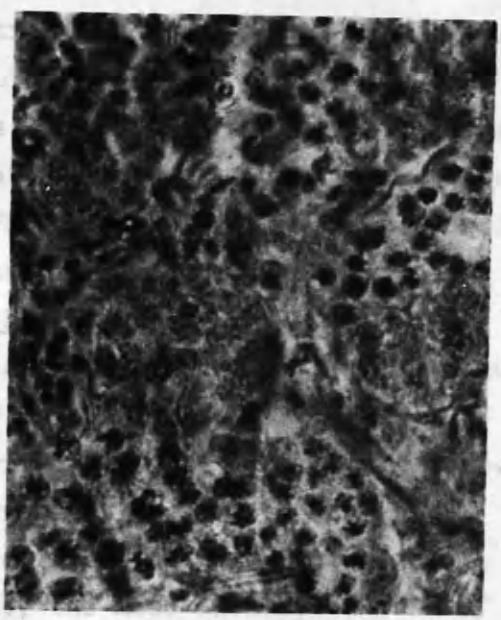


b

spawned on the 28th day of treatment i.e. after 14 injections, and another one spawned on the 30th day. The ovaries of the remaining five fish were all in Stage IV. The HCG treated fish also had the highest GSI (Table 22).

Pregnant Mare Serum was also very effective in stimulating the ovaries of Methallibure-treated Tilapia. Five of the seven fish treated were mature (Stage IV) and the remaining two had ovaries in the period of vitellogenesis (Stage III). The GSI was also high (Tables 21 and 22). The control fish receiving saline only had protoplasmic oocytes (Stage II) and a low GSI (Tables 21 and 22).

(b) Effects on the Testes.

The testes of all the HCG-treated fish were strongly stimulated (Table 23). Microscopic examination of slides of the testes revealed empty lobules among cells at all stages of spermatogenesis in two of the seven fish treated. The latter observations are characteristics of testis in the post-spawning condition. The GSI was also high(Table 24).

Tables 23 and 24 reveal that PMS stimulated spermatogenesis and brought about high GSI in Methallibure-treated fish. Eighty percent of the PMS-treated fish had testes in Stage IV. The remaining fish had testis in Stage III.

(c) Effects on the Pituitary

The basic staining affinity of the gonadotrophic cells of the pituitary which was lost as a result of Methallibure treatment was reinstated with intraperitoneal injections. All the hormones used gave positive results.

In those fish that had reached Stage IV at the end of the experiment, the meso-adenohypophysis of their pituitaries was dominated by strongly-staining, granulated basophil cells. Degranulation in some of the cells had occurred confirming that the fish were in the spawning phase (Plate 34(b)).

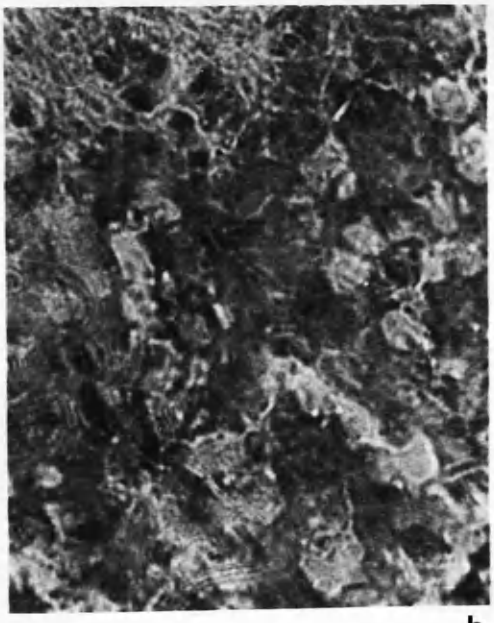
PLATE 34

Effects of exogenous gonadotrophins on the pituitary of Methallibure-treated T.mossambica

a) Sagittal section of pituitary of Methallibure-treated male, injected for 30 alternate days with FSH: absence of basophil cells; nuclei of chromophobic cells in meso-adenohypophysis inconspicuous; cells without distinct cytoplasm. The testis of the fish was in the immature phase.

b) Sagittal section of pituitary of Methallibure-treated male, injected for 30 alternative days with a combination of FSH and LH: presence of numerous granulated basophils in meso-adenohypophysis, some basophils are degranulated. The testis of the fish was in the spawning phase. All stained with Azan, Bouin fixation, and X.620.

a



b

The staining intensity of the basophils of the pituitaries of fish in Stage III was comparatively less, and much less was that of the pituitaries of fish in Stage II (Plate 34(a)). In this way, there was a gradual increase in the basophilia of the gonadotrophic cells of the pituitary of Methallibure-treated fish as a result of injection of exogenous gonadotrophins. The treated fish at different stages of maturation showed different affinities not only for basic dyes, but also for histochemical tests. The PAS method, adopted for testing the glycoproteinaceous nature of the basophilic secretion, although proving positive in all cases, showed some degrees of varying intensities of the reaction.

Table 25. Effect of Saline on oogenesis in Methallibure-treated T.aurea.

Wt.of fish (gm)	Length of fish (cm)	Mean size of advanced oocytes (μ)	Stage of maturation
8.0	8.0	549	III
18.0	10.3	400	II
15.1	9.8	466	II - III
10.0	9.0	516	III
10.8	9.2	483	III
13.8	9.5	455	II - III

Table 26 Effect of Carp pituitary on oogenesis in Methallibure-treated T.aurea

Wt of fish (gm)	Length of fish (cm)	Mean size of advanced oocytes (μ)	Stage of maturation
10.0	8.5	533	III
9.8	8.5	433	II
11.1	8.9	400	II
10.5	8.6	450	II
9.5	8.2	521	III
12.1	9.5	481	II - III

The Effects of Carp pituitary and Testosterone propionate on the Secondary Sex characters and Gonads of Methallibure-treated Tilapia

Thirty six male and female T. aurea, treated for 40 days with Methallibure, were divided equally into three groups and injected with different hormones. The first group was injected with 0.25 mg. of carp pituitary, the second group received 0.42 mg. of testosterone propionate, while the third group was injected with 0.1 ml. of 0.75% saline, and served as the control. Injections were given six times per week for 30 days.

Results.

(i) Controls: (a) Secondary Sex Characters.

The greenish-blue coloration of the upper part of the body which disappeared as a result of Methallibure treatment did not re-appear in the control males after 30 days of saline-treatment. However, slight red coloration of the borders of the fins was evident in the males. It was also possible to differentiate the sex according to the genital papilla, even though the latter, at this stage, was not greatly developed.

(b) The Ovary

With the exception of one fish, the ovaries of all the controls, receiving injections of saline were in the early stage of vitellogenesis (Plate 35(a) and Table 25). Oocytes measuring 455-549 μ with a nuclear diameter of 100-167 μ were observed in the ovaries. In these fish, a mean GSI of 0.9 was recorded (Table 28).

(c) The Testis

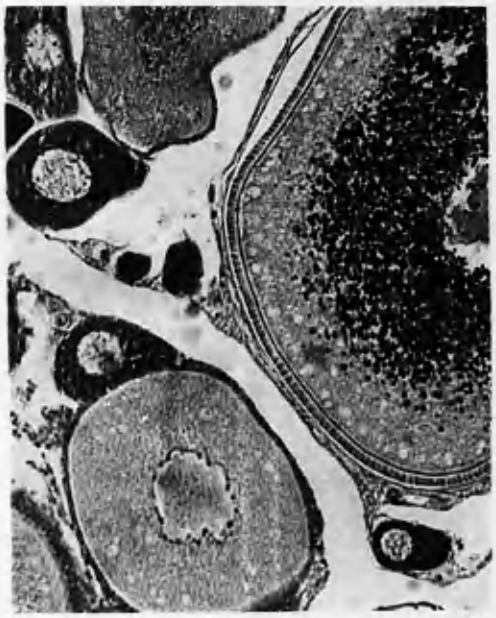
Intensive spermatogenesis was observed in the testis of the saline-treated controls which had been treated previously with Methallibure. As a result, spermatogonia, spermatocytes and spermatids were present in the testis (Plate 35(b), Fig 8 and Table 28). Spermatozoa however, had not been formed, but the active spermatogenetic state of the testis left no doubt that spermiogenesis would soon have ensued. There was a mean GSI of 0.6 in these control males (Table 29).

PLATE 35

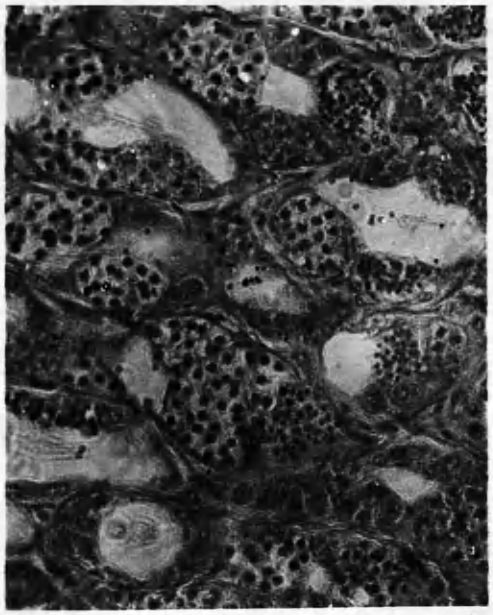
Effects of extracts of carp pituitary (CP) and testosterone propionate (TP) on maturation in Methallibure-treated T. aurea

a) T.S. of ovary of Methallibure-treated control female, injected for 30 days with saline: presence of vitellogenetic oocyte. X.155.

b) T.S. of testis of Methallibure-treated control female, injected for 30 days with saline: presence of all germinal cell types except spermatozoa. X.310. All stained with Heidenhein's iron haematoxylin and Bouin fixation.



a



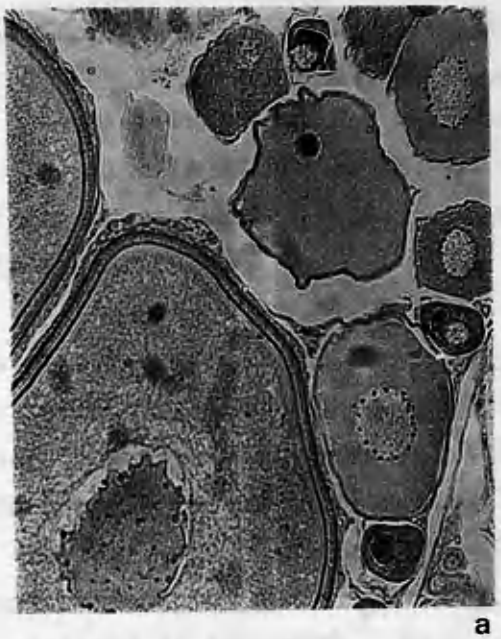
b

PLATE 36

Effects of carp pituitary (CP) on maturation in Methallibure-treated T.aurea.

a) T.S. of ovary of Methallibure-treated female, injected for 30 days with CP: advanced cells in transformation phase into vitellogenetic growth. Stained with Azan X.155.

b) T.S. of testis of Methallibure-treated male, injected for 30 days with CP: retardation of spermatogenesis (compare with controls in Plate 35, Fig.(b)). Stained with Heidenhein's iron haematoxylin. X.310. All fixed with Bouin's fixative.



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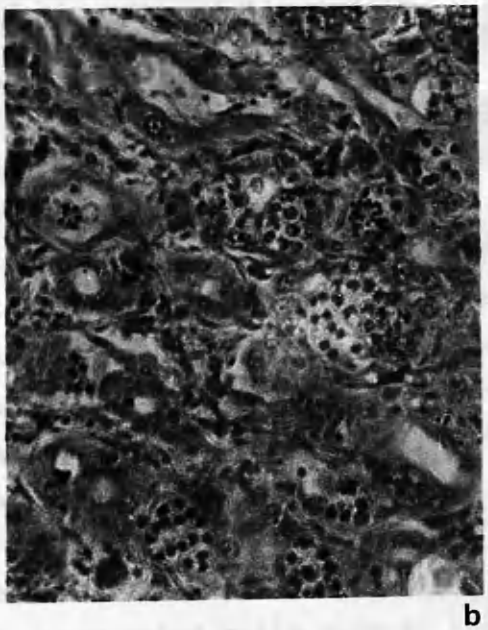


b

(ii) Carp pituitary (CP): (a) Secondary Sex Characters

There was no re-instatement of the secondary sex characters as a result of CP-administration. Sex differentiation was difficult among individuals of the group, and even the genital papilla, except for those females with ovaries in Stage III (Table 26), was still hardly discernible.

(b) The Ovary

Administration of CP into female T.aurea, previously treated with Methallibure, did not have any stimulatory effect on the ovary. After 30 days of treatment only three of the six females had ovaries with oocytes in the initial stage of the period of vitellogenetic growth (Plate 36(a) and Table 26). However large oocytes measuring between 400 and 533 μ with a nuclear diameter of 133-160 μ were observed in the ovary. As compared with the controls (Plate 35(a) and Table 25) there was less response in the CP-treated fish. The mean GSI of 0.6 in the treated females was also less than that in the controls (Table 27).

(c) The Testis

After 30 days of CP-treatment, the testis of the males presented a most intriguing picture. The Sertoli cells, occupying the cysts which they once shared with the germinal cells, and being one of the cells not affected by Methallibure treatment, were found in a hyperfunctional condition. They were encountered in large numbers in each testicular lobule and also in the sperm duct, and were enveloped within a thin sheet of connective tissue, forming a cyst. These large cysts containing Sertoli cells, found in the testis of CP-treated fish, were scattered in all parts of the testicular tissue, except the periphery (Plate 37(a)). The usually inconspicuous cell membrane becomes even more difficult to define, as the cytoplasm of the aggregated cells seem to fuse with each other, leaving distinctly, their nuclei, which measure 4-6 μ. However, in the cells which were lying singly in the testicular lobule, the cell

Table 27. Effect of Carp pituitary (CP), and Trestosterone propionate on the ovary of Methallibure-treated T.aurea

Group	Mean body weight (gm)	Treatment for 30 days CP (mg)	TP (mg)	S* (ml)	Mean GSI±SE
1	(6)** 10.5	0.25	0	0	0.658±0.018
2	(6) 14.9	0	0.42	0	0.468±0.018
3	(6)a 12.6	0	0	0.1	0.911±0.039

* Saline (treatment for controls)

** Number of fish in parenthesis

a. Controls

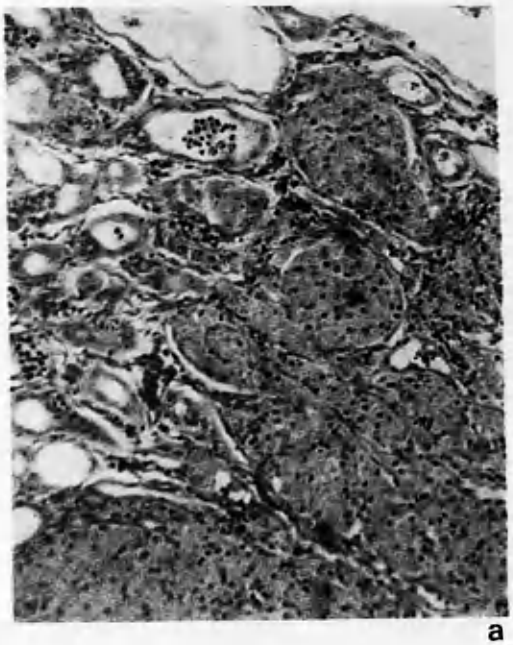
p values calculated by Students's t test for GSI between groups: 1 and 3 $p < 0.001$; 2 and 3 $p < 0.001$

PLATE 37

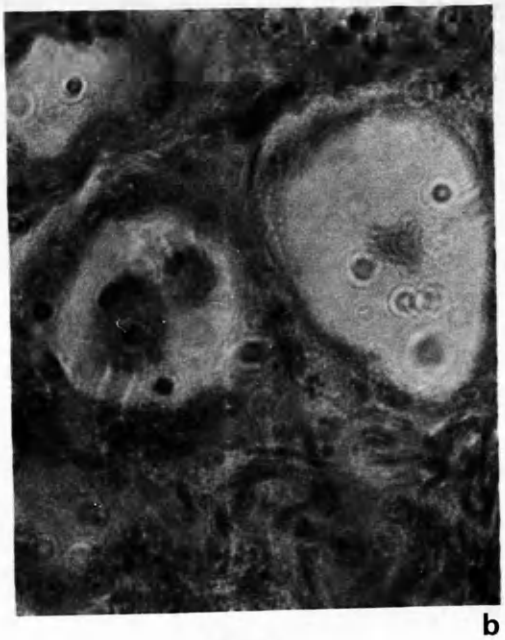
Detrimental effect of Carp pituitary on the testis of Methalliburе-treated T.aurea

a) T.S. of testis of Methalliburе-treated male, injected for 30 days with CP: presence of cysts containing Sertoli cells. X.155.

b) Two magnified Sertoli cells: oval-shaped with nucleus at one end of cell. X.620. All stained with Heindenhein's iron haematoxylin and Bouin fixation.



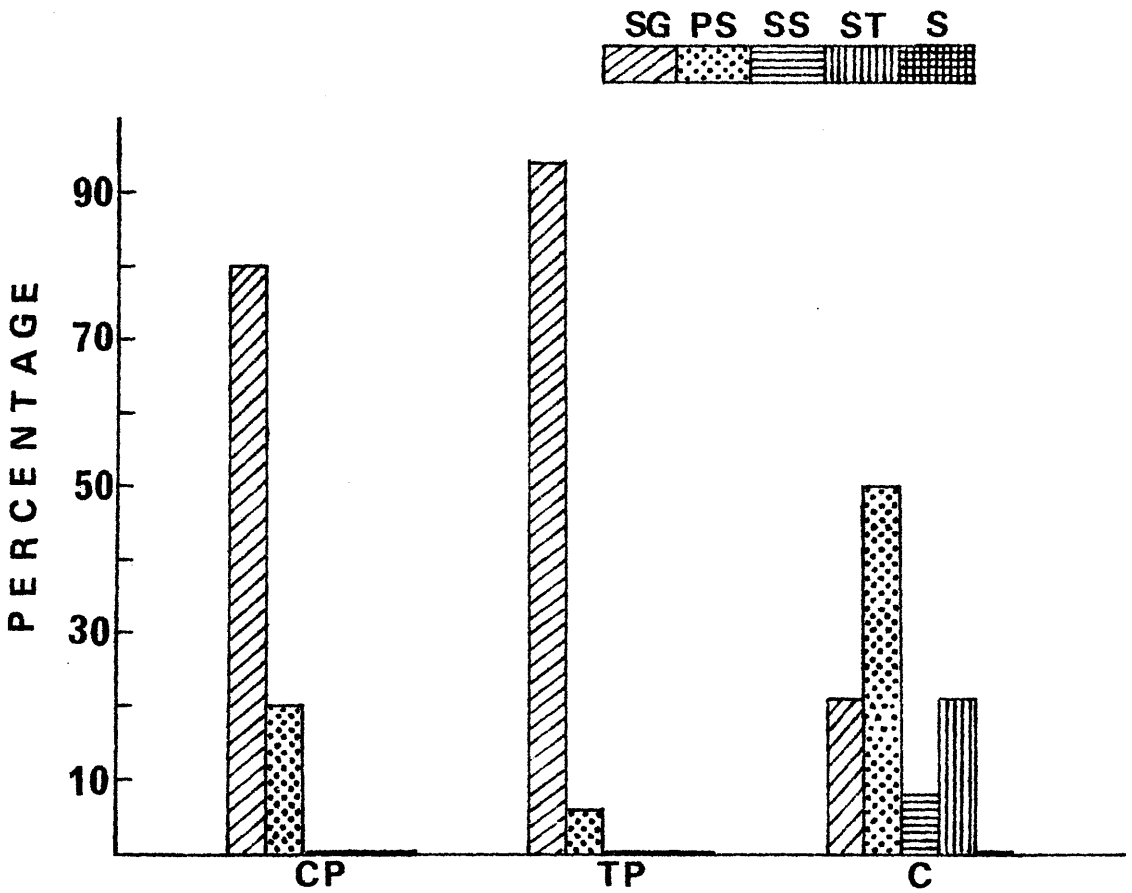
a



b

FIGURE 8

Effects of extracts of carp pituitary and testosterone propionate on the testis of Methallibure-treated T. aurea. CP - carp pituitary treatment; TP - testosterone propionate treatment; C - controls treated with saline.


SG PS SS ST S
PERCENTAGE
90
70
50
30
10
CP
TP
C

membrane becomes clear and gives the hypertrophied Sertoli cell, its oval shape (Plate 37(b)). At this stage, the nucleus of the densely granulated cell is found on one side of the cell. The largest cells found measured 16-20μ.

With regard to the germ cells in the testis of the CP-treated fish, only spermatogonia and a few primary spermatocytes were present (Plate 36(b); Fig 8 and Table 28). They were commonly found at the cortex of the testis even though a few of them were observed in the other parts of the testicular tissue. As compared with the saline-treated controls, the spermatogonia and primary spermatocytes were more numerous in the controls (Plate 35(b); Fig 8 and Table 28). Probably necrosis of the spermatogonia was taking place. The mean GSI of 0.3 in the CP-treated group was also less than that of the controls (Table 29).

(iii) Testosterone propionate (TP): (a) Secondary Sex Characters.

During the 30 days of experimentation, the previously Methallibure-treated T.aurea, without secondary sex characters, became beautifully coloured as a result of administration of testosterone propionate. In the males, the greenish-blue coloration of the upper half of the body was unmistakably reinstated, and the borders of the fins, especially those of the dorsal, caudal and anal, attained a bright red coloration. Even in the females, there was a slight coloration of the tips of the fins.

The genital papilla in both sexes of fish were greatly developed and sex differentiation, according to this character presented no difficulty.

Similar observations were made on T. mossambica.

(b) The Ovary

In spite of the fact that the genital papilla were greatly developed in the females, the treatment did not have any stimulatory effect on the ovaries. As compared with the controls, there was even a retardation of oogenesis in the TP-treated fish.

Table 28. Effects of Carp pituitary (CP), and Testosterone propionate (TP) on spermatogenesis in Methallibure-treated T. aurea

Treatment	No.of Fish	Mean wt. of fish (gm)	Mean length of fish (cm)	Spermatogenetic condition of testis SG±SE	PS±SE	SS±SE	ST±SE	S±SE
CP	6	14.5	9.7	12±3	3±0	-	-	-
TP	6	19.2	10.2	17±4	1±1	-	-	-
S*	6	14.0	9.5	14±2	33±7	5±1	14±4	-

Values for spermatogenetic condition are averages of each germinal stage in six cross-sectioned lobules.

* Saline (treatment for controls).

Table 29. Effect of Carp pituitary (CP) and Testosterone propionate (TP) on the testis of Methallibure-treated T.aurea.

Group	Mean body weight (gm)	Treatment for 30 days CP (mg)	TP (mg)	S* (ml)	Mean GSI±SE
1	(6) ** 14.5	0.25	0	0	0.314±0.048
2	(6) 19.7	0	0.42	0	0.150±0.018
3	(6)a 13.0	0	0	0.1	0.629±0.107

* Saline

** Number of fish in parenthesis.

a. Controls

p values calculated by Student's t test for GSI between groups:

1 and 3 $p < 0.02$: 2 and 3 $p < 0.001$

Table 30. Effect of Testosterone propionate on oogenesis in Methallibure treated T.aurea

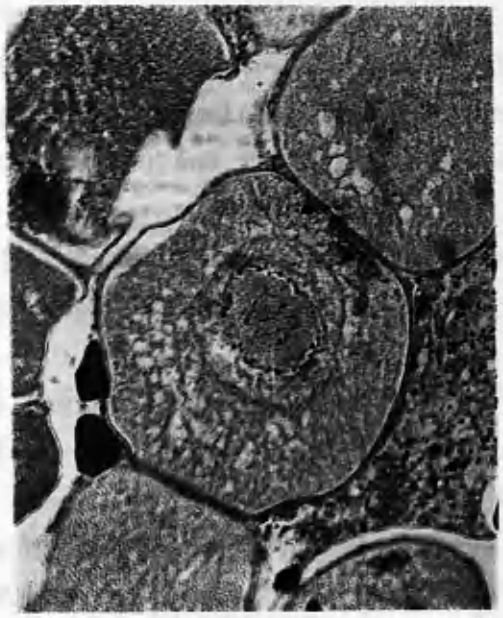
Wt. of fish (gm)	Length of fish (cm)	Mean size of advanced oocytes (μ)	Stage of maturation
20.0	10.7	500	II
18.0	9.9	533	II
12.5	9.5	450	II
15.0	9.8	416	II
10.6	9.5	500	II - III
13.5	9.6	350	II

PLATE 38

Effects of testosterone propionate (TP) on maturation in Methallibure-treated T.aurea

a) T.S. of ovary of Methallibure-treated female, injected for 30 days with TP: advanced oocytes still in the period of protoplasmic growth, retardation of oogenesis (compare with Plate 35 (a)). X.155.

b) T.S. of testis of Methallibure-treated male, injected for 30 days with TP: retardation of spermatogenesis (compare with Plate 35 (a)) X.310. All stained with Heidenhein's iron haematoxylin and Bouin fixation.



a

b

Only one out of the six females injected with this steroid hormone for 30 days had ovary in the transformation phase, into the period of vitellogenesis. The remaining fish had ovaries in Stage II of maturation (Plate 38 (a) and Table 30). The size of the larger oocytes in the ovaries ranged between 350 and 533 μ (Table 30.) and the diameter of the nucleus was 100-165 μ The least GSI among the females was recorded in this group -0.4 (Table 27).

The Testis

There was no stimulation of the testis as a result of TP-administration. The majority of the germinal cells were in the spermatogonial stage, while very few of them had entered into the first stage of the first maturation division - meiotic prophase (Plate 38(b), Fig 8 and Table 28). The TP-treated fish also revealed reduced testicular weights over controls, as evidenced by the low GSI of 0.15, as compared with 0.629 in the controls (Table 29). However, no peculiarity in the development of the testis, as those evidenced in the CP-treated fish regarding the morphology of the Leydig and Sertoli cells, was found.

Steroid and Succinate Dehydrogenase Reactions in the Gonads of Normal and Methallibure-treated T.aurea.

Thirty normal, mature T.aurea (15♂ and 15 ♀) and the same number of methallibure-treated fish were used for these investigations.

Table 31 (below) shows the enzymes tested as substrates for the reaction. The control medium lacked the substrates.

Table 31. Enzymes tested for Histochemical reactions.

Enzymes	Substrate	Cofactor + Carrier
3 Bol-DH	Dehydroxyepiandrosterone (DHA) (Δ^5 - androsten-3B-ol-17-one)	Diphosphopyridine nucleotide (NAD or DPN)
11 B ol-DH	Androstenedione (11 B-dehydroxy-5α- androsten-17-one)	NAD
17 Bol-DH	Testosterone (17B-hydroxy-4-androsten-3-one	NAD
S.DH	Sodium Succinate	NAD + Menadione

Results

After several attempts, successful results were obtained only with the use of 3 Bol-DH and S.DH.

Histochemical Localization of 3 B-hydroxysteroid dehydrogenase

In the normal ovary : 3 B-hydroxysteroid dehydrogenase activity was found to be limited to the follicular cells of the ovary. This was demonstrated by the presence of formazan deposits in the follicles of mature ovaries incubated in NAD (Plate 39(a)). Sections of the controls incubated without the steroid substrate did not show any reaction.

In the normal testis : the visualization of 3B-hydroxysteroid dehydrogenase activity was characterized by a reaction in the Leydig cells (Plate 40(b). No reaction was detected from the sections of the controls lacking the steroid substrate.

PLATE 39

Histochemical reactions in the steroid-producing cells in the gonads of normal T.aurea

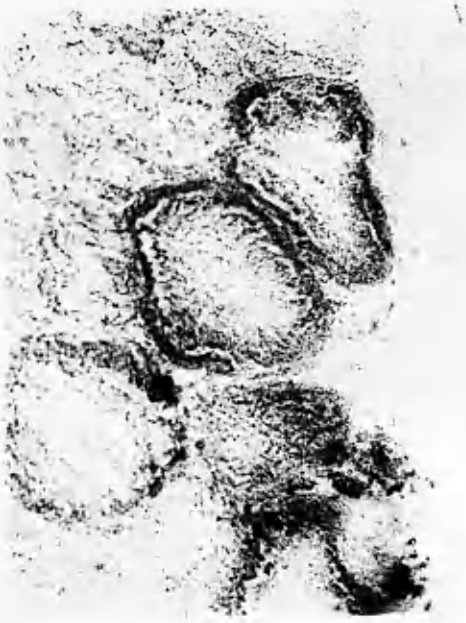
Frozen sections of ovary of fish: showing

a) 3B-hydroxysteroid dehydrogenase activity in the follicular cells. X.400. Tissue incubated with ^{5} androsten-3B-ol-17-one)

b) succinate dehydrogenase activity in the follicular cells

c) enlarged section of (b) X.500. Incubated with sodium succinate.

a



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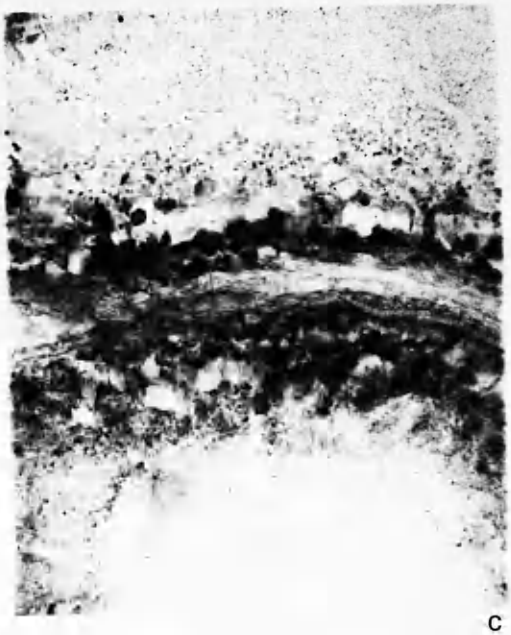


c

PLATE 40

Histochemical reactions in the steroid-producing cells in the gonads of normal and Methallibure-treated *T. aurea*

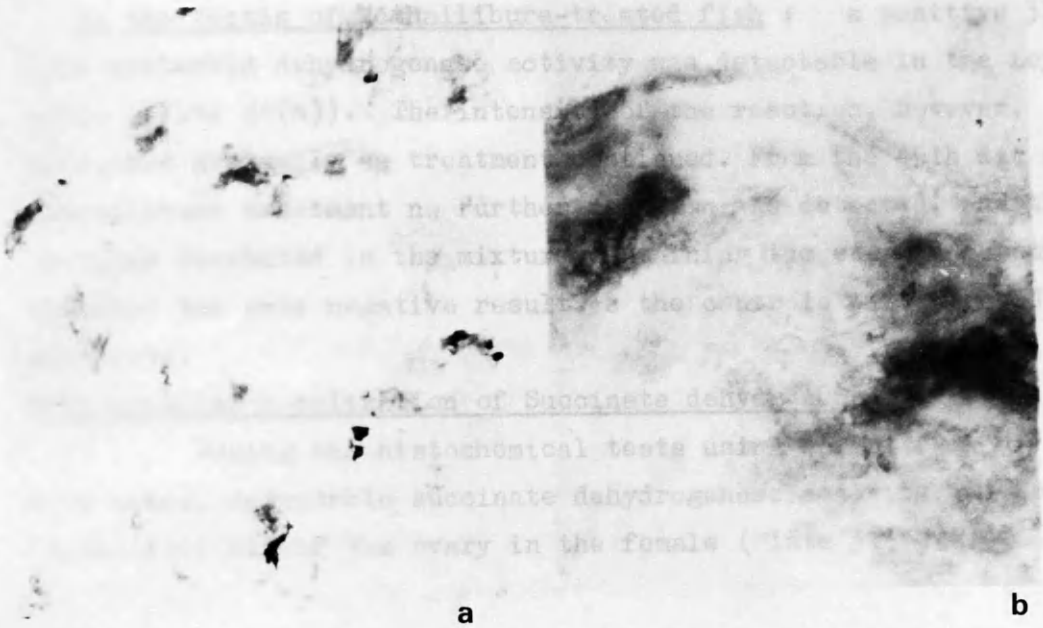
a) Frozen section of testis of 10 day Methallibure-treated fish: showing positive, but less intensive, 3B-hydroxysteroid dehydrogenase reaction in Leydig cells. X.400.

b) enlarged section of (a) X.1200

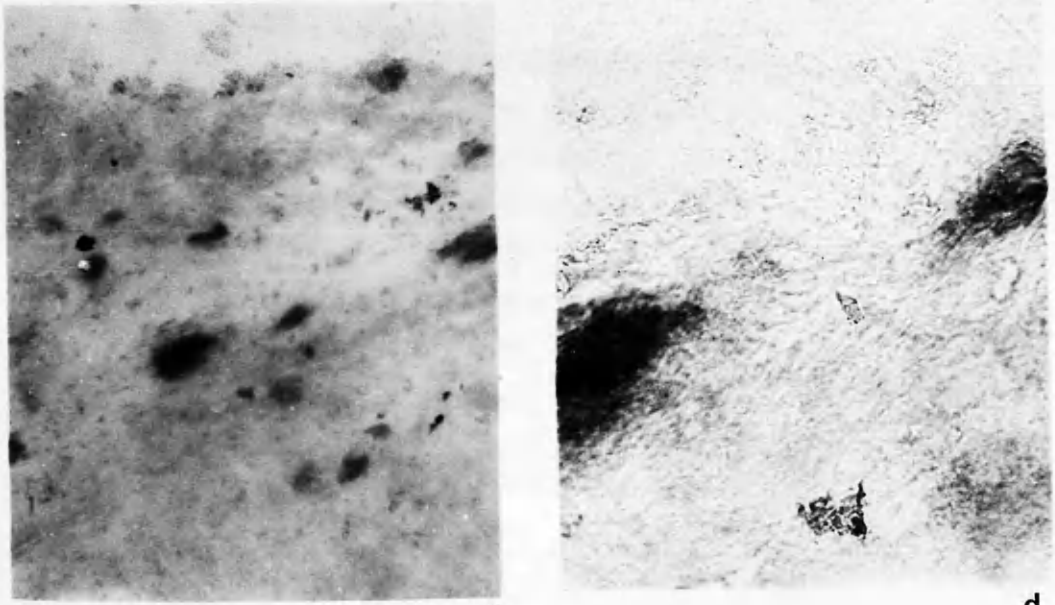
c) Frozen section of testis of normal fish, showing positive 3B-hydroxysteroid dehydrogenase reaction in Leydig cells. X.400.

d) enlarged section of (c) X.1200.

All tissues incubated with ^{5}-androsten-3B-ol-17-one)



a b



c d

In the testis of Methallibure-treated fish : a positive 3B-hydroxysteroid dehydrogenase activity was detectable in the Leydig cells (Plate 40(a)). The intensity of the reaction, however, decreased gradually as treatment continued. From the 45th day of methallibure treatment no further reaction was detected, and the sections incubated in the mixture containing the steroid substrate revealed the same negative result as the controls which lacked the substrate.

Histochemical Localization of Succinate dehydrogenase

During the histochemical tests using normal fish of both sexes, detectable succinate dehydrogenase activity was found in the follicle of the ovary in the female (Plate 39(b)(c)).

Fish Culture in Israel

Israel today has a total of 480,000 acres of fish ponds with an average yield of 2.050 kg. and a maximum yield of 3.500 kg per 100 acres. Shortage of freshwater restricts the construction of fish ponds. Thus, brackish water is generally used for fish culture, but freshwater is also used wherever available, or where it is found in abundance. The total pond-fish production is about 10,000 metric tons per year, which is 60% of the entire fish production of the country, including that of sea fisheries.

Types of Fish Raised.

The common carp, Cyprinus carpio is the main fish raised in ponds. Successful attempts have been made lately to introduce some other species to be cultivated together with the carp. Two of these, the grey mullet, Mugil cephalus and Tilapia aurea have proved to be economical, and when stocked in the right proportion to carp, contribute towards higher yields and better exploitation of the food in the pond.

The grey mullet is in very limited supply as it is caught only during its natural anadromous migration at the beginning of Winter. Transportation difficulties, at the present time, restrict the distribution of this fish, and it is therefore bred mainly in the farms on the coastal areas.

Tilapia, which is becoming increasingly important as a supplementary fish in the ponds, also has its limitations. These arise chiefly from its prolific nature, and so a perfect system of stocking this fish together with carp has not yet been established. So far, Tilapia is cultured during the period beginning in June and ending in late autumn. Thus its marketing is strictly limited.

Breeding Methods : Carp

Fish used for spawning are carefully selected, the sexes separated and kept in special ponds. Selection is based simply on the choice of the nicest and fast-growing specimens.

Spawning occurs about the middle of April. The size of the spawning ponds varies from 10 to 200 acres and the number of spawners varies accordingly. The ratio between males and females is 3:2. Usually, spawning occurs on the next day after the fish have been introduced into the ponds. The bottom of the pond is covered, in several places, with pine branches to serve as substrates for the eggs. After three to five days the eggs are hatched. The average number of fry per female is 100,000.

After 10-14 days, the fry are removed from the spawning ponds and put into nursing ponds of different sizes at a density of 100,000 frys per 100 acres. After a month they grow to about 10 grams and are transferred into storage ponds, at a density of 500,000 fry per 100 acres. Before the attainment of a weight of 10 grams the fry are very vulnerable to gill parasites, especially Gyrodactylus and Dactylogyrus. Therefore they are stocked at a higher density to ensure that they reach a weight of 10 grams as quickly as possible.

Spawning may continue until October, according to the requirements of the farmer, if the spawners are well kept. However, where warm water is available, spawning can be completed at an earlier date. Fry are kept in the storage ponds for various periods of time, according to the production programme. The amount of fry calculated at the start of the season is 10,000 per 100 acres of production pond.

Stocking of Ponds.

The stocking programme of the production ponds is determined by the following factors :

1. Fertility of the pond
2. Size of marketable fish desired.
3. Length of breeding season.

Generally, a growing season lasts about 100 days and the pond is emptied two or three times a year. Complete emptying

of the ponds makes it possible to eliminate undesirable fish which may have been introduced into the pond through the water supply. After the pond is refilled, it is restocked.

There is no rigid system of stocking the ponds. There are variations in the number of fish stocked, and the size of fish used. Much depends on the water supply, fertility of the ponds, etc. However, the stocking scheme most frequently used is described below :

The pond is stocked with 3,000 fish per 100 acres, each fish with an average weight of 100 grams. After 20 to 30 days when the fish reach 200 grams, another 3,500 fry, with an average weight of 10 grams are added. This technique is based on the assumption that the food consumed by small fish is different from that of the larger ones, and in this manner, better exploitation of the pond's food potentiality is obtained. Furthermore, under such conditions a pond is able to serve both as a production and nursery pond at the same time. When the large fish reach an average weight of 500-600 grams and are ready for marketing, the same pond is emptied and restocked with the small fish. When the fish reach an average weight of 350 grams, 500 fish per 100 acres are removed and marketed; and after some time, another 500 - 1,000 fish are removed and sold. This system of thinning out the larger fish is practised in order to ensure continuous growth of the fish according to the holding capacity of each pond. Manipulation with the density of the population and the size of the fish is important for the proper exploitation of the pond.

Feeding

In order to obtain high yields, artificial feeding is practised. Carp thrives well on a wide variety of cheap grain or oil cakes. Usually, a second or third grade of this commodity, which is cheap, is used for this purpose, and makes feeding profitable. The food is distributed once a day in the morning hours with the help of a mechanical food distributor.

The amount of food depends upon the fertility of the pond and the size of the fish. The larger the fish the more food is required. In well-fertilized ponds, the average food coefficient is about 2.5-3 : 1, i.e. 2.5 - 3 kg. of food are needed for the production of 1 kg. of fish. This is the average coefficient but the ratio varies throughout the growing season, according to the size of the fish. A 100 gm. fish requires a coefficient of 1:1, whereas a 500 gm. fish requires a doefficient of 1:3.5.

The amount of food needed is determined every two weeks when a sample weighing of the fish is done, the growing rate determined and, in accordance with the right coefficient, the final amount of food for the next two weeks is set.

Fertilization

Fertilization may increase the growth of phytoplankton for the phytophagous fish, or more abundant food for zooplankton, or insect larvae, on which, in turn, feed the fish. The role of fertilization in enriching the natural food is twofold :
(1) it enables a denser stocking rate of fish per unit area, and
(2) it makes possible better exploitation of the additional feed given to the fish, and thus lowers the coefficient of the feed.

Reports of experiments conducted in different parts of Israel some years ago revealed that natural fertility produces 200 - 400 kg. of fish per 100 acres. By adding fertilizers, without supplemented food, production can be raised to 800 - 1000 kg.

Chemical Fertilization

Fertilization with Lime. This is of special importance where the water supply and soil are acidic, and consequently, the pH of the pond water will also be acidic. Waters with a pH as low as 4.0 are lethal to pondfish.

The desirable range of pH for fish culture is 6.5 to 9.0 at sunrise. At a pH of 4.0 - 6.0, fish production is low and reproduction of carp does not occur. The addition of limestone or

or calcium carbonate ($CaCO_3$) releases nutrients from the soil, promotes bacterial breakdown of waste material and increases productivity of the water.

In waters and soils with a pH below 4.2, preliminary fertilization of soil and pond water (two weeks before stocking of fish) with calcium hydroxide ($Ca(OH)_2$) is necessary. An amount, approximately of 1500 kg/100 acres sometimes increases the pH to 6.5. If limestone ($CaCO_3$) is used, an amount of 4000-6000 kg/100 acres is required for soils with a pH of 4.0; and 2000 kg/100 acres if the pH is 5.0. Where pondwater is more alkaline than 6.5 before daybreak and bottom soils more than 6.0, no liming is needed.

Phosphate fertilizers : As phosphate is the nutrient usually present in minimal quantity, it is the most important fishpond fertilizer.

In acid waters the best fertilizer used is basic slag, which contains 40% of lime. When the water is limed to neurtral, the effectiveness of phosphate fertilizers in fishponds increases substantially. In waters rich in sulphur, triple superphosphate is preferable, as ordinary 18% superphosphate already contains sulphur.

In limy ponds phosphate fertilizers are applied at least once a fortnight at a dosage level of 600 kg/100 acres.

Nitrogen fertilizers : Nitrogen is the second most important nutrient for the establishment of a good plankton growth in fishponds. The important natural sources of supply of nitrogen to the ponds are known, and have been taken into consideration when planning the fertilization with nitrogen. The first is the natural source of water. In most of Israel waters, the nitrogen-phosphorus ratio has been found to be 90:1 to 325:1. Thus, with the supply of freshwater, a considerable amount of nitrogen is added to the pond. The other source are the blue-green algae which are abundant in most fishponds of the tropical regions, and which can fix considerable quantities of nitrogen from the air.

Usually four parts of nitrogen to one part of phosphorus, is the desirable ratio for good growth of plankton. In Israel, a fortnightly dose of 600 kg/100 acres of 20% nitrogen containing ammonium sulphate (fertilizer) is used. Nitrogen fertilization stops two to three weeks after the filling of the pond with freshwater, and in peak summer (mid June to mid September) when there is a bloom of blue-green algae.

Other fertilizers used are ammonium sulphate, suitable mainly for alkaline ponds; calcium nitrate, suitable for acidic ponds, aequeous ammonia (20%) for acidic ponds.

Organic Fertilization

The following organic manure is used :

Stable manure (cattle, horse or sheep) at 5000 to 7500 kg/100 acres. In order to avoid excess absorption of oxygen the manure is not spread over the whole pond bottom, but in heaps of 20 - 30 per a 100 acre pond. Manuring is done in the cool season of the year.

As the decomposition of chicken manure is very rapid, it is added in small amounts also in heaps, along the embankments of the pond, just beneath the water level.

Tilapia

Tilapia aurea is the popular species which is cultured in ponds together with carp and grey mullet. The standard method was to populate carp ponds with small Tilapia fingerlings, a few weeks afrer hatching, usually at the beginning of June, giving a marketable-size tilapia of 200 gm at the end of October.

Since this was the common method in Israel, it resulted in concentrating the whole production of tilapia in a very short period (October-December), leaving the market without tilapia for the rest of the year. The expansion of tilapia production demanded development of new growing methods enabling the sale of this fish all year round.

The extension of the growing season of tilapia beyond October is complicated by two main factors : a) frost - tilapia is very susceptible to temperatures below 10°C and may die during the winter; b) spawning - unlimited and continuous spawning of tilapia starting early in summer may easily block the growth of the fish in a pond because of overpopulation with small fry.

Keeping tilapia in deep ponds during the winter with addition of warm water during the cold days avoided the danger of frost. To eliminate overpopulation by continuous spawning, monosexed populations were employed. Tilapia males are known to grow faster than females when grown separately, and even together.

Initial Experiments in Growing Male Tilapia (Report from Ein Hamifratz Fish Farms)

The first sexing of tilapia was carried out in 1961 (Mires 1969). A group of 10-12 month old T.aurea was sexed by hand. Initial weight at the time was only 30 gm. and identification, at this size was quite difficult. A 400-acre pond containing carp was stocked with 1000 tilapia per 100 acres. The results of the experiment are given in Table 32.

The outstanding daily increment of individual male tilapia and the total yield per 100 acres was very encouraging.

Table 32. Growth of male T.aurea together with carp

Days of growth	142
No. of tilapia per 100 acres	920
Individual wt.of tilapia at harvest	540
Total wt. kg/100 acres	496.8
Daily increment per fish, gm	3.7
Total yield of carp	700

Table 33. Nursing of Tilapia hybrids and their sexing in 1966.

	Pond H	Pond G
Size of pond, acres	4.1	4.5
Date of stocking	12.July	8.August
Date of harvest	7.October	11 November
Days of growth	86	76
No. of fish stocked, per 100 acres	8800	11350
Initial individual wt. gm.	12	5
No. of fish harvested, per 100 acres	9630	12200
Individual wt. at harvest, g	155	75
Total increment, kg/100 acre	1387	866
Daily increment, kg/100 acres	16.10	11.40
Daily increment, gm/fish	1.68	0.93
Males at harvest, %	61	62
Individual wt. of males at harvest, gm	179	86
Individual wt. of females at harvest, gm	119	70

In the next series of experiments, 600-700 male T.aurea per 100 acres were introduced into five ponds of 300-400 acres each in 1962. The experiments failed for the following reasons :

1. The sexing of fish of only 30 gm. weight was difficult and not reliable. The few females included by error in the male stock spawned in the fattening ponds and at harvest a total of almost 1000 kg/100 acres of small tilapia fry was found in each pond. This limited the normal growth of the males.

2. The early date (March) of stocking caused high mortality because the tilapia had still not recovered from the winter cold.

Observations of Growth of Tilapia Hybrids.

Tilapia hybrids (crosses of male T.aurea and female T.nilotica) are thought to give a higher percentage of males in their offspring (Fishelson, 1966).

Spawning took place in June and nursing of the fry was carried out in two 400-acre ponds in which 1140 and 1300 carps per 100 acres with an initial weight of 350 and 400 gm were stocked. At harvest, the increment of the carp was 2.95 kg/100 acres in pond H, and 103 kg/100 acres in pond G. The individual weights of the carps at harvest were 589 and 505 gm respectively. Results of tilapia growth during the nursing stage in those ponds are given in Table 33.

The results of the nursing were quite satisfactory. The hybrids weighed over 80 gm. at harvest so that sexing was easy and most of the females were of marketable-size and could be sold. Sexing was done by hand on a sorting table, the average number of males separated per work day (out of a 60% male population) was 2000.

During the winter the hybrids were stocked in 2-meter-deep ponds at a density of 10,000 fish/100 acres. When temperatures fell below 12°C, warmer water from a stream or a well was added. Rearing of male hybrids started in April the following year in 3 different combinations :

1) 1500 male hybrids together with 1600-1900 carp/100 acres;

Table 34. Growth of male hybrid tilapia (1500/100 acres), together with 1500-1600 carp/100 acres of 18 gm. initial weight.

	Pond No. (Size in acres)		
	3(450)	53(400	55(100)
Date of stocking	24.April	24.April	24.April
Date of harvest	11.July	15.July	26.August
Days of growth	78	82	124
No. of tilapia stocked/100 acre	1500	1500	1560
Initial individual wt.gm	210	210	210
No.of tilapia harvested/100 acres	1500	1570	1560
Individual wt.at harvest, gm	460	440	500
Total increment,kg/100 acres	388	395	452
Daily increment, kg/100 acres	4.98	4.80	3.64
Daily increment, g/fish	3.33	3.06	2.34

Table 35. Growth of male hybrid tilapia (2000 and 2270/100 acres) together with 1500 and 2500 carp/100 acres of 200-340 gm initial weight

	Pond No. (Size in acres)	
	5(410)	51(500)
Date of stocking	14.May	3.May
Date of harvest	30.July	1.September
Days of growth	77	121
No.of tilapia stocked /100 acres	2000	2270
Initial individual wt.gm	60	60
No.of tilapia harvested/100 acres	1980	2270
Individual wt.at harvest.gm	490	750
Total increment, kg/100 acres*	858	1570
Daily increment,kg/100 acres	11.100	13.000
Daily increment, gm/fish	5.55	5.73

* "Wild spawning" in pond 51 gave an additional increment of 560 kg/100 acres of 7000 tilapias /100 acres of 80 gm average weight

Table 36. Growth of male hybrid tilapia (1000/100 acres) together with 1600-1900 carp/100 acres of 30-150 gm size and 600 mullet of 12-18 gm. size.

	Pond No. (Size in acres)					
	41 (350	4 (450)	6 (440)	49 (150)	18 (280)	54 (300)
Date of stocking	1.May	1.May	24.Apr.	3.May	24.Apr.	1.May
Date of harvest	20.Jly.	6.Aug	10.Aug	20.Aug.	1.Sep.	2.Sep.
Days of growth	80	97	108	109	130	124
No. of Tilapia stocked 100/acres	1050	1000	1000	1000	1000	1000
Initial individual wt. gm	157	75	210	75	210	105
No. of tilapia harvested 100 acres	1020	1000	1000	1000	1000	1070
Individual wt. at harvest, gm	400	500	500	500	455	500
Total increment, kg/ 100 acres	280	425	290	426	260	416
Daily increment,kg/100 acres	3.50	4.38	2.68	3.90	2.00	3.35
Daily increment, g/fish	3.44	4.38	2.68	3.90	2.00	3.13

2) 2000 - 2200 male hybrids together with 1500-2500 carp/100 acres;
3) 1000 male hybrids together with 1600-1900 carp and 600 grey mullet yearlings.

All the ponds were fertilized biweekly with 60 kg superphosphate and 50 kg ammonium sulphate/100 acres. Additional feed (sorghum) was given according to the carp population only. The conversion rate of food calculated on the carp yield only was 0.5 - 2.5 accordingly in various ponds. Though tilapia "wild spawning" was found in all the ponds, no serious interference with the growth was observed. The results of the experiments are given in Tables 34, 35 and 36.

In ponds 3, 53, and 55 (Table 34) the carp gave an additional weight of 380, 440 and 580 gm respectively. In the ponds 5 and 51 (Table 35) the increment by carp was 363 and 514 kg/100 acres, the average weight being 635 and 457 gm. In Table 36, the additional increment of carp was between 680 and 950 kg/100 acres (410-515 gm. average weight), 178 to 286 kg/100 acres in grey mullet with an average individual weight of 700-750 gm.

In summary (Table 37) the following conclusions were drawn :

1. The combination of the three species (carp, tilapia and grey mullet) gave better results in yield per 100 acres than the combination of carp with 1500 tilapia only. The best result was obtained with the higher densities of tilapia in the carp ponds, where the addition of 200-2200 tilapia/100 acres gave the highest result of 1648 kg/100 acres.
2. The daily increment per 100 acres increased with the increasing density of tilapia. This shows that the densities utilized best the pond fertility. It was still not clear whether that was the maximal density.

The daily increment of each tilapia was almost the same in the first two combinations and higher in the third one.

Table 37. Summary of data on growth of male hybrid tilapia in mixed culture and in various combinations with carp and grey mullet.

	1600-1900 carp, 1000 tilapia, 600 mullet				1600-1900 carp 1500 tilapia			1500-2500 carp 2000-2200 Tilapia		
	Carp.	Tilapia	Mullet	Total	Carp	Tilapia	Total	Carp	Tilapia	Total
Daily increment, kg/100 acres	4.0	3.3	1.22	8.52	3.5	4.22	7.72	4.75	12.20	16.95
Daily increment, g/fish	2.4	3.0	2.4		2.4	2.7		3.1	5.7	
Total, kg/100 acres	800	350	227	1377	650	410	1060	438	1210	1648
Days of growth	200	108	185		184	97		92	99	

Tables 34 and 35 show that when tilapia with lower initial weight were introduced, a higher daily increment per fish and a higher net additional daily weight per 100 acres were reached.

DISCUSSION

Oogenesis

The process of the development of the female sexual cells in T. aurea does not seem to differ greatly from that in T.mossambica described by Dadzie (1969), and the same process, in the two Tilapia species, does not differ, in broader aspects, from that in most teleosts. Primary germ cells proliferate through mitotic divisions and transform into protoplasmic then vitellogenetic oocytes. The process ends with the maturation of the oocytes.

Interspecific studies, however, have revealed certain peculiarities in the development differing from those of other fish. Dadzie (1968) described the simple structure of the chorion of the egg in T. mossambica and correlated it with the mouthbrooding characteristics of the species. The present study in T.aurea, another mouthbreeder, has confirmed the previous findings, that under normal conditions, the zona radiata is present as a thin, homogeneous layer (Pl.1(d). The usual radial canals from which originated the name of this layer and which provides channels for exchange of materials between the oocyte and the follicle, are not clearly visible even under the highest magnification of the light microscope. However, Kraft and Peters (1963) presented a picture of the zona radiata with radial canals in T.mossambica. Typical zona radiata with distinct radial canals have been found in many species of Mugil (Abraham, et al (1966). Apart from providing a passage for exchange of materials between the oocyte and the follicle, the zona radiata also plays a protective role, in substrate-spawning fish, against outer influence, at the time of fertilization and incubation of the egg, by becoming firm and compact on coming in contact with water. It therefore becomes clear why this layer is not prominent in the two mouth-incubating species of Tilapia - it has no functional role.

The gelatinonous membrane which is situated between the

zona radiata and the zona granulosa in most fish and swells when it comes into contact with water during fertilization, is responsible for sticking the egg to the substrate (Ivanov, 1956). In T.mossambica Dadzie (1968) reported of the absence of this membrane from the chorion of the egg. Kraft and Peters (1963) in studying the structure of the ovarian cell wall in the same species did not mention the presence of the gelatinous membrane. In the present study, there was no evidence of the presence of the membrane in the chorion of the egg of T. aurea. The same explanation, that incubation takes place in the mouth of the female parent, accounts for the absence of the layer of gelatinous membrane.

The follicle of the oocyte of T. aurea like that of T.mossambica consists of the zona granulosa which is situated immediately after the zona radiata, and then the connective tissue layer or the theca which follow the zona granulosa. These two layers, normally, consists of a single layer of cells each. Thus, in the two species of Tilapia of the present study, an intraspecific adaptation of the ovarian cell wall has been observed in connection with the ecology of reproduction of the species.

During oogenesis, the appearance of peripheral vacuoles in the oocytes, marks the end of the period of protoplasmic growth and the beginning of vitellogenesis. These vacuoles are rich in polysaccharides (Yamamoto, 1956, a, b, c, d, e, f). In T.mossambica Dadzie (1966) did not observe the typical peripheral vacuolization in the oocyte. The author, however, did not rule out completely, the absence of polysaccharides from the vitellogenetic oocytes of this species, but suggested that they might be present in small quantities since they participate in the formation of the perivitelline space, at the time of fertilization, and also their quantity in an oocyte determines the degree of swelling of the egg during, and after fertilization. In T. aurea peripheral vacuolization is a distinct feature of the oocytes during their transformation

into the yolk stage (Pl.2a). The presence of peripheral vacuolization in the oocytes of T. aurea and its absence in that of T.mossambica suggests a difference in rythm and degree of formation of the perivitelline space and swelling of the egg at the time of, and after fertilization. Thus, two species of Tilapia with the same adaptive ecological morphology in connection with reproduction, can differ, in some aspects, in their reproductive physiology.

Spermatogenesis

Observations on the cyclical activities in the testis of T. aurea in the present study, were similar to those made by Dadzie (1969) in T. mossambica. Similar reports have been given by numerous workers on various aspects of spermatogenesis, the latest having been received from Rai (1965) in Barbus, Stanley, Chieffi and Botte (1965) in Gobuis, Ahsan (1966) in Couesius and Rastogi (1968) in Macroganthus.

The existing two views regarding the origin of the germ cells in teleosts were also discussed by Dadzie (1969) who subscribed to the information provided by Eckstein and Spira (1965) on the migration of gonocytes from the splanchnopleura along the lateral part of the somatopleura into the germinal tissue in T. aurea. In the present report, however, apart from the germ cells, information is also given regarding the histology of the other cells of the testis - the Sertoli cells and the interstitial cells of Leydig.

Sertoli cells have been described in the mature testis of Gasterosteus (Craig-Bennett, 1931; Follenius, 1953). The authors, however, did not report the functions of these cells; while Bowers and Holliday (1961) and Ham and Leeson (1961) believed that the Sertoli cells were concerned with the nourishment of the germ cells. During the present study, the development of the Sertoli cells was not strictly followed to establish whether they undergo seasonal changes during maturation development and to elucidate their functional significance. However observations made on the Sertoli

cells of T. aurea previously treated with a pituitary inhibitor and then injected with alcohol-preserved carp pituitaries (see pages 127-128 for details and Pl. 37(b)), make it tempting to ascribe the role of nourishment of the germ cells, as has been reported by Bowers and Holliday (1961) and Ham and Leeson (1961), to the Sertoli cells. The subject will be discussed under the appropriate section (viz: the effects of carp pituitary and testosterone propionate on the gonads of methallibure treated T.aurea)

In the early literature, the presence of Leydig cells in the testis was controversial (see reviews by Craig-Bennet, 1931; and Hoar, 1955). Marshall and Lofts (1956, 1957) gave convincing evidence on the presence of two types of endocrine cells lying in the periphery of the lobules of the testis, which were described as Leydig-cell homologues - the lobule - boundary cells" and the typical Leydig-cells conforming in form and appearance with those usually found in vertebrates and to which the endocrine tissue of the testis of Tilapia, according to the authors, belong. These interstitial cells are believed to be precursors of the male hormone. In T. aurea and T. mossambica the Leydig - cells conformed in appearance and localization with the vertebrate pattern as reported by Marshall and Lofts (1956) especially for Tilapia species, even though it is not clear whether the authors investigated these cells in all species of Tilapia before making this inference, from the observations on T. mossambica and T. aurea it seems to be true.

(a) Cytology of the Pituitary of Tilapia

The Pro-adenohypophysis : The eta or Prolactin cells.

Two cell types have been identified in the pro-adenohypophysis of T. mossambica. The first and more prominent type are the eta cells. In their staining affinities they resemble the prolactin - secreting cells in mammals (Herlant, 1960; 1964). The presence of eta cells in the pro-adenohypophysis of teleosts has been reported by Ball and Pickford (1964), Ball (1965), Emmart et al (1966), in the Fundulus, Ball and Olivereau (1964; 1965), Olivereau and Ball (1964; 1966), Ball (1965 b; c), Ball et al (1965) in Poecilia spp, Ball and Olivereau (1964), Olivereau (1966; 1967; 1968; 1969a) in Anguilla, Sage (1966) in Xiphophorus, Olivereau (1969b) in Rutilus. ~~These authors, besides describing their staining properties also described the response of~~ The eta cells of T.mossambica adapted to freshwater and also to media with varying salinities were described by Dhamamba and Nishioka (1968) who observed a hypertrophy of the eta cells when the fish were maintained in freshwater; and a hypotrophy of the same cells on being transferred into hypertonic media. Results of the present studies on the eta cells of T.mossambica and T. aurea are in agreement with those of the earlier workers.

In the hypophysectomized Fundulus, Pickford and Phillips (1959) and Pickford et al (1965) observed that only prolactin was able to promote the survival in freshwater. Since then, numerous works have substantiated this hypothesis of the important role played by a prolactin like hormone in osmoregulation. Such reports were given by Ball and Ensor (1967) in the cyprinodouts, Lam and Hoar (1967) and Olivereau and Chartier-Baraduc (1968) in Gasterosteus, and Dhamamba et al (1967) in T. mossambica.

The hypertrophy of the eta cells encountered in the pro-adenohypophysis of T. mossambica and T. aurea of the present study, maintained in freshwater, and the hypotrophy observed in the cells of those fish maintained in half-seawater, suggests an

increased secretion, in the first instance, and a reduced secretion, in the second instance, of a factor involved in osmoregulation. This factor, is probably a prolactin-like hormone, since this hormone has been shown to maintain freshwater survival in hypophysectomized teleosts. The eta cells observed in the pro-adenohypophysis of T. mossambica are identical, in their tinctorial properties and localization, with the deeply-staining acidophils of the same region of the pituitary in T.aurea and probably, have the same functional significance.

The α_1- or ACTH cells.

Studies on the pro-adenohypophysis of teleosts, have revealed the presence of, apart from the eta cells, the or ACTH (corticotrophic cells (Olivereau, 1963, 1965, 1967; Hanke et al, 1967) in Anguilla, (McKeown and Van Overbeeke, 1969) in Onchorhynchus (Olivereau, 1969) in Leuciscus. Metuzals et al (1968) identified two cell types in the same region of the pituitary of Aequidens, one of which, in distribution and staining affinities could be equated with the α_1- cells. This cell type (the α_1- cells) has been reported to be present in the pro-adenohypophysis at the border with the neurohypophysis, and its association with corticotrophin secretions has been demonstrated by immunohistochemical studies (McKeown and Van Overbeeke, 1969) and cytochemical analysis (Olivereau, 1963, 1965, 1967, 1969).

The only contradictory report on the localization of the α_1- cells was presented by Molnár and Szabó (1968) who observed this cell type in the meso-adenohypophysis.

Metropirone (methyl - 2 bis (3-pyridyl) -1,2 - propanone -1) is known for its suppressive effect on the adrenal cortex (Chemical Abstract, 1960). Olivereau (1965) by injecting eels with this drug for periods up to 21 days observed hypertrophy, hyperplasia, degranulation and vacuolization in the ACTH cells of the pituitary. Simultaneously, the adrenocortical cells, according to the author, were strongly stimulated. Also as aresult of this treatment Olivereau reported

that the thyrotrophs were often degranulated and the thyroid gland might be stimulated.

Treatment of T. mossambica, under different environmental conditions (see Page 61) with the hope of obtaining histological modifications in two cell types of the pituitary, i.e. the ACTH cells, and possibly, the TSH cells, yielded positive results only in one cell type. The ACTH cells reacted to metropirone treatment by hypertrophy and hyperplasia (Pl.7 b,c). However, degranulation and vacuolization of these cells as observed in the eels by Olivereau (1965) was not observed in Tilapia. The absence of these features could possibly be due to the period of treatment (12 days) which, in T. mossambica, may not have been long enough, or the dosage (0.5 mg) which was not sufficient enough, to cause degranulation within the 12 days of treatment. However, the daily dosage of 0.5 mg per fish of the size used, could be regarded as a threshold. Longer periods of treatment, therefore, were probably required for the degranulation effect in the ACTH cells to be realized, and for the hypertrophy and subsequent degranulation in the TSH cells to be observed; i.e. if such changes in the thyrotrophs could be induced with metopirone treatment.

The interrenal tissue was not examined histologically to study the correlative changes in the adrenocortical cells enabling a precise identification of the α_1- cells as the corticotrophin secreting cells, and to attribute the cause of the hypertrophy of the α_1- cells to a disturbance in the feed-back mechanism as a result of metopirone treatment. However, relying on documented reports by previous workers, especially that of Olivereau (1965), on the localization, function, and staining propertiesof the ACTH cells, coupled with the present histochemical studies, it could be suggested that the α_1- cells in the pro-adenohypophysis of T. mossambica, bordering the neurohypophysis, are concerned with the secretion of corticotrophin. Furthermore,

the presence of increased number of α_1- cells in the pro-adenohypophysis of Tilapia subjected to low temperatures for three months (Pl.7d) is suggestive of their corticotrophin function since Finerty and Briseno-Castrejon (1949) saw demonstration of the corticotrophic function of the - cells in the fact that these cells increased in numbers after adrenalectomy and that this phenomenon was accelerated by the effect of low temperature.

The Meso-adenohypophysis : The α-or STH cells

The presence of only one acidophilic cell type in the meso-adenohypophysis of teleosts has been reported by Olivereau (1963, 1967, 1969a, b), Metuzals et al (1968), Molnar and Szabo (1968) Blanc (1969). The cells are classic acidophils and often have precise localizations. In the reports of the previous workers as well as the present one, the α - cells are concentrated in the caudal region of the meso-adenohypophysis, and in an undulating manner they border the neurohypophysis.

The little evidence existing on the functional significance of the α - cells have been given on vertebrates other than fish. In the pituitary of the mutant dwarf discovered by Snell (1922), Smith and McDowell (1930), Ortman (1956) and Elftman and Wegelins (1959) discovered that no α - cells were present in the meso-adenohypophysis. On the other hand Koneff et al (1958) reported that administration of purified somatotrophin results in the inactivation of the α - cells and eventually leads to their involution. Marlier, Smith and Smith (1923) had observed greater stimulation of growth in hypophysectomized tadpoles with injections of extracts from the lateral regions of the bovine pituitary - where α - cells predominate, than did extracts of the median region - poor in acidophils (α - cells).

The influence of STH on fish is not known except that Olivereau (1963) observed pathological growth of the dorsal fin in Carassius and an increased body growth in Oncorhynchus. In

Anguilla. Olivereau (1967) reported that the somatotrophic cells are much more numerous and generally more active in the female whose growth is prolonged, and in 1969 the author suggested that the α - cells of the meso-adenohypophysis of Leuciscus secrete the growth hormone.

In T. mossambica and T. aurea, hypertrophy of the α- cells and an increased secretive activity was observed following gonadectomy. Simultaneously a greater increase in somatic growth was observed in the castrates, as compared with the sham-operated controls (Pl.22d and Table 11). This observation, though not conclusive, could possibly suggest the α - cells to be responsible for the increased growth in gonadectomized Tilapia, by the production of growth hormone. More convincing evidence of the secretion of growth hormone by the α - cells is the presence of these cells in an inactive condition with less cytoplasm (hypotrophy) in the pituitary of T. mossambica, subjected to temperatures of 13 - 15°C. These features of the α - cells are correlated with a significantly reduced somatic growth in these fish. In the absence of other experimental proof regarding the functional significance of the - cells in teleosts, it could further be possible, at this stage, comparing these cells with those in other vertebrates, to speculate, on grounds of staining affinities and localization, on the association of the α - cells in the meso-adenohypophysis of T. mossambica and T. aurea with the production of growth (somatotrophic) hormone. The view of Metuzals et al (1968) in considering the α- cells of the meso-adenohypophysis of the cichlid Aequidens to be prolactin - secreting cells, could quite possibly, be discarded.

The B - or Gonadotrophic cells

The B-cells essentially correspond to the classic basophils and are localized in the meso-adenohypophysis in teleosts.

Halmi (1950) and Purves and Griesbach (1951a, b) distinguished a category of basophil cells which displayed vacuolar hypertrophy in the pituitary of gonadectomized rat. This category was later divided by Purves and Griesbach (1954, 1955) into two distinct classes and considered to be gonadotrophs.

Studies on the pituitary of teleosts have produced evidence in support of the results obtained in rats. Two of the three basophils identified in the meso-adenohypophysis of Protopterus spp. were regarded as gonadotrophs (Kerr and Van Oordt, 1960). Making use of multiple staining techniques, Olivereau (1966, 1967) identified two types of basophils in the meso-adenohypophysis of Anguilla, and associated them with gonadotrophin secretion. In the pituitary of the cichlid fish, Aequideus, Metuzals et al (1968) reported the presence of two types of basophil (B_1 and B_2) cells in the meso-adenohypophysis. The author suggested that the B_1-cells may be connected with FSH secretion, while the B_2-cells may produce LH. The gonadotrophic function of two types of basophils in the pituitary of Leuciscus was demonstrated by Kerr (1948) and confirmed by Olivereau (1969). Blanc (1969) reported the presence of two types of basophils in the meso-adenohypophysis of Mugil and associated them with the production of gonadotrophic hormones. However, Atz (1953) found sufficient evidence for the report of the existence of only one cell type in Astyanax. This cell type showed changes related to the physiological state of the gonad and was therefore associated with gonadotrophin secretion.

In the present study, two types of basophil (B_1 and B_2) cells have been identified in the meso-adenohypophysis of T. mossambica and T. aurea. During the reproductive cycle, a gradual increase in the quantity of the B_1-cells, reaching a peak at the prespawning period, takes place. At this time secretive activity in the cytoplasm of the cells becomes evident, and degranulation ensues at the spawning phase. This phenomenon of

granulation - degranulation in the basophils in connection with gonadal maturation makes it possible to associate this cell type (the B_1-cell) with gonadotrophin secretion.

The B_2-cells identified in the meso-adenohypophysis of the two species of Tilapia studied, are slightly less basophilic than the B_1-cells. Differences in distribution and staining intensities became apparent when the Alcian Blue - PAS - Orange G method was used to stain the pituitaries. The polygonal shape of the B_2-cells was more than ever apparent, and their light red coloration was undoubtedly distinct from the dark red coloration of the B_1-cells. However, both cell types displayed the same staining affinity when the PAS technique was used alone. It was not until a depletion of basophilia and a very low PAS response was observed in the meso-adenohypophysis of 46 - 50 days gonadectomized fish, that the B_1-cells were thought to be a second type of basophil which could also be regarded, possibly, as a gonadotrophic cell type.

Depending on size, distribution and physiological state of the two types of basophils in the meso-adenohypophysis of Tilapia it is tempting to associate the first type - the B_1-cells, with the secretion of LH, and the second type - the B_2-cells, with the production of FSH. However, in the literature a great controversy exists regarding the secretion, in the fish pituitary, of two gonadotrophins - FSH and LH. The discussion of this subject will be dealt with in the appropriate section in this thesis.

The gamma (γ-) or Chromophobe cells

The functional significance of the fourth cell type in the meso-adenohypophysis of teleosts is not established. In the pituitary of T. mossambica and T. aurea these chromophobic cells undergo a small numerical variation during the various stages of the reproductive cycle (Pl. 9 and Table 7).

Of all the cell types in the pituitary treated so far,

functional significance has been ascribed to all of them, with the exception of the chromophobe (γ-) cells. In the meso-adenohypophysis, where the gonadotrophic function of the pituitary is localized, four cell types have been identified. Olivereau, 1963, 1967, 1969,; Metuzals et al , 1968; Kerr, 1948; Kerr and Van Oordt, 1966; Molnar and Szabo, 1968; Blanc, 1969. All these workers, with the exception of Kerr and Van Oordt (1966) reported one acidophilic cell type in the meso-adenohypophysis of the teleosts studied, and the association of this cell type with somatotrophin production was suggested in some of the species (Olivereau, 1963, in Carassuis and Onchorhynchus; Olivereau, 1967 in Anguilla; Olivereau, 1969 in Leuciscus; Blanc, 1969 in Mugil). The formation of basophils frmm acidophils in the meso-adenohypophysis as suggested by Matthews (1936), Bretschneider and de Wit (1947), D'Ancona (1951) and Sathyanesan and Singh (1963) does not therefore seem likely.

It is generally agreed that, at the initial stage of ontogenesis, chromophobes and acidophils were the only cell types in the meso-adenohypophysis. In the course of development, chromophobes became less numerous and basophils made their appearance, while acidophils remained and dominated this zone for some time until the onset of the prespawning phase, when basophils become the dominating cell type. It is further agreed, that in some fish, at least, chromophobes increase in number once more, soon after spawning.

From the works of Gerbilskii (1940) Sathyanesan (1960) and from the observations of the present study in T.mossambica and T. aurea, it has been found that chromophobes take the place of the depleted basophils soon after spawning. Castration, and the treatment of normal mature fish with a pituitary inhibitor (Methallibure) have each resulted in a depletion of basophilia and an emergence of chromophobe cells in the area once occupied by the secretive basophils.

At this stage, it could be suggested that, in T.mossambica and T. aurea, basophils are formed from chromophobes. During the transformation of the chromophobes into basophils, an intermediate cell is possibly formed with amphoteric properties, appearing at times, depending on the physiological state of the fish, as a pink-blue colour. These intermediate cells are not acidophils, and their presence is only transitory. The (γ-) or chromophobe cell type of the meso-adenohypophysis of the two species of Tilapia studied, could therefore be regarded as the precursors of the basophils.

The meta-adenohypophysis

The presence of two cell types, both acidophilic, in the meta-adenohypophysis of teleosts has been reported by Olivereau (1967, 1969) in Anguilla and Leuciscus, Kerr and Van Oordt (1966) in Protopterus spp., Molnar and Szabo (1968) in Eudontomyzon, Metuzals et al (1968) in Aequideus and Blanc (1969) in Mugil. None of these workers provided evidence relating to the functional significance of these cells.

In the meta-adenohypophysis of T.mossambica and T.aurea two types of acidophlic cell types, similar, in distribution and staining affinities, to those observed by previous workers, were observed. The larger one was slightly PAS positive. The function of these cells is yet to be established.

In summary, eight cell types have been distinguished in the pituitary of T. mossambica and T. aurea. The pro-adenohypophysis contains 2 cell types : (1) acidophilic cells which are similar to the prolactin-secreting cells of other teleosts; (2) cells which are normally less acidophilic and less numerous and which are similar to the corticotrophic-secreting cells of other teleosts.

In the meso-adenohypophysis four cell types were identified: (1) acidophilic cells which probably secrete the

somatotrophic hormone; (2) first basophil cell types (B_1 cell) which is gonadotrophic and secretes probably the luteinizing hormone; (3) second basophil cell type (B_2 cell) which is also gonadotrophic and secretes, probably the follicle stimulating hormone; (4) chromophobic cells which are possibly the precursors of the basophils. The meta-adenohypophysis is composed of two cell types, the larger one being slightly PAS positive. The function of these cells remain to be established.

Correlative Cyclical Changes in the Gonads and Pituitary of T. mossambica

It is largely believed that the basophils of the meso-adenohypophysis of the pituitary are associated with reproduction in fish. However, Matthews (1936) suggested that the acidophils may be the producers of gonadotrophin, as he observed the domination of acidophils in the meso-adenohypophysis of Fundulus during the prespawning period. Olivereau (1954) reported the production of FSH by the basophils and LH by the acidophils of the meso-adenohypophysis. In the pituitary of the catfish, Mystus Sathyanesan and Singh (1963) reported that the site where acidophils were situated in the resting phase were occupied by basophils during the prespawning and spawning phases; and they were PAS positive.

Qualitative and quantitative studies on the cells of the meso-adenohypophysis in relation to reproduction have been carried out by several workers and the results produced by most of them agree with those found in T. mossambica in the present research. Scruggs (1951) recorded a 2:1 proportion of basophils to acidophils in the meso-adenohypophysis of Carrasius during the prespawning season. An increase in the quantity of the basophil cells was observed by Bretschneider and de Wit (1947) in Rdodeus at the time of spawning. The authors reported a transformation of acidophils into basophils at the spawning time, and then a return, again, to acidophility. They stated that if this transformation was true in all fish, the acidophils were to be considered as potential basophils, depending on their physiological state. The intermediate basophil stage, according to the authors, was "probably the actual working phase of the cell and represents the restitution phase of the products".

From further information provided by Copeland (1943) on Triturus, Buchmann (1940) on Clupea, Lee (1942) on Xiphias, Kerr (1948) on Leuciscus, Sathyanesan (1960) on Mystus, Sokol

(1961) on Fundulus, Poecilia, and Lebistes, Chieffi (1962) in Torpedo and Scyliorhinus, Robertson and Wexler (1962) on Salmo and Oncorhynchus, Sathyanesan (1963) on Hilsa, Barbus, Heteropneustes and Mystus, Lagios (1965 on Embiotoca, a general pattern of cyclical activity in the meso-adenohypophysis of fish can be drawn and be correlated with the cyclical changes in the gonads, in connection with maturation. The results obtained from the present study confirm those of previous workers that during the reproductive cycle there is a gradual accumulation of basophils in the meso-adenohypophysis of the pituitary. In T. mossambica the peak accumulation is reached at the prespawning phase (Fig.1 and Plate 14c),at which time the cytoplasm of the cells is packed with secretive granules. Soon after spawning, a considerable depletion in the basophils occur and the chromophobic or weakly acidophilic cells are found in place of the basophils. However, not all the basophils get depleted after spawning. Moreover a rapid recrudescence takes place resulting in an increase in the basophils.

While a general agreement exists regarding the increased accumulation of basophils at the time of spawning, there is a less agreement on the origin of these cells and their mode of transformation into the secretive basophils. Two views are held by workers on this issue. Gerbibkii (1940) provided evidence of the transformation of chromophobes into acidophils, which, at the time of spawning are transformed into basophils. On the other hand, Matthews (1936) D'Ancona (1951) and Bretschneider and de Wit (1947) maintain that basophils are formed from acidophils. Recently Sathyanesan and Singh (1963) gave evidence in support of the latter view. This subject, however, has been discussed in the previous chapter.

The Effects of Low Temperature and Total Darkness on the Reproductive System in T. mossambica

The role of environmental temperature and light regimes in the regulation of the reproductive cycle of teleosts has been a subject of investigation by numerous workers. The results obtained so far are so equivocal that it is still difficult to make general conclusions covering all the teleostean species. In preparing the review on the subject, a whole range of light intensities, photo-periods, and temperatures have been considered, even though in the experimental work, these two factors, i.e. temperature and photo regime, were investigated only on one extreme case in which there was little existing information.

With this point in view, Bullough (1939) could be regarded as one of the earliest workers in this field. The author maintained Phoxinus in constant darkness for 58 days at a temperature of 17°C, and failed to observe any difference in the reproductive activity of the experimental fish from those of the controls maintained at the same temperature in daylight. In contrast, Rasquin (1949) reported that 2-month old Astyanax maintained in darkness for 2 - 10 months retained the same ratio of basophils to acidophils, in the meso-adenohypophysis, as those of immature fish, and the gonads of these fish also reduced in size. Rasquin and Rosenbloom (1954) observed an endocrine imbalance in Astyanax kept in complete darkness. The pituitaries, according to the authors, were hypofunctional and contained fewer basophils than those found in normal fish of the same age but reared under conditions of recurring light and darkness. Similar information was given by Atz (1953) who also observed degranulation in the basophils of Astyanax reared in continuous darkness. All the three fish studied by the worker, had completely undifferentiated gonads.

Oryzias seemed so sensitive to light that there was a lack of gain in weight of the ovary when the fish were maintained

only for 17 days in total darkness (Egami, 1959). Sequin (1957) observed a 2 month delay in the spawning of Salvelinus maintained in dark hatchery.

Contradictory reports similar to that provided by Bullough (1939) on the failure of complete darkness to affect the reproductive activity in Phoxinus, have also been presented by Bjorklund (1958) who kept immature male Carassius in darkness for 10 months and females nearly 4 months and, still observed maturation. Recently, Pang (1969) has given striking information on male Fundulus maintained in complete darkness for nearly 10 months at 20°C: the gonadosomatic index of the experimental fish was significantly higher, correlated with higher nuptial coloration and flowing sperm. Histological studies of the testis, according to the author, indicated a reproductively active testis compared to the controls which were on the onset of sexual regression. Pang (1969) however, did not explain why the testis of the controls were on the onset of sexual regression. It is therefore not clear whether the control males matured first, but due to the absence of spawning partners (females) the spermatozoa in their testes showed signs of resorption at the time of sampling, or the presence of recurring light and darkness in the controls actually had retardatory effects on spermatogenesis. The main point in Pang's report, which is undisputed and interesting, is the failure of complete darkness to abolish the development of the testis in Fundulus. But by going further in comparing the gonadosomatic index of the experimental group with the controls, as well as the level of spermatogenesis in the two groups, without taking into consideration, the circumstances analysed above, that the regressive signs noticed in the testis of the fish, which could also have resulted, simultaneously, in a low GSI and might have been the result of the males having waited for too long without a spawning partner, does not permit a comparison at that level.

The evidence presented above, by different workers, demonstrates clearly the presence of species specificity in the reaction of fish towards an environment of complete darkness.

Low temperature, however, seems to have a fairly consistent effect on gametogenesis in teleosts. Egami (1954) observed an almost complete inhibition in growth of oocytes and a delay in egg-laying in Oryzias subjected to a low temperature of 4 - 8°C for only 2 - 24 hours; the optimum temperature of the fish, in its natural habitat, being 20 - 25°C. Carassuis which usually breeds at a temperature of 22 - 23°C did not spawn when Butler (1940) kept it at 9°C. However, pituitary injection into another group of fish maintained at the same low temperature resulted in (as compared with untreated Carassuis, also at 9°C), spermatogonial and first maturation divisions.

Misgurnus was induced to spawn at a temperature of 2°C (lower than the usual spawning temperature of 10 - 12°C) with a gonadotrophin (Kazanskii and Persov, 1949). Kazanskii (1951, 1952) maintained Gasterosteus at 4 - 6°, 9 - 13°, and 20 - 22°C under continuous illumination and observed spawning in the warmest group after three weeks when the other groups did not show any signs of approaching the spawning condition. Another experiment with two groups of fish at 12 - 13° and 17 - 18°C for two months, yielded similar results - spawning only in the warmer group. However, females injected with pituitaries ripened at 10°, 12-13°, and 16.5°C.

From the evidence outlined above, it seems that the pituitary is the target organ which is primarily affected by low temperature. This is evidenced by the fact that fish maintained at low temperatures have been induced to ripen with extracts of gonadotrophin. This indicates that gonadotrophin secretion was abolished in these fish, at low temperatures.

In the present study gametogenesis has been halted under conditions of low temperatures and total darkness. Mitotic divisions of the germ cells and their transformation into oocytes and spermatocytes were abolished. These observations are in agreement with those of the previous workers. Yolky oocytes underwent atresia and protoplasmic ones entered into a resting state. In the protoplasmic oocytes the accumulation of organelles around the nucleus - the circumnuclear ring; and at the side of it, could be regarded as a physiological adaptation of the oocytes in connection with the persistance of unfavourable environmental conditions. That these accumulations are organelles, was demonstrated by Gerbilskii (1937) who also observed an arrest of gametogenesis in Cyprinus, subjected to low temperatures.

The similar observations made on the gonads of fish maintained at low temperatures and total darkness in the present study, indicate a physiologically parallel effect caused by ecologically different environments.

Low temperatures of 13-15^{o}C also retarded the formation of basophils in the meso-adenohypophysis, while the same region of the pituitary of the control fish was packed with granulated basophils. In the meso-adenohypophysis of the fish maintained in total darkness, the same retardatory effect, but even to a greater degree, was observed. As a result, there were no basophils at all in any of the pituitaries examined. Endocrine imbalance, as observed by Rasquin (1949) and Rasquin and Rosenbloom (1954) in Astyanax was in evidence in the present case involving T.mossambica subjected to low temperatures of 13-15^{o}C and total darkness for 3 months.

At this stage there is evidence for the suggestion of a deficiency of gonadotrophin for the cessation of gametogenesis in T.mossambica.

It can therefore be concluded that in the context of reproduction, low temperatures (13-15^{o}C) and total darkness affect T.mossambica, by possibly, blocking the release of gonadotrophins from the pituitary which, subsequently, retards gametogenesis.

Effects of Gonadectomy on the Pituitary of Tilapia

The adoption of the method of surgical castration to determine the influence of the gonads over the gonadotrophic activity of the pituitary gland has, until recently, not been investigated. However, a review of the scanty literature has revealed unequivocal results that in teleosts, total gonadectomy results in modifications in the meso-adenohypophysis (Sokol, 1955; Screibman, 1962; Robertson and Wexler, 1962; McBride and van Overbeeke, 1969). In all cases, the basophils were found to be the cells which reacted to the operation as evidenced by hepertrophy, hyperplasia and degranulation in these cells. The low basophil count made in the pituitary of gonadectomized Lampetra at the height of the spawning season (Evennett and Dodd; 1963) supports a post-vacuolatory condition of the pituitary. Sokol (1955) even used this approach to demonstrate the presence of gonadotrophs and thyrotrophs in the meso-adenohypophysis of Lebistes reticulatus.

In the present study, gonadectomy in Tilapia resulted in gradual degranulation and eventual depletion of the meso-adenohypophysial basophils. Concomittantly, the acidophils of the same region were characterized by hypertrophy, hyperplasia and intense granulation. These features, which are similar to those reported by other workers in this field, demonstrate a break in the feedback mechanism which maintains the pituitary-gonadal relationship. In this way, the control which the gonad exerts over the gonadotrophic activity of the pituitary gland is established.

Effects of Gonadectomy on Secondary Sexual Characters and Behaviour.

Castration has varying effects on the secondary sexual characters and behaviour in fish. Numerous workers have reported an abolition of all traces of secondary sexual characters as a result of this operation, but others have observed the persistance of these characters after the operation. Pickford and Atz (1957), Dodd (1960) have extensively reviewed the literature and a few cases have been analysed in the review of the present study.

In Tilapia macrocephala, Aronson (1948, 1951), Levy and Aronson (1955) reported that castration abolished the secondary sexual characters, but nest building continued. Observations on other gonadectomized teleosts have revealed that behaviour is affected not in all fish as a result of the operation (Noble and Kumf, 1936, Baggerman, 1957). "Heightened awareness" has been reported in castrated fish (Noble and Kumf; 1936) in Hemichronus bimaculatus, Aronson (1951) in T.macrocephala, Tavolga (1955 in Bathygobius soporator.

In T. mossambica and T. aurea gonadectomy results in abolition of all the secondary sexual characters. This agrees with Aronson's observation, but cessation of spawning behaviour which was also observed in the two Tilapia species in the present study is in contrast to Aronson's report. However, a heightened awareness to the environment found by the previous worker in T.macrocephala was also found in T. mossambica and T. aurea.

These results suggest that in T. mossambica and T. aurea the gonadal steroids are responsible for the maintenance of the secondary sexual characters and spawning behaviour, whereas in T. macrocephala the secondary sexual characters may be steroid dependent but not the spawning behaviour; or specific androgens are required, in the latter species, for the maintenance of the secondary sexual characters, on the one hand, and the spawning behaviour, on the other hand.

Effects of Gonadectomy on Growth

Weight differences, following castration have been considered only in a few teleostean species. Aronson (1951) did not find any significant weight gains in castrated males and females over intact males but intact females gained considerably less weight. In Kokanee Oncorhynchus, Robertson (1961) reported of growth induced by castration. Similar observations were made on the sockeye Oncorhynchus (McBride, 1963; McBride and Overbeeke, 1969).

In the present study the two groups of gonadectomized T.mossambica both gained more weight than the sham-operated controls. However the mean weight gains of 16.3 gm in the males and 15.0 gm in the females of the first group of operated fish were far greater than the 5.9 gm per male and 9.2 gm per female in the second group. The cause of this difference in weight gained between the two operated groups could possibly be found in the conditions of the environments in the two groups of fish. However the weight gains in the totally castrated fish in both groups were significant ($p < 0.001$ for each group and $p < 0.001$ for each sex in each group) as compared to the controls, which gained only 1.7 gm per male and 2.0 gm per female.

It could be suggested, according to the above observations, that the hypertrophy, hyperplasia and granulation of the acidophils in the meso-adenohypophysis of the gonadectomized Tilapia reflected in the increased somatic growth of the fish, by the production of growth hormone.

Effects of Replacement Therapy on the Secondary Sexual Characters and Behaviour in Gonadectomized Tilapia

In elucidating the relations of the sex steroids to the sex accessories and secondary sexual characters, most workers have concentrated on the injection of homologous or heterelogous sex steroids into fish with intact gonads. Numerous reports, reviewed by Pickford and Atz (1957), Dodd (1960), have yielded successful results. It must be emphasized that since these experiments were performed on fish with intact gonads, they lack the critical quality which could be provided only by experiments involving castration and replacement therapy.

As a result of castration and androgen treatment Hoar (1962,a,b) concluded that the gonad (probably its steroid) was essential for nest-building and associated reproductive behaviour in Gasterosteus. Androgen treatment in the female was less effective (Ogunro, 1958; Hoar, 1962), as only two females showed this activity.

However, a more pronounced morphological sex reversal was obtained in gonadectomized Misgurnus anguillicaudatus (Kobayashi, 1951) by heterologous sex steroids.

In the present report, the reduced genital papilla in both sexes of castrated T. aurea became greatly increased in size as a result of TP treatment. However the male sex hormone caused a greater increase in size of the papilla in the males than in the females, 1.8 mm as opposed to 0.5 mm. The nuptial coloration was restored in the males, and even in the females masculinization which could only be attributed to the treatment, was observed. The males built nests, courted the females and showed aggressive behaviour.

In the castrates treated with Oestradiol benzoate, the size of the genital papilla similarly increased. In this instance, OB caused a greater increase in the length of the genital papilla in females, than in the males, 0.8 mm as opposed to 0.6. The usual male secondary sexual characters did not appear in the castrated OB-treated males. Spawning behaviour was not noticed even though the fish continued to show heightened activity.

The results of the present experiments indicate that the secondary sexual characters and spawning behaviour in T.aurea are androgen dependent, the absence of which, following gonadectomy, results in the abolition of these sexually dimorphic characters. On the "Methallibure" section, it has been proved that inhibition of the gonadotrophic factor in the pituitary also results in the disappearance of these characters, which are also reinstated with male sex steroids. Taking the results of these two different experiments into consideration, it can be concluded that in T. aurea the regulation of the secondary sexual characters and spawning behaviour by the male sex steroid is mediated through the pituitary. The results further indicate that the administration of heterologous sex steroids into gonadectomized Tilapia causes morphological sex reversals.

The Effects of Methallibure on the Reproductive System in Tilapia

1) Effects on Secondary Sexual Characters and Behaviour

Secondary sexual characters, such as nuptial coloration, and sex accessories, such as gonoducts failed to develop or showed a considerable degree of regression in hypophysectomized fish (Vivien, 1938, 1941; Burger, 1942; Pickford, 1953). Pandey (1969) however, reported that in the hypophysectomized male Poecilia, the gonadopodium (modified anal fin) remained unaffected after hypophysectomy, but the patches of bright lipophores (yellow and red pigments) present on the sides of the body became faint or entirely disappeared. In the cyclostome, Lampetra fluviatilis, Dodd et al (1960) reported that hypophysectomy in both sexes as in Actinopterygii, completely abolished the secondary sexual characters.

Like the effects of surgical hypophysectomy, Methallibure inhibited the growth of the border segments of the kidney tubules in Gasterosteus, a secondary sexual character which increase in thickness under the influence of male gonadal hormones (Hoar, et al, 1967). The fleshy modifications on the male anal fin (secondary sex structures which also develop under the influence of the male steroid) were in a state characteristic of immature fish, after treatment of Cymatogaster with Methallibure (Wiebe, 1968).

In the present study, Methallibure abolished the secondary sexual characters in the two species of Tilapia. Only after a few days of treatment the males became sexually indistinguishable from the females or from immature fish of both sexes. The genital papilla also reduced greatly in size. These results are in agreement with those of the earlier workers who conducted surgical hypophysectomy or treated the fish with Methallibure.

Spawning behaviour - courtship and aggresiveness were also abolished as a result of the treatment. Similar observations were also made by Tavolga (1955) in the hypophysectomized Bathygobius.

2) Effects on the Gonads

Surgical hypophysectomy has been attempted in a relatively few species of fish, and even fewer studies have been made on these fish in the context of reproduction. The older literature includes the work of Vivien (1938, 1941) on Gobius, Matthews (1939) on Fundulus and Burger (1941, 1942), also on Fundulus. More recent investigations are those of Barr (1963) on Pleuronectes, Ahsan (1966) on Coyesius, Sundararaj and Nayyar (1967) on Heteropneustes, Lofts et al. (1966, 1968) on Fundulus, Sundararaj et al (1968) on Heteropneustes, Yamazaki and Donaldson (1968) on Carassius, Pandey (1969) on Poecilia and Larsen (1969) on Lampetra.

All investigators believe that hypophysectomy is followed by regressive changes in the gonads. However, the precise stage of development which is affected by the operation seems to vary considerably in different species. Barr (1963) suggested that the first meiotic division is the one that is inhibited, while Ahsan (1966) believed that the critical stage is the division immediately preceeding the transformation of secondary spermatogonia into primary spermatocytes. Sundararaj and Nayyar (1967) observed an arrest of spermatogenesis at the spermatogonial level, while Pandey (1969) subscribed to the observation of Sundararaj and Nayyar. In Lampetra, Dodd et al. (1960) reported that hypophysectomy acts mainly on the spermatogonial stages of the testis, and, possibly, also on those cells which are in the process of transformation into primary spermatocytes.

It is also clear that when maturation is well advanced at the time of hypophysectomy the process may continue to the end (Dodd, 1960; Barr, 1963; Ahsan, 1966; Sundararaj and Nayyar, 1967; Pandey, 1969). With time, as a result of necrotic changes, the testes become depleted of spermatocytes, spermatids, and eventually, spermatozoa. These stages may persist for long periods of time, as Barr (1963) observed spermatozoa in regressed testes in three of the

10 hypophysectomized Pleuronectes, 130, 175 and 222 days after hypophysectomy. Sundararaj and Nayyar (1967) observed residual sperms in the testis of Heteropneustes 337 days after hypophysectomy. In Lampetra, hypophysectomized in January, Dodd et al (1960) reported the presence of sperm when the testes were examined in April.

The results of Methallibure treatment on the reproductive activity of the testes of fish appear to be entirely comparable to those of surgical hypophysectomy. It is agreed, however, that this approach is new and reports on the treatment of fish with this compound are extremely few, but the results, obtained so far, leave little doubt regarding its pituitary-gonadotrophic-inhibiting function.

The data for Carassius, Gasterosteus and Cymatogaster were consistent in showing that methallibure effectively blocks the pituitary gonadotrophic action (Hoar et al., 1967). In all the three species studied, Hoar and his collaborators observed a regression of the testes and an interference with their differentiation as a result of Methallibure treatment.

Wiebe (1968) presented similar reports on the inhibition of pituitary gonadotrophic activity in Cymatogaster. This time 1 ml. of approximately 10% methallibure in suspension was added to the 100-litre aquaria five or six times each week. While Hoar et al.(1967) recorded high mortality when the compound was added to the ambient water or implanted intraperitoneally as pellets in Carassius and Gasterosteus, Wiebe (1968) recorded a 100% survival when the compound was added to the aquaria.

Reproductively, spermatogenesis was effectively halted after 4-6 weeks of treatment, and most of the lobule cells, according to Wiebe, were in the premeiotic stages. The author further noticed that on the 40th day of treatment, 96% of the cell type of the lobule area of the testes was composed of spermatogonia.

The remaining 4% was shared, more or less equally, by primary spermatocytes, secondary spermatocytes and spermatophores. Spermatids and spermatozoa were completely absent.

On the effects of Methallibure on the testes of T.mossambica and T.aurea, results similar to those produced by Dodd et al. (1960), Barr (1963), and Sundararaj and Nayyar (1967) were obtained, regarding the persisting spermatozoa in the testis of hypophysectomized fish. In Methallibure-treated T.mossambica, sperm persisted in the testis until the 35th day of treatment. In T.aurea sperm was present in the testis on the 30th day of treatment, and judging from their number it was obvious that they would survive even longer than those in the testis of T.mossambica did.

That spermatozoa persist for some time in the testis of Methallibure-treated Tilapia is certain, but their periods of persistance, probably, depend on the temperature of the water. A similar suggestion was made by Lofts et al. (1968) regarding the persisting sperms in the testis of hypophysectomized Pleuronectes, observed by Barr (1963).

The effect of Methallibure on the testes of Tilapia was already recognizable on the 10th day as many intermediate cells had disappeared from the testis and spermatogonia had become numerous. By the 20th day of treatment almost all the primary spermatocytes, and, definitely, all secondary spermatocytes and spermatids had degenerated from the testis; and on the 25th day the organ was dominated only by numerous resting spermatogonia and some spermatozoa.

The observation on Tilapia leads to the conclusion that Methallibure causes degeneration of all cells in the testes beyond the spermatogonial stage. However, ripe sperm, already present in the testis at the time of treatment persist for some time before degeneration.

Hypophysectomy in female fish has yielded unequivocal results. Ovarian involution and follicular atresia following the removal of the pituitary has been recorded by Vivien (1939) on Gobius, Matthews (1939) on Fundulus, Barr (1963) on Pleuronectes and Sundararaj et al. (1968) on Heteropneustes. In all the reports, the period of transformation of protoplasmic oocytes into vitellogenetic ones, seems to be the critical stage at which the effect of the operation is felt. This period is essentially the same as the early yolk-vesicle stage observed as the critical stage in other species (Regnier, 1938; Vivien, 1952, on Xiphophorus; Belsare, 1965 on Ophiocephalus). In Lampetra, however, Dodd et.al (1960) and Larsen (1965; 1969) reported the presence of yolky oocytes in the ovary of the hypophysectomized fish. In fact, the authors did not see any signs of atresia. In any case, growth of the oocytes was abolished and ovulation never occurred.

In female Tilapia Methallibure caused a regression of the ovaries. All yolky oocytes underwent atresia and only oogonia and protoplasmic oocytes eventually remained in the ovary. Hoar et al. (1967) observed similar atretic changes in the ovaries of the three teleostean species he treated with Methallibure. The observations on female Tilapia were also in agreement with those made in the ovaries of hypophysectomized fish already discussed, with the only exception of those of Dodd et.al (1960) and Larsen (1963; 1965) on Lampetra, one of the two living representatives of the first vertebrate class to appear in the fossil record.

It can therefore be concluded that Methallibure acts on the ovaries of Tilapia by abolishing all stages of development beyond the protoplasmic stage.

3) Effects on the Pituitary

The effects of hypophysectomy on the gonads of fish have now been clearly established. The few reports on the effects

of Methallibure on the gonads of fish seem to be similar to that of a surgical hypophysectomy. In the second approach, it is believed that the compound (Methallibure) acts on the pituitary by blocking its gonadotrophic action, resulting in the regression of the target organs (the gonads). Observations on the histological changes in the meso-adenohypophysis of Methallibure-treated fish, which have not been reported by any worker before, have, to some extent, thrown light on the process, and possibly, the degree, of inhibition.

In the pituitary of Tilapia, the changes in the basophils as a result of Methallibure treatment are correlated with regressive changes in the gonads. This confirms once more, the evidence that the basophils of the meso-adenohypophysis of Tilapia are gonadotrophs. Secondly, the depletion of the basophilia of these cells as a result of Methallibure treatment, accompanied not by degranulation and vacuolation, but by a gradual reduction in the staining intensity possibly indicates that the compound (Methallibure) prevents the release of the gonadotrophins already present in the cells at the time when treatment began, and also, prevents their further synthesis.

The Effects of Different Modes of Methallibure Treatment on the Gonads in Relation to the Gonadosomatic Index

From the graph presented in Fig 4 it is evident that oral Methallibure treatment of T.aurea had a quicker effect on the gonads than external treatment. The results were as expected since the compound, as a result of oral treatment, was probably absorbed immediately and went into the circulation more quickly than when it was added to the aquaria.

The increase in the GSI of the orally-treated fish within 10 days could be correlated with the hypertrophied and hyperplasic condition of the atretic oocytes in the ovaries. In the externally-treated fish the peak GSI was reached only on the 30th day of treatment, when the ovaries revealed conditions similar to that observed in the 10-day orally-treated fish with a high GSI.

In Fig 5 the GSI of the orally-treated fish was on the declining side 10 days after treatment; while that of the externally-treated fish was at its peak. It must be emphasised that the fish used in this group were smaller than those in the previous group (compare weights of fish in Appendix XI with Appendices XII and XIII) It is therefore possible that the same dose of the compound had a more rapid effect on the smaller fish than the big ones. If this is the case, then it could follow that, the peak GSI in the orally-treated, smaller fish was reached before the 10th day when the first sample in the treated groups was taken, after which, as a result of advanced atresia in the ovary (resorption of yolk), the GSI declined. In the externally-treated, smaller fish, the same reason, that the same dose of Methallibure might have acted quicker in the smaller fish, might have accounted for the earlier attainment of the peak GSI which was correlated with hypertrophied and hyperplasic condition of the atretic oocytes in the ovaries.

The graph in Fig. 6 demonstrates clearly the direct decline in the GSI of the testes as a result of Methallibure treatment. In the testes of these fish necrotic changes, which resulted in the

abolition of all the intermediate spermatogonial cells, and eventually, the spermatogonia, were evidenced on microscopic examination of slides of the testes.

The results of these investigations suggest that (1) oral-treatment of Tilapia with Methallibure has a more rapid effect on the gonads than external treatment, (2) smaller fish are affected sooner by the compound than larger fish at the same dosage.

The Secondary Effect of Methallibure on Growth in T.aurea in Ponds.

Methallibure treatment of fish, even though it has been reported only in three species, has apparently never been investigated in the context of growth.

The evidence presented on Table 19 revealed that after 40 days of Methallibure treatment in tanks, the experimental fish, only after two weeks of transfer into ponds grew three times bigger than the controls - by 45 gm per fish as compared with 15 gm in controls. The experiment, unfortunately, had to be terminated because of the mortality which occurred in the experimental pond.

Experiments 2a and 2b (Table 19) could not be conducted for reasons beyond my control, earlier than Autumn, and the fish had to be treated for 3 months in tanks before being transferred into the ponds. The water temperature of 26-21°C at that time, was not conducive to Tilapia for spawning. The fall in temperature could possibly have accounted for the small weight increase of 52 gm. per female in the experimental group, as compared with 27 gm. in the controls during a period of seven weeks. The experimental males seemed to have been more susceptible to the declining temperature than the females as, in spite of the bigger weight increase, the comparative weight gain of 81 gm. was less than in the controls which gained 93 gm. per fish. It must be borne in mind that Tilapia males grow faster than females.

The second experiment was conducted in the same ponds as the first. Since there was a 100% survival, it could be suggested

that Methallibure was possibly not the cause of mortality in the first experiment.

During the experiments the control fish spawned frequently. The spawning of one experimental female fish occurred only at the end of the seventh week.

The results suggest that Methallibure had a secondary effect on growth in female T.aurea and postponed the onset of spawning by some seven weeks after the treatment has stopped. So long as treatment continues spawning never occurs.

The ~~imposing~~ important question now is how the increased growth was brought about ?

It is not likely that the increased growth was brought about through an excess secretion of growth hormone. The answer could, most likely be found in the energy utilization in the fish. According to Smith (1969, personal communication), working on energy utilization in Tilapia, the energy available for somatic growth in normal Tilapia is drastically reduced by gametogenesis. The interesting point is that Smith found the reduction in the energy for somatic growth to be more drastic in the females than in the males. It therefore seems that, in the present study, the increased somatic growth realized in the females after Methallibure treatment was, most likely, due to the divertion of a substantial part of the energy available for gametogenesis, towards somatic growth.

The Effects of Unilateral Ovariectomy on the Remaining Gonad in T. aurea.

Hemicastration in female teleosts has been reported in a number of species (Bock, 1928b; Craig-Bennet, 1931, in Gasterosteus, Robertson, 1958, in Salmo gavidnerii, Goswami and Sundararaj, 1968 in Heteropneustes fossilis) All the workers agree that the operation is followed by a compensatory hypertrophy of the remaining ovary. In the detailed experiments of Robertson (1958) and Goswami and Sundararaj (1968) the workers even investigated those periods of development during which the compensatory hypertrophy of the remaining ovary is greatly realized.

The present study on T. aurea has provided information in support of the existing data, that as a result of unilateral ovariectomy the remaining ovary has the ability to grow rapidly and compensate for the loss of its contralateral counterpart. The present investigations have been concerned not only with an account of general weight increases and quantitative studies of the cell types of the ovary, but also with an analytical description of the functional mechanisms of the ovary contributing towards the manifestation of compensatory hypertrophy.

The first striking feature observed in the ovary of hemicastrated T. aurea was the large number of dividing oogonia. Mitotic division in the normal ovaries of Tilapia, unlike the testis, is rarely observed under the microscope. The presence therefore, of a number of mitotic figures in the oocytes of the unilaterally castrated fish could have been induced as a result of the operation, as a means of recruiting new oocytes to compensate for the loss of the contralateral ovary.

The second feature was the presence of protoplasmic oocytes with polymorphic nuclei. Increase in the nuclear metabolism, as a result of compensatory hypertrophy, could probably, have reflected on the morphology of the nucleus. It is obvious that

as a result of nuclear polymorphism, the surface area of the nucleus is increased, and consequently, the rate of nuclear metabolism increases enhancing increased growth of the protoplasmic oocytes - hence the presence of oocytes with polymorphic nuclei in the ovary of unilaterally gonadectomized Tilapia.

The ovarian cell wall of the hemcastrated fish, most likely, contributes towards the manifestation of compensatory hypertrophy. The zona radiata becomes unusually conspicuous, with visible radial canals. Mitotic figures in the granulosa cells and the appearance of a second layer of cells in the same zone, as well as the zone of connective tissue reveals the hypertrophic and hyperplasic activities of the cells. These features of the cell wall of the vitellogenetic oocytes of hemicastrated fish could be regarded as adaptations enhancing the additional supply of trophic subtances to the oocyte, to cope with the ovarian compensatory hypertrophy.

From the evidence presented above, it could be concluded that in T. aurea compensatory hypertrophy of the remaining ovary, as a result of unilateral ovariectomy, is manifested in the following ways :

(1) increase in the number of dividing oogonia
(2) increase in the metabolic activity of the protoplasmic oocytes as reflected in the polymorphism of the nuclei.
(3) hypertrophy of the ovarian cell wall enhancing the additional supply of trophic substances to the oocyte.

Effects of Methallibure Treatment of Unilaterally Ovariectomized T. aurea.

In the present study, partially ovariectomized female T. aurea were used to test the prolonged pituitary blocking effect of Methallibure against the compensatory hypertrophy of the remaining ovary.

The data on Table 20 and Fig 7 show that beginning with day 15, Methallibure inhibited the compensatory hypertrophy

of the remaining ovary. However, histological examination of slides of the ovaries of fish sampled at five day intervals revealed that the compensatory hypertrophy resulted from the operation lasted between the fifth and 10th post-operative day. From about the 10th day regressive changes, due to Methallibure treatment, started. As Methallibure usually affects the ovaries by an initial increase followed by subsequent decreases in ovarian weights, in relation to the stages of atresia, so did it affect the remaining ovary of the unilaterally-gonadectomized fish - hence the presence of gonads with increased weights (not as a result of compensatory hypertrophy) in the remaining ovary, in connection with atretic changes.

These observations indicate that Methallibure has a prolonged blocking effect on the pituitary against compensatory hypertrophy of the remaining ovary, as a result of unilateral ovariectomy in T. aurea.

The Effects of Exogenous Hormones on the Gonads, Secondary Sexual characters and Behaviour in Tilapia

It is well documented that the pituitary gland of higher vertebrates elaborates two different hormones which act on the gonads, and may also catalyze the behaviour associated with reproduction. Follicle stimulating hormone stimulates spermatogenesis in the male and ovarian development in the female. Luteinizing hormone stimulates the androgen-producing cells of the testis and oestrogen-producing tissue of the ovary.

The evidence in support of the presence of two gonadotrophic hormones is satisfactory in all higher vertebrates, but a distinct FSH has not yet been recognized conclusively in fish (Hoar, 1965a,b) However, LH when injected into fish has frequently been shown to produce effects which are attributed to two distinct hormones in the higher vertebrates (Pickford and Atz, 1957; Dodd, 1960; Ahsan and Hoar, 1963).

Ahsan (1966) on the basis of experiments on hypophysectomized Coquesius, found mammalian LH to be equally effective as a combination of FSH and LH or purified fish pituitary, and concluded therefore that the pituitary control of reproductive processes in the male depended only upon an LH-like protein. These results suggest the similarity of fish gonadotrophin and mammalian LH.

The subject is further complicated by the fact that when extracts of fish pituitaries are injected into higher vertebrates, they show effects usually attributed to LH, as well as FSH- like action. Results of bioassays, using different fish pituitaries on higher vertebrates (the rat vaginal cornification test and the weaver-finch test) revealed the presence of both FSH and LH. However, the FSH activity was always lower than LH (Witschii, 1955). There are other reasons for suspecting the presence of two gonadotrophins in fish pituitary. The fact that pituitaries when injected into fish with immature eggs result, frequently, in ovulation (Pickford

and Atz, 1957), indicates the presence of two different gonadotrophins, since FSH stimulates ovarian development and LH induces ovulation. Similarly, the induction of vitellogenesis in the primary oocytes of hypophysectomized fish as a result of injection of pituitary material (Barr, 1963), suggests the presence of an FSH-like hormone.

Ball (1960) considered the phenomena of follicular growth and ovulation in fish and suggested that two pituitary gonadotrophins would provide a better control of these processes than a single factor. The very process of ovulation, which, in most species, is in response to a particular stimulus provided by the environment or by the male partner (Pickford and Atz, 1957) would demand a separate gonadotrophin for a better control. The frequency of ovulation, which is usually low in an isolated Tilapia macrocephala, increases greatly at the sight of a male (Aronson, 1945, 1948). Ball (1960) compared this phenomena of frequency of ovulation in Tilapia, with other cases in other species and concluded that a two-component control system, with two pituitary gonadotrophins appears to be necessitated by the need to retain fully-grown eggs in the ovary until the appropriate situation for their ovulation is presented. Finally, the presence of two gonadotrophins in the meso-adenohypophysis of teleosts has been demonstrated histochemically. The subject has been already discussed in the appropriate chapter.

The results of the present study seem to be in support of the view on the elaboration of two gonadotrophins in the pituitary of teleosts. It could be seen from Tables 21 & 23 that in the Methallibure-treated T.mossambica, a combination of FSH and LH consistently stimulated the regressed gonads in fish of both sexes. As a result, all the gonads, after 30 alternate days of treatment, were in the spawning condition. LH and FSH when injected singly were less effective. However, the fact that two males and two

females also matured under LH-treatment, is suggestive of a more pronounced effect of this hormone than FSH. Maturation in the three females and a male in the FSH-treated group might have been due to the small amounts of LH which may be expected even in the purified FSH preparation. That might even be the reason why the effect was better realized in the females, in which the two hormones play distinct roles, than in the males. However, the results are highly suggestive that the pituitary of T. mossambica elaborates FSH and LH as two separate gonadotrophins.

2. The Chorionic Gonadotrophins

The administration of chorionic gonadotrophins into intact fish has resulted in ovulation and spawning in a number of species. The literature has been exhaustively reviewed by Pickford and Atz (1957). Experimentally, the action of HCG in mammals is similar to that of LH (Hoar, 1966). Therefore the stimulatory effects of this hormone on maturation could well be ascribed to its LH-properties. However, Atz in Pickford and Atz (1957) pointed out that HCG is not identical in action to LH, and that it sometimes seems to act as a complete gonadotrophin.

Pregnant Mare Serum is generally considered to resemble FSH in its actions, although Evans and Simpson (1950) reported that it is also, like HCG, a complete gonadotrophic stimulant. The presence of an action similar to a mixture of FSH and LH in PMS has also been reported by Hoar (1966), who also pointed out that the predominant effect of PMS depends on the dosage.

In Gasterosteus with depressed gonadotrophic activity, Ahsan and Hoar (1963) reported that neither of the two chorionic gonadotrophins was as effective as pituitary LH in incuding maturation. However, the authors observed a comparatively higher activity in PMS than HCG. On the contrary, Sundararaj and Nayyar (1967) saw sufficient evidence in the effectiveness of HCG in restoring spermatogenesis in hypophysectomized Heteropneustes.

The presence of LH and FSH factors in both HCG and PMS was reflected in the results of the present experiments, in which the two chorionic gonadotrophins were both effective in stimulating the regressed gonads of T. mossambica to maturation. Ovulation, observed in two HCG treated fish might have been due to the dosage, which could have been greater than that of PMS.

3. Crude Carp Pituitary Extracts.

Several attempts have been made to bring sexually immature fish into full spawning conditions by the injection of extracts of fish pituitaries. While successful results have been achieved in many instances, there still exists a high degree of specificity. Most experiments of this nature were conducted on fish with intact pituitaries and so the results could always be open to criticisms. The literature has been extensively reviewed by Pickford and Atz (1957) Dodd (1960), Hoar (1957; 1963).

Injection of fish pituitary extracts into hypophysectomized, or reproductively inactive fish, has been a subject of recent investigations. Ahsan and Hoar (1963) maintained Gasterosteus under a photoregime which depresses the gonadotrophic activity and treated the fish with crude salmon-piauitary extracts. The authors observed varying degrees of stimulation of gonads of both sexes beyond the autumn condition of the controls.

The results obtained by injecting T.aurea, previously treated with Methallibure, with extracts of carp pituitaries, were entirely unexpected. Although there was no abnormal development of the ovary, oogenesis was unmistakably suppressed, as compared with the controls (Plates 35 and 36 (a) and Tables 25 and 26). The ovarian weights were also suppressed as evidenced by a much higher GSI in the controls over the experimental fish.

Surgical hypophysectomy, followed by replacement therapy, has proved that, in fish the beginning of vitellogenesis marks the stage beyond which the oocyte is dependent on pituitary

gonadotrophins. The phenomenon of species specificity also exists among fish themselves and also between fish and other vertebrates (Gerbilskii, 1938; Kazanshiii, 1940: Pickford and Atz, 1957; Dodd, 1960). In T.aurea which had been previously treated with Methallibure, it could be that fish pituitary extracts are ineffective in restoring maturation, at that regressed state of the ovary, or the organ, in that condition is possibly species-dependant in response to its stimulation with crude pituitary extracts.

Most incredible of all, was the histological picture of the testis of the carp-pituitary treated males (Plates 37(a)(b). For the first time, the actual morphology of the Sertoli cells was revealed. This could be due to the abnormal process of hypertrophy of the Sertoli cells and possibly, the necrotic changes in the germ cells. These were hypertrophied and granulated and were found, as usual, in the lobules, but more conspicuously in those lobules in which the germ cells had degenerated. Their physiological condition at the time, i.e. their state of hypertrophy and granulation, suggested a break in contact between them (the Sertoli cells) and the cells they nourish (the germ cells). It is therefore tempting, at this stage, to subscribe to the evidence of Lam and Leeson (1961) and Bowers and Holliday (1961) that the Sertoli cells nourish the germ cells.

Carp pituitary treatment of Methallibure treated fish also resulted in the suppression of spermatogenesis and of testicular weights. A high GSI, twice as much as that of the experimental fish was recorded in the controls. The suppression of spermatogenetic activity coupled with the abnormal development of the testis may well suggest inability to respond to foreign gonadotrophins, resulting in the formation of antihormones. The phenomenon of anti-hormones formation is not clearly explained in fish. However, Pickford and Atz (1957) described a few cases. Among them, the following could serve as examples : (1) the anomalous results obtained by

Palmer et.al.(1954), in which there was an actual reduction in the proportion of ripe Oncorhynchus during the course of one experiment, (2) the failure of Hasler, Meyer and Field (1939) to obtain ripening, even when control fish were becoming ripe, might indicate antihormonal activity. Immunological processes are known to occur in fish, and Pickford and Atz (1957) reported that it is obvious that formation of antihormones may have been one of the reasons for the failure of the treatment with gonadotrophins that involved several injections or implantations extending over an appreciable length of time.

In general, the suppression of gametogenetic activity, and the abnormal development of the testis in previously Methallibure treated T.aurea, as a result of carp pituitary injections could be attributed to the possible formation of antihormones.

4. Testosterone propionate

Numerous reports exist on the effects of androgens on gametogenesis in fish with intact pituitaries. These reports, which are so conflicting, have been reviewed in several places (Pickford and Atz, 1957, Dodd 1960; Hoar 1965) Hypophysectomy, followed by androgen treatment gives a much more satisfactory approach since the effects of gonadotrophins are eliminated, and the stage of gonadal development predetermined. However, the few reports available, with this approach in view, vary to such an extent that more investigations are required before a general statement can be formulated.

Burger (1942) disclosed that hypophysectomy in Fundulus led to a decrease in testis size by about 50%, with abolition of spermatogenesis and involution of the duct system. Androgen treatment caused testicular weight increase of one-fifth heavier than those of hypophysectomized controls. There was no striking spermatokinetic effect, but the duct system showed less severe involution. The author concluded that spermatogenesis was little affected by the male sex hormone. In contrast, Lofts et al(1966)

observed a marked stimulation of spermatogenetic activity after the administration of methyl testosterone. The efferent duct system was also strongly developed. Similar positive results were obtained by Sundararaj and Nayyar (1967) in the hypophysectomized Heteropneustes with the use of TP; while Pandey (1969) saw proof of stimulation of spermatogenesis in the hypophysectomized Poecilia treated with methyl testosterone.

In the higher vertebrates, the effect of androgen on the regressed gonads also vary. Bocabella (1963), Clermont and Harvey (1966) observed stimulatory effect of testosterone on the testis of hypophysectomized rats. On the contrary, Baser and Handi (1965) Basu (1968) observed a testosterone inhibition of spermatogenesis in hypophysectomized Rana pipiens and R. hexadoctyla respectively.

Recently Wiebe (1969) obtained a regression of the testis of Cymatogaster by Methallibure treatment and then administered methyl testosterone into these fish. According to the author, spermatogenesis was not stimulated by the male steroid hormone.

In T.aurea, the gonads are in a regressed state after 40 days of Methallibure treatment. A threshold dosage of 0.42 mg Testosterone propionate administered into the Methallibure treated fish resulted in a retardation of gametogenesis in fish of both sexes. In the control females, in contrast to the TP-treated fish, the ovaries contained yolky oocytes. The ovarian weights were also suppressed as a result of TP-treatment. In the Methallibure-treated males, TP also retarded spermatogenesis as evidenced by the comparatively few primary spermatocytal cells in the testis after 30 days of TP administration, whereas the germ cells in the testes of the controls were in the spermatid stage. Testicular weights were similarly suppressed as the testes of the controls were four times heavier than those of the TP-treated fish. These results are in agreement with those of Burger (1942), Wiebe (1969) in teleosts,

Basu and Nandi (1965), Basu (1968) in amphibians, and are contrary to those of Lofts et.al (1966), Sundararaj and Nayyar (1967), Pandey (1969) in teleosts, Boccabella (1963) Clermont and Hervey (1966) in rats.

5) The Secondary Sexual Characters and Behaviour

Full secondary sexual characters (the nuptial coloration in males and the genital papilla in both sexes) soon developed as a result of treatment with HCG, PMS, and a combination of FSH and LH. These observations strengthen the suggestion that the pituitary of Tilapia possibly elaborates two gonadotrophins. LH when injected singly was slower than the chorionic gonadotrophins, in restoring the secondary sex ual characters. However, these characters were restored quicker in the LH-treated group than the FSH-treated group. This observation, also augments the suggestion that LH, produced in the pituitary of Tilapia has probably a more pronounced effect than FSH. Spawning behaviour resumed in all the males which showed the secondary sex characters.

Unlike the purified gonadotrophic hormones, carp pituitary did not restore the secondary sexual characters in Methallibure-treated T.aurea. This could probably be due to the unusual observations made on the gonads, which are hard to reconcile except to suggest its connection with antihormone formation. Spawning behaviour was not noticed in any of the fish treated with carp pituitary.

Testosterone propionate restored the secondary sexual characters in fish of both sexes beyond the premethallibure treatment stage. The appearnace of slight red coloration on the borders of the fins of the females, suggests morphological feminization effect of the male sex hormone on the female. The males actively engaged in spawning behaviour and courted the females.

The results on the effects of exogenous hormones on the gonads, secondary sexual characters and behaviour in Methallibure-treated fish have yielded definite evidence regarding the extent and

nature of pituitary and gonadal control of some aspects of reproductive physiology in fish.

Dodd (1964) suggested that it is unlikely for a specific cellular process to be stimulated equally by a steroid and a protein. Consequently, it was suggested by Dodd and Wiebe (1968) that the gonadotrophic influence on spermatogenesis may be through the production of a specific steroid rather than directly. Sundararaj and Nayyar (1967) saw proof for the suggestion that the gonadotrophins act on the gonads indirectly, through the interstitial cells, since HCG-injection into hypophysectomized fish caused hypertrophy and secretive activity in the Leydig cells and resulted also in induction of spermatogenesis. Similar results were obtained when the workers injected TP into the hypophysectomized fish.

Wiebe (1969) observed an increase in the demonstrable hydroxysteroid dehydrogenases as a result of LH treatment and subscribed to the hypothesis that the pituitary controls androgen biosynthesis in the testis. The author, however, observed no stimulation of spermatogenesis as a result of methyl testosterone treatment, and concluded that it may well be that only a very specific androgen or a different dose is required to evoke the response.

In the present study, the stimulation of the regressed testes and ovaries to maturation in Tilapia, with gonadotrophic hormones, and the failure of a steroid hormone to bring about the same results, makes it possible to conclude that in Tilapia the pituitary exerts a direct influence on the gonads. It must be recalled that the best replacement therapy results were those obtained with the use of HCG, PMS, and FSH ~~and~~ + LH. The presence of both FSH and LH factors in the chorionic gonadotrophins has already been discussed. It is therefore most likely that the FSH present in the injected hormones would stimulate ovarian development directly and, subsequently, the LH, will, also directly, cause ovulation.

This hypothesis, on the one hand is in complete agreement with that of Dodd (1964) that it is unlikely for a protein and a steroid to mediate the same biological process. On the other hand, the hypothesis is in sharp contrast with the conclusion of Sundararaj and Nayyar (1967) and with the subsequent suggestion of Dodd and Wiebe (1968) of the indirect influence of the pituitary on maturation; viz, through the production of a specific steroid which acts of the gonads.

The views of Sundararaj and Nayyar (1967) and Dodd and Wiebe (1968) are shared in considering the effects of gonadotrophins on the appearance of the secondary sexual characters. These characters, which are androgen-dependent were also elaborated on the administration of gonadotrophins into Methallibure-treated fish, suggesting that the gonadotrophins stimulated the Leydig cells to produce specific androgens which caused the appearance of the secondary sexual characters, including the growth of the genital papilla.

It could therefore be concluded that while the gonads (most likely their steroids) are responsible for the elaboration of the secondary sexual characters in Tilapia, the whole process may be regulated by the pituitary.

Steroid and Succinate Dehydrogenase Reactions in the Gonads

The results of the present research into the 3B-hydroxysteroid dehydrogenase activity in the ovary of T. aurea is in agreement with the few reports in the literature. Bara (1965) on Scomber and Wiebe (1969) on Cymatogaster detected this histochemical activity in the follicular cells of the ovaries of the species studied. In T. aurea the presence of formazan deposits was found also in the follicular cells.

The presence of 3B - hydroxysteroid dehydrogenase activity in the teleostean testis has been identified, histochemically, in the interstitial cells (Lupo and Chieffi, 1963; Stanley et.al. 1965; Yaron, 1966; Bara, 1966; Livni, 1969, Wiebe, 1969). Histochemical analysis of the testis of T.aurea yielded evidence in support of the presence of 3B-hydroxysteroid dehydrogenase activity in the interstitial cells of Leydig.

A decrease in the histochemically demonstrable 3B - hydroxysteroid dehydrogenase activity detected in the testis of Methallibure-treated Tilapia agrees with the observation of Wiebe (1968,1969) on Methallibure-treated Cymatogaster. Niemi and Ikonen (1962), Baillie, Ferguson and Hart (1966) described similar reductions of steroid dehydrogenases in rat testes following hypophysectomy.

Succinate dehydrogenase activity found in the follicular cells of the ovary agrees with the findings of Livni (1969) on Mugil.

The results of the histochemical investigations are only suggestive and serve simply as a preliminary report.

GENERAL DISCUSSION ON THE IMPLICATION ON THE USE OF METHALLIBURE IN TILAPIA CULTURE

From the report presented on the growth of Tilapia in Israel, it can be seen that the nursing of two groups of Tilapia hybrids with initial weights of 5 and 12 gm. for 76 and 86 days respectively, yielded an average daily increase of only 0.93 and 1.68 gm per fish respectively (see Table 33). The selection and rearing of male hybrids either with carp or with carps and mullet together, for periods between 77 and 130 days yielded weight increases between 2.7 and 5.7 gm. with an average of 3.9 gm. per fish per day (see Tables 32 and 34 - 37).

During the growth periods of Methallibure treated Tilapia in ponds, the best results were obtained from an experiment lasting two weeks during which the environmental conditions favoured growth of the fish. The weight increases realized were 45.5 gm per fish, representing an average daily increase of 3.3 gm per fish. These results compare favourably with those obtained through the difficult method of monosex culture. Moreover, the latter results were obtained as a result of years of experimentation, during which the whole fish culture management was maintained at its optimum.

It is therefore encouraging that the preliminary experiments on the use of Methallibure in Tilapia culture have yielded such excellent results. No doubt, proper management of the ponds (optimal stocking rate, feeding, fertilization, etc) will be required in order to realize better growth. It is interesting to note here, that worse growth is realized not only from an overstocked pond, but also from one that is understocked - as the case was (understocking) in the present Methallibure experiments in ponds.

The fact that Tilapia readily accepts feed mixed with Methallibure (oral treatment) presents another advantage, which, together with the points analysed in the present section and observations made in the present research, makes it possible to make the

following suggestions on the use of the compound in Tilapia culture for the postponement of the onset of maturation and the rearing of larger fish.

Methallibure could be added directly to the pond as one of the ingredients of the pellets, thereby abolishing the restriction of the treatment only to tanks, before the fish are transferred into the ponds. In this way, the regressive effects of Methallibure on the gonads will be realized directly in the pond through feeding.

Investigation will need to be made into the time which the gonads will take to undergo complete regression, as a result of the treatment, and their recuperation to maturity as a result of cessation of the treatment. From such data a system of "on and off" periods of treatment can be calculated. During the "on" period the fish could be fed with Methallibure-included pellets. As a result, maturation would be abolished but normal growth of the fish would continue. During the "off" period, the fish could be fed with Methallibure-free pellets, and significant weight increases would be realized during the period of recuperation. Just before maturation, the treatment could be resumed, and stopped at the appropriate time again. These "on and off" periods of treatment could continue within a season, until the desirable size of the fish is achieved.

The Economy of Methallibure.

The compound has proved to be economical in use. Wiebe (1968) treated Cymatogaster with a 1.0 ml. volume per 150-litre aquaria of 10 gm. Methallibure suspended in 100 ml. distilled water. In the present study, the dosage was reduced to 1.0 ml. volume per 100-litre aquaria of only 1 gm. Methallibure suspended in 100 ml. distilled water. The rate of the effects on the gonads were the same at the different dosages.

Apart from the daily treatments of the fish, twice weekly

treatments were also tried, but the experiments, for reasons beyond my control, could not be carried out to the end. However, histological slides of the gonads of the fish sampled at the primary stages of the experiment revealed regressive changes similar to those found in the gonads of the daily treated fish. This suggests that it is even possible to cut down the dosage used, or the frequency of treatment.

Side Effects.

The possibility of side effects which Methallibure treatment might have on other tissues, were not investigated, but they are not ruled out. However, the fact that the fish is capable of spawning after the cessation of the treatment, is an indication that a greater amount (if not all) of the compound is excreted from the body.

In any case, the possible use of Methallibure in Tilapia culture, as a result of the present investigations, is being considered by the Inland Fishery Biologists of the Food and Agriculture Organisation of the United Nations, and so problems such as side effects (on fish and man) and palatability of the fish, as a result of the treatment, will be thoroughly investigated by them.

CONCLUSIONS

Laboratory and field studies in T.mossambica and T.aurea have revealed the absence of species specificity, on the broader aspects of gametogenesis. However, differences, such as the time one species takes to go through a particular stage in development, exist, and are determined by the age at which sexual maturity is attained.

As a result of histological and cytochemical studies, eight of the cell types of the pituitary have been identified. In the pro-adenohypophysis, the eta or prolactin cells were identified and their role in osmoregulation demonstrated. In the same region, the α_1-cells were identified and were associated with corticotrophin secretion.

In the meso-adenohypophysis, the only acidophilic cell type - the α- cell, was ascribed the functional significance of somatotrophin secretion. The two basophils were identified as gonadotrophs, while the chromophobes were speculated to be the percursors of the basophils.

Two acidophilic cell types were found in the meso-adenohypophysis. Their functional significance is not known.

During the reproductive system correlative cyclical changes takes place in the pituitary and the gonads of Tilapia. These changes are associated with maturation.

Low-temperatures between 13° and 15°C, and total darkness have an inhibitory effect on the development of the pituitary and gonads. Somatic growth is also retarded.

Total gonadectomy results in modifications of two cell types (the acidophils and the basophils) in the meso-adenohypophysis. The operation also results in increased somatic growth. Testosterone propionate restores the secondary sexual characters and spawning behaviour in males and reveals a masculinization effect on the females. Oestradiol benzoate stimulates the growth of the genital

papilla in both sexes but does not restore the nuptial coloration and spawning behaviour in the males.

Methallibure inhibits the pituitary gonadotrophic activity by blocking the release, and probably preventing the synthesis, of the gonadotrophic factors. The gonads undergo regressive changes as a result of Methallibure treatment. Injection of commercially prepared gonadotrophins stimulate the gonads to maturation.

Methallibure also abolishes the secondary sexual characters and spawning behaviour. These features are also restored with the administration of exogenous gonadotrophins, and also with androgen. The male steroid, however, does not stimulate maturation. The latter, therefore, is directly dependant on the pituitary. On the other hand, the secondary sexual characters and spawning behaviour are androgen dependant but regulated by the pituitary.

Methallibure postpones maturation in Tilapia and enhances somatic growth in the female after the treatment has stopped.

Unilateral ovariectomy results in compensatory hypertrophy of the remaining ovary. Methallibure treatment of unilaterally ovariectomized fish results in suppression of the compensatory hypertrophy, but only beginning from 5-10 days after the operation.

The follicular cells of the ovary and the interstitial cells of Leydig of the testis are probably the hormone-producing cells in Tilapia.

Methallibure could possibly be used in Tilapia culture to restrict spawning and for realization of increased growth. However, a thorough system of application and management in ponds should be investigated, together with a research into the side effects, which the compound might bring about.

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Appendix I. Effects of Low Temperature and Total Darkness on Growth and Maturation in Female T.mossambica

Group	Wt. of Fish (gm)	Length of fish (cm)	Mean size of advanced oocytes ( )	Stage of maturation
Initial Control	6.9	7.7	713	III
	3.2	6.2	285	II
	5.5	6.8	502	II-III
	4.0	5.2	310	II
	5.8	7.0	665	III
	3.3	6.0	290	II
Final Control	7.5	8.0	968	IV
	7.9	8.1	993	IV
	8.8	8.9	1102	IV
	5.2	6.6	896	IV
	8.5	8.6	1008	VI-III
	7.5	7.7	932	VI

NOTE: Values for oocyte sizes represent mean measurement per ovary present over an area of six cross sections.

Appendix II Effects of Low Temperature and Total Darkness on Growth and Maturation in female T.mossambica

Group	Wt. of Fish (gm)	Length of fish (cm)	Mean size of advanced oocytes ( )	Stage of Maturation
Low Temperature	2.2	5.5	427	II
	2.3	5.8	447	II
	6.8	8.0	1005	Atretic
	1.2	4.4	325	II
	1.3	4.6	347	II
	1.4	4.4	444	II
Total Darkness	1.6	4.9	236	II
	1.4	4.4	175	II
	2.3	5.5	283	II
	2.5	6.0	227	II
	2.4	5.0	362	II
	2.2	5.0	250	II

NOTE: Values for oocyte sizes represent mean measurement per ovary present over an area of six cross sections.

Appendix III. Effects of Low Temperature and Total Darkness on Growth and spermatogenesis in individual *T.mossambica*

Group	Wt.of Fish (gm)	Length of fish (cm)	Spermatogenetic condition of testes				
			SG	PS	SS	ST	S
Initial Control	1.6	4.9	46	30	12	34	-
	1.8	4.9	36	27	21	56	-
	2.4	5.3	36	36	16	44	-
	3.5	6.3	60	28	24	68	-
Low temperature	1.1	4.5	108	2	-	-	-
	1.2	4.4	100	-	-	-	-
	2.1	5.5	96	3	-	-	-
	1.8	5.0	73	2	-	-	-
Total Darkness	1.3	4.9	124	-	-	-	-
	1.7	5.2	89	-	-	-	-
	2.0	5.2	103	-	-	-	-
	1.8	5.0	112	-	-	-	-
Final control	7.5	8.2	8	36	7	13	56
	7.6	8.4	9	20	20	40	39
	9.0	8.7	3	4	5	12	64
	10.7	9.4	1	8	6	10	76

NOTE: Values for spermatogenetic types are averages of each germinal stage in five cross sectioned lobules.

Appendix IV. Effect of Gonadectomy on Growth in male T.mossambica

Group	Before operation		50 days after operation			
	Wt.of Fish (gm)	Length of fish (cm)	Wt.of Fish (gm)	Length of Fish (cm)	Weight gain (gm)	Increase in in length (cm)
1	19.7	11.2	34.0	12.8	14.3	1.6
	14.7	9.8	32.4	12.5	17.7	2.7
	18.5	10.1	36.0	13.1	17.5	3.0
	18.4	10.6	34.0	13.0	15.6	2.4
	19.7	11.4	35.9	13.0	16.2	1.6
2	6.3	8.0	8.8	8.4	2.5	0.4
	11.0	9.0	17.6	10.5	6.6	1.5
	12.2	9.3	18.0	10.6	5.8	1.3
	19.4	11.4	26.5	12.4	7.1	1.0
	15.4	9.8	22.9	10.9	7.5	1.1
3*	8.9	8.5	10.1	9.3	1.2	0.8
	9.6	8.7	10.7	9.0	1.1	0.3
	14.1	9.8	17.0	10.4	2.9	0.6
	11.0	9.2	13.7	9.6	2.7	0.4
	5.9	7.5	6.4	7.6	0.5	0.1

* Control group

Appendix V. Effect of Gonadectomy on Growth in female T.mossambica

Group	Before operation		50 days after operation			
	Wt.of Fish (gm)	Length of Fish (cm)	Wt.of Fish (gm)	Length of Fish (cm)	Wt.gain (gm)	Increase in length (cm)
1	10.0	9.4	23.0	11.4	13.0	2.0
	12.9	9.6	28.0	12.0	15.1	2.4
	14.6	9.5	30.2	12.1	15.6	2.6
	13.5	10.0	30.0	12.3	16.5	2.3
	11.0	9.3	25.8	11.8	14.8	2.5
2	12.5	9.3	20.0	10.9	7.5	1.6
	10.2	8.8	16.5	9.9	6.3	1.1
	14.0	9.2	25.1	11.9	11.1	2.7
	15.6	10.1	27.1	12.1	11.5	2.0
	12.8	9.5	22.1	11.1	9.3	1.6
3*	8.5	8.3	10.5	9.2	2.0	0.9
	9.9	8.5	12.1	9.3	2.2	0.8
	5.5	7.2	7.1	8.0	1.6	0.8
	6.0	7.5	8.3	8.5	2.3	1.0
	6.7	7.7	8.4	8.6	1.7	0.9

* Control group

Appendix VI Effects of Gonadectomy and of Steroid administration on the Genital papilla in male T. aurea

Wt. of fish (gm)	Length of fish (cm)	Treatment	Length of genital papilla (mm)			
			Before operation	40 days after operation	30 Days after treatment	Increase/loss in length of papilla after treatment
89.0	17.5	TP	2.95	2.80	4.90	2.10
89.5	17.5	"	3.65	3.05	5.60	2.55
77.0	16.9	"	3.10	2.60	5.40	2.80
96.5	18.0	"	2.00	1.70	2.80	1.10
88.0	17.5	"	3.25	3.05	4.15	1.10
84.0	17.4	"	3.90	3.55	4.70	1.15
83.0	17.3	OB	3.50	2.95	4.05	1.14
90.0	17.8	"	3.00	2.85	3.20	0.35
90.0	17.5	"	3.10	2.95	3.32	0.37
119.0	18.6	"	2.40	2.00	2.45	0.45
100.0	19.2	"	2.90	2.75	3.00	0.25
116.0	19.3	"	2.50	2.25	3.05	0.80
90.0	18.0	AO*	2.85	2.70	2.51	-0.19
80.5	17.5	"	2.91	2.72	2.50	-0.22
92.0	17.4	"	2.95	2.70	2.60	-0.10
72.0	16.8	"	2.60	2.44	2.30	-0.14
82.5	17.8	"	2.50	2.35	2.35	0
88.0	18.0	"	2.55	2.45	2.40	-0.05

* Arachis oil (treatment for controls)

Appendix VII Effects of Gonadectomy, and of Steroid administration on the Genital papilla in female T. aurea

Wt. of Fish (gm)	Length of Fish (cm)	Treatment	Length of genital papilla (mm)		
			Before operation	40 days after operation	30 days after treatment	Increase/loss in length of papilla after treatment (mm)
46.0	14.3	TP	2.18	2.00	2.15	0.15
60.0	15.6	"	2.20	2.00	2.30	0.30
57.6	15.5	"	2.00	1.80	2.05	0.25
53.0	14.6	"	2.53	2.20	3.40	1.20
50.0	14.5	"	1.65	1.40	2.20	0.80
54.0	15.4	"	1.20	1.00	1.40	0.40
45.0	14.7	OB	2.30	1.90	3.85	1.95
50.0	14.6	"	2.20	1.95	2.95	1.00
41.0	13.5	"	1.95	1.60	2.30	0.70
40.0	13.7	"	1.55	1.40	1.96	0.56
40.0	13.3	"	1.70	1.65	1.85	0.20
39.0	13.4	"	1.85	1.70	2.05	0.35
41.0	14.2	AO*	2.30	2.00	2.00	0
40.0	13.5	"	1.85	1.70	1.60	-0.10
43.3	13.7	"	1.20	1.00	0.95	-0.05
41.0	13.0	"	1.50	1.30	1.15	-0.15
36.0	13.3	"	1.80	1.65	1.60	-0.05
31.0	13.1	"	1.35	1.20	1.15	-0.05

* Arochis oil (treatment for controls)

Appendix VIII Detailed Results on the Effect of Methallibure on the length of the Genital Papilla of male T.aurea

Experiment No.	Wt.of Fish (gm)	Length of fish (cm)	Length of Genital Papilla (mm) Before treatment	After treatment	Length (mm)
1	156.0	20.7	4.4	3.3	-1.1
	140.0	20.0	3.5	2.2	-1.3
	94.0	17.5	2.1	1.3	-0.8
	83.0	16.1	2.6	1.2	-1.4
	68.0	15.5	2.6	1.5	-1.1
	80.0	17.2	2.5	1.4	-2.1
	88.2	17.3	2.5	1.7	-0.8
	52.0	14.4	1.6	0.8	-0.7
	40.0	13.4	1.5	0.7	-0.8
	48.0	14.2	1.5	0.8	-0.7
Total	849.2	166.3	24.8	14.9	-10.8
Mean	84.9	16.6	2.48	1.49	-1.08

Appendix IX Detailed Results on the Effect of Methallibure on the length of the Genital Papilla of male T.aurea

Experiment No.	Wt. of Fish (gm)	Length of Fish (cm)	Length of Genital Papilla (mm)		Length (mm)
			Before treatment	After treatment	
2	50.0	14.4	1.5	0.7	-0.8
	72.0	16.5	2.7	1.5	-1.2
	69.0	15.3	2.6	1.4	-1.2
	50.0	14.6	1.6	0.7	-0.9
	58.0	15.0	1.9	1.0	-0.9
	99.0	17.8	2.3	1.3	-1.0
	82.0	16.1	2.6	1.3	-1.3
	89.0	17.6	2.4	1.2	-1.2
	66.0	15.0	2.3	1.3	-1.0
	80.0	16.0	2.6	1.6	-1.0
Total	715.0	158.3	22.5	12.0	-10.5
Mean	71.5	15.8	2.25	1.20	-1.05

Appendix X Detailed Results on the Effect of Methallibure on the length of the Genital Papilla of male T.aurea

Experiment No.	Wt. of Fish (gm)	Length of fish (cm)	Length of Genital Papilla (mm)		
			Before treatment	After treatment	Length (mm)
3 (Control)	97.0	19.0	2.2	3.1	+0.9
	131.0	18.6	2.6	4.2	+1.6
	104.0	18.9	3.8	4.6	+0.8
	48.0	13.8	1.4	2.1	+0.7
	74.0	17.3	2.7	3.1	+0.4
	73.0	16.5	2.8	3.5	+0.7
	86.5	18.0	3.3	4.2	+0.9
	51.5	15.0	2.9	3.5	+0.6
	42.0	13.6	1.6	2.0	+0.4
	45.5	14.0	1.5	2.0	+0.5
Total	752.5	164.7	24.8	32.3	+7.5
Mean	75.3	16.5	2.48	3.23	+0.75

Appendix XI Details of length, gravimetric analysis and GSI of methallibure -treated T. aurea

Experment No.	Treatment	No. of Fish	Mean wt of fish (gm)	Mean length of fish (cm)	Mean wt. of gonads (gm)	GSI	Treatment period (days)	Significance
1	External							
	Oral							
	Control	4	75.2	16.5	0.77	0.928	0	-
	External	4	68.8	15.9	0.89	1.294	10	0.01
	Oral	4	48.3	14.3	0.74	1.515	"	NS
	Control	4	56.3	15.4	0.93	1.648	"	-
	External	4	74.3	16.1	1.11	1.443	20	0.01
	Oral	4	56.9	15.1	0.49	0.849	"	0.002
	Control	4	74.2	16.4	2.35	3.881	"	-
	External	4	75.8	15.6	1.23	1.531	30	0.001
	Oral	4	56.2	15.0	0.24	0.426	"	0.001
	Control	4	66.1	15.3	2.82	4.339	"	-
	External	4	85.0	18.2	0.43	0.417	40	0.001
	Oral	4	75.0	15.5	0.25	0.330	"	0.001
	Control	4	73.0	16.4	2.35	4.445	"	-

Appendix XII Details of length, gravimetric analysis, and GSI of female Methallibure-treated T.aurea

Experiment No.	Treatment	No. of Fish	Mean wt of fish (gm)	Mean length of fish (cm)	Mean wt. of Gonads (gm)	GSI	Treatment Period (Days)	Significance
2a	External							
	Oral							
	Control	4	8.5	8.0	0.058	0.678	0	-
	External	4	12.5	9.2	0.197	1.572	10	NS
	Oral	4	11.5	9.4	0.07	0.605	"	0.01
	Control	4	11.5	9.0	0.14	1.214	"	-
	External	4	10.1	6.7	0.079	0.781	20	0.001
	Oral	4	6.0	7.5	0.022	0.371	"	0.001
	Control	4	11.0	9.0	0.27	2.454	"	-
	External	4	13.8	8.7	0.073	0.53	30	0.001
	Oral	4	8.0	8.0	0.024	0.303	"	0.001
	Control	4	19.6	10.7	1.024	5.225	"	-

pendix XIII Details of length, gravimetric analysis, and GSI of male Methallibure-treated T. aurea

periment	Treatment	No. of Fish	Mean wt. of fish (gm)	Mean length of fish (cm)	Mean wt. of gonads (gm)	GSI	Treatment Period (days)	Significance
	External Oral Control	4	16.5	10.2	0.063	0.382	0	-
	External	4	16.0	10.0	0.059	0.371	10	NS
	Oral	4	17.0	10.2	0.036	0.211	"	0.01
	Control	4	14.5	9.7	0.058	0.401	"	-
	External	4	16.2	10.0	0.022	0.133	20	0.001
	Oral	4	14.4	9.7	0.015	0.105	"	0.001
	Control	4	20.9	10.8	0.17	0.814	"	-
	External	4	17.3	10.2	0.02	0.115	30	0.001
	Oral	4	13.0	9. 5	0.008	0.06	"	0.001
	Control	4	24. 9	11.6	0.729	3.038	"	-

Appendix XIV. Effect of Unilateral Ovariectomy on the remaining ovary in T.aurea

Period (days)	Wt.of fish (gm)	Length of fish (cm)	Initial wt. of ovary (gm)	Final wt. of ovary (gm)	Wt.gained by remaining ovary (gm)	% wt.gain over initial ovarian wt. (gm)
	47.8	14.5	0.719	1.225	0.506	70
	47.0	14.5	0.872	1.169	0.297	34
	45.5	14.0	0.690	1.170	0.480	70
5	38.9	13.8	0.655	1.160	0.505	77
	60.0	15.7	-	1.450*		
	57.3	15.1	-	1.700*		
	40.0	13.3	0.595	1.649	1.054	177
	39.0	13.4	0.630	1.822	1.192	189
	45.0	14.2	0.655	1.465	0.810	123
10	42.8	13.8	0.600	1.175	0.575	96
	50.0	14.6	-	1.055*		
	54.5	15.4	-	0.950*		
	60.0	15.5	0.531	1.728	1.197	225
	51.0	15.0	0.453	1.808	1.355	299
	55.5	15.0	0.905	1.800	0.895	99
15	44.0	14.2	0.680	1.725	1.045	154
	54.0	15.3	-	1.422*		
	44.0	14.1	-	1.251*		

Appendix XV (Contd) Effect of Unilateral ovariectomy on the remaining ovary in T. aurea

Period (days)	Wt. of Fish (gm)	Length of fish (cm)	Initial wt. of ovary (gm)	Final wt. of ovary (gm)	Wt.gained by remaining ovary (gm)	% wt.gain over initial ovarian wt.
	49.0	15.0	0.075	1.612	1.537	205
	68.5	18.2	0.617	2.343	1.726	280
	50.1	15.1	0.605	2.175	1.570	260
20	59.0	16.0	0.615	2.665	2.050	333
	47.8	14.5	-	1.405*		
	61.9	16.3	-	1.307*		
	60.0	15.7	0.334	2.822	2.488	745
	63.0	16.0	0.432	2.637	2.205	511
	60.0	15.5	0.505	2.950	2.445	484
25	51.0	15.2	0.455	3.100	2.645	581
	60.0	15.5	-	1.633*		
	64.5	16.5	-	1.564*		

* Sham-operated controls. Weight of both ovaries presented

Appendix XVI. Effect of methallibure on the remaining ovary of unilaterally ovariectomized T.aurea

Period (days)	Wt. of fish (gm)	Length of fish (cm)	Initial wt. of ovary (gm)	Final wt. of ovary (gm)	Wt.lost/ gained by remaining ovary (gm)	% wt.lost/ gained over initial ovarian wt.
	28.1	11.7	0.348	0.479	0.131	37
5	35.2	12.1	0.480	0.656	0.176	37
	83.0	17.2	1.143	3.265	1.122	98
10	38.0	13.5	0.145	0.438	0.293	202
	48.0	13.7	0.931	0.438	-0.493	-53
15	55.0	13.7	0.545	0.280	-0.265	-49
	52.0	15.0	0.782	0.328	-0.454	-58
20	57.0	15.2	0.805	0.166	-0.639	-79
	53.5	14.4	0.895	0.066	-0.827	-92
25	42.8	13.2	0.620	0.025	-0.595	-96

NOTE: See appendix for controls: unilaterally ovariectomized non-methallibure-treated fish in Appendix served as controls.

Spermatogenesis and the stages of maturation in the male cichlid fish *Tilapia mossambica*

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Spermatogenesis and the stages of maturation in the male cichlid fish *Tilapia mossambica*

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(With 3 plates in the text)

Cyclical changes in the histology of the testis of *Tilapia mossambica* are described. The immature virgin testis contains primary germ cells and primary spermatogonia lying within a body of connective tissue. Just before spawning the testis is packed with sperm but cells at all stages of spermatogenesis are also present. After spawning the unexpelled spermatozoa undergo phagocytosis. No quiescent period during spermatogenesis has been observed though there are two periods of increased spermatogenetic activities. The stages of maturation of the testis are also reported. Five stages are distinguished.

Contents

Introduction

In recent years *Tilapia* has attained great economic importance as a productive source of animal protein for human consumption. One of the most serious disadvantages in *Tilapia* culture is the ease with which the population "runts" or "dwarfs" (de Bont, 1948)—the pond-raised fish become mature and start to breed when they are still very small. Investigations are therefore being carried out into the control of maturation in this fish.

Materials and methods

The fish used in these investigations were raised in aquaria in the laboratory. Tanks of dimensions 90 × 30 × 38 cm were used and the fish were maintained at 25°–30°C under a normal daylight cycle and fed *ad lib.* on dry food and fresh lettuce.

Forty-five fish measuring from 1·5 to 10·7 cm were examined and their testes were fixed in Bouin's solution. Sections were cut at 4–6 μ and stained with Heidenhein's iron haematoxylin and Ehrich's Haematoxylin with Eosin.

Spermatogenesis

The testes of *Tilapia mossambica* are paired, thin, elongated and usually of equal length. Each testis is enclosed in a thin peritoneum and has a fairly thick fibrous tunica. Along the periphery a layer of primary germ cells and spermatogonia is usually present.

Primary germ cells (gonocytes) are present in the testis throughout the reproductive cycle of *Tilapia* but the number varies at different periods of the cycle. They are, by far, the largest cells in the germinal tissue of the testis. The diameter of the cells measure 10–15 μ and that of the nuclei, 8–10 μ (Plates I(a) and II(d)). Each germ cell is spherical in outline and bears a large central nucleus. The nucleolus is also large and lies in the centre of the nucleus. These cells divide mitotically, forming spermatogonia.

The spermatogonia are smaller than the primary germ cells. Each has a spherical nucleus with a central nucleolus. Primary spermatogonia repeatedly divide mitotically, forming spermatogonia of subsequent generations (Plates I(a) and II(b)). During the period of spermatogonial proliferation microscopic slides of the testes reveals numerous mitotic figures (Plate I(b)). After the last mitotic division, the daughter spermatogonial cells, which are considerably smaller in size, enter a short phase of growth and are finally transformed into spermatocytes. Some of the spermatogonia do not divide but become enlarged and form "resting" spermatogonia, a reserve fund for the next crop of spermatogonia. There is a synchronization in the division of cells which originated from the same spermatogonium. These cells are enveloped in a common covering of connective tissue and constitute a "cyst".

Primary spermatocytes enter into a period of meiotic prophase. The proleptotene stage in *Tilapia mossambica* seems to proceed very quickly and it is therefore difficult to locate on microscopic slides. The stages of conjugation of chromosomes—synapsis and parasynapsis; the bouquet or leptotene and pachytene stages are all visible (Plates I(c),(d) and II(a)); as well as the subsequent stages of the first meiotic division (Plate II(b)).

Secondary spermatocytes are formed by reductive division of primary spermatocytes and are comparatively smaller in size (Plate II(c)). At the end of the second meiotic division secondary spermatocytes are transformed into spermatids.

Spermatids are yet smaller in size than the secondary spermatocytes. The diameter of the nucleus of the former is only 2 μ. It is represented by a compact chromatic mass and usually occupies one side of the nuclear space, sometimes attaining the shape of a half-moon (Plate II(c),(d)). They have no distinct cell boundary or nuclear membrane.

During spermiogenesis, the spermatids are gradually transformed into spermatozoa. The head of a spermatozoon is round and small, the diameter measures only 1·5 μ (Plates II(d) and III(a)).

Stages of maturation (Table I)

Stage I

At this stage the testis is not fully developed, and in the form of two tiny transparent threads. Macroscopically, sex differentiation is impossible. Microscopic examination of slides of the testes reveals primary germ cells and spermatogonia. This condition is characteristic only of the young, virgin fish and lasts a very short time.

PLATES I to III. Testicular tissues of *Tilapia mossambica* stained with Heidenhein's iron haematoxylin and Bouin fixation.

PLATE I. T. S. of testis: (a) showing primary germ cell (pgc) cysts of primary spermatogonia (psg) and secondary spermatogonia (ssg); (b) showing the metaphase (m) and anaphase (a) of mitotic divisions of spermatogonia; (c) showing the synaptic and parasynaptic stages of meiotic prophase; (d) showing the bouquet or leptotene stage of meiotic prophase. All $\times 938$.

PLATE II. T. S. of testis: (a) showing the pachytene stage of meiotic prophase; (b) showing late anaphase of meiotic prophase (la); ssg, secondary spermatogonia; (c) of fish in the II stage of maturation; ss, secondary spermatocytes; st, spermatid; (d) showing primary germ cells (pgc), spermatids (st) and spermatzoa (s). (a), (b), (d) $\times 938$; (c) $\times 417$.

PLATE III. T. S. of testis: (a) showing ripe spermatozoa; (b) of fish in the III stage of maturation; (c) of fish in the IV stage of maturation; numerous lobules filled with spermatozoa; (d) of fish in the V stage of maturation (spent fish); unexpelled sperms still present, as well as cells at all stages of spermatogenesis. (a) $\times 417$, (b) $\times 500$, (c) $\times 83$, (d) $\times 208$.

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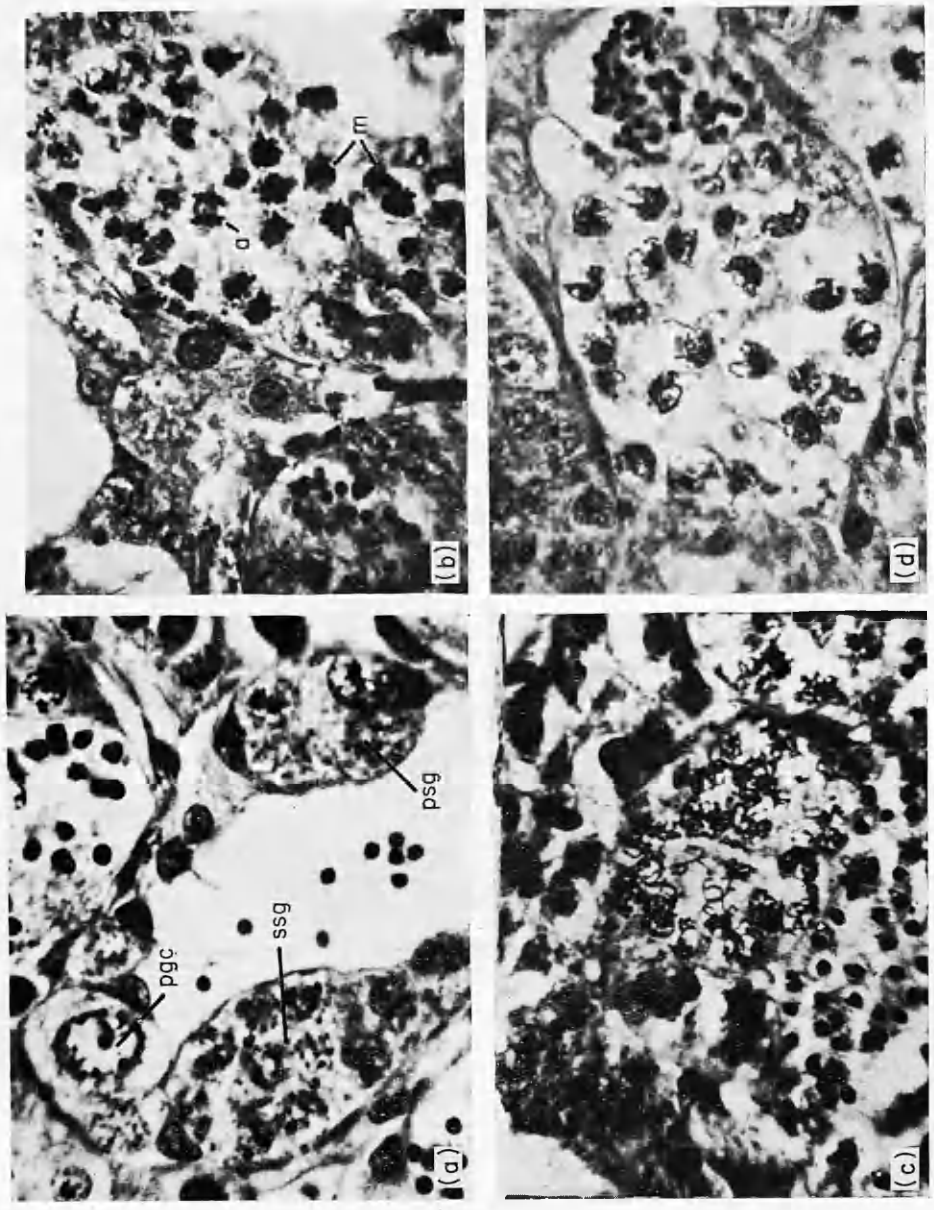

PLATE I

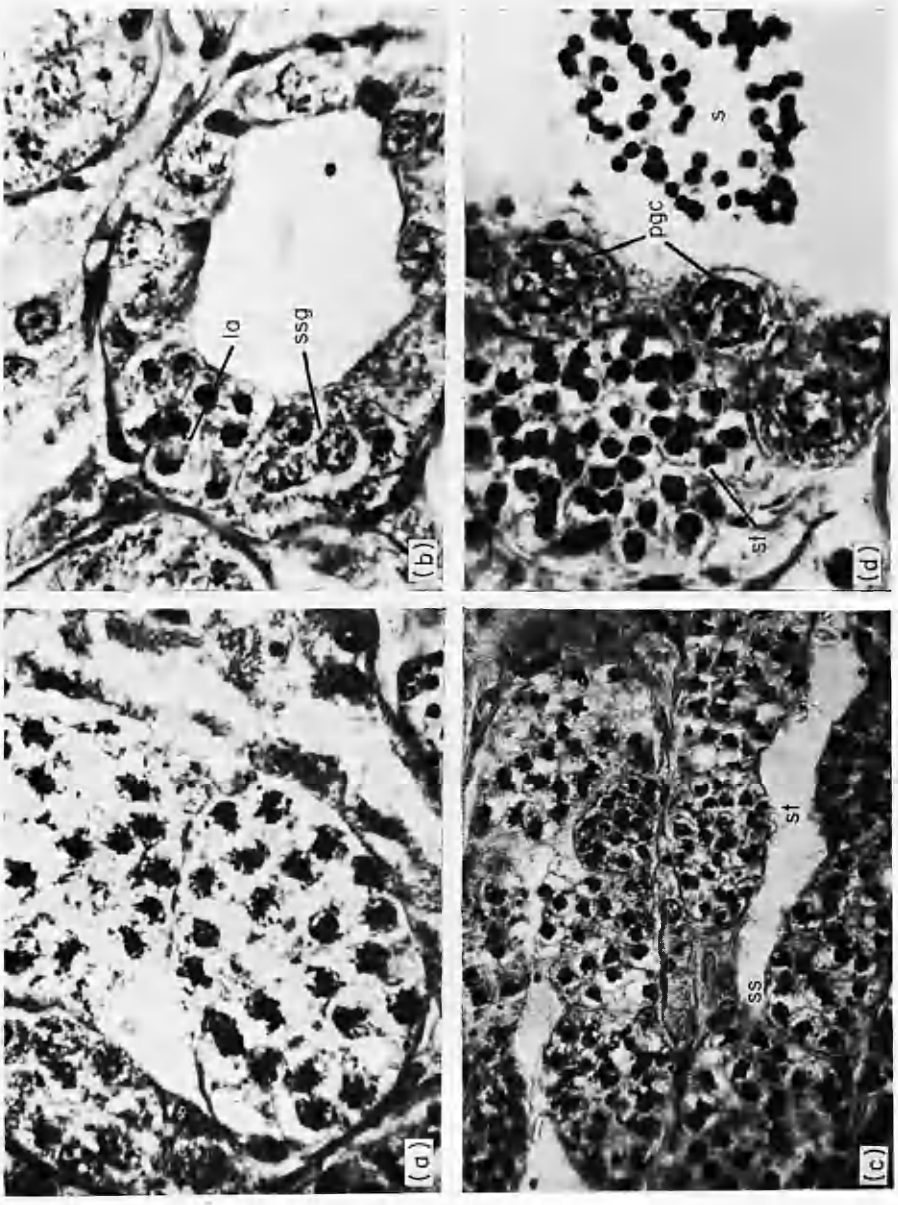

PLATE II

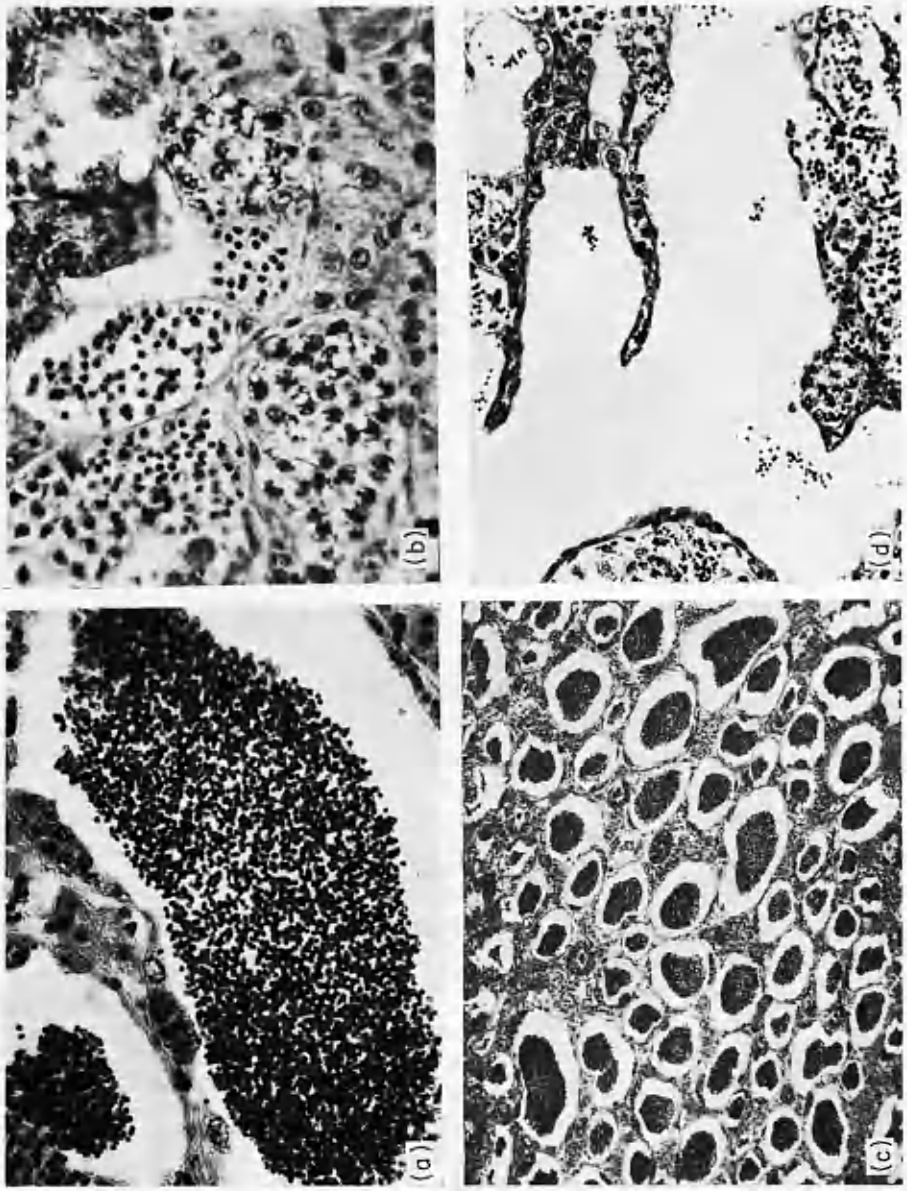

PLATE III

Stage II

The testis at this stage is filled with primary germ cells, spermatogonia and spermatocytes and a few spermatids (Plate II(c)). It is slightly longer than that in Stage I because of the presence of numerous cells but retains its transparency. The borders of the fins of the fish attain slight reddish colouration.

Stage III

Intensive spermatogenesis takes place during this period. Cells at all stages of development are visible—primary germ cells, spermatogonia, spermatocytes and spermatids. Groups of ripe spermatozoa appear only at the end of this stage (Plate III(b)). There is a sudden increase in the weight and volume of the testis which now looks opaque. Apart from the red colouration of the tips of the fins of the fish, full secondary sexual characters appear on the body—black colouration of the skin and brilliantly shining opercula.

TABLE I

Relation of age, weight and length to stages of maturation in ***Tilapia***

Age (weeks)	Weight (g)	Length (cm)	Stages of maturation	No. of fish
<6	1·8–2·0	1·5–5·0	I	9
6–12	2·4–4·2	5·2–5·5	II	9
8–12	2·2–8·7	5·4–8·2	III	9
10–12	7·4–10·5	7·9–10·7	IV	9
12–13	8·0–9·8	8·5–9·0	V	9

Stage IV

This stage marks the end of the first wave of spermatogenesis. The testis is filled with lobules containing ripe spermatozoa (Plate III(c)). Since *Tilapia* is a polycyclic fish, cells at all stages of spermatogenesis are found in the testis, apart from the ripe sperm, but the latter is present in greater quantities. With the formation of large quantities of spermatozoa, the testis attains a whitish colour and maximum weight. The secondary sexual characters become intensified. Nest-building ensues, and the fish is sexually mature.

Stage V

This is the period after the male has discharged most of the milt. The unexpelled sperms seen in the empty lobules soon undergo phagocytosis (Plate III(d)). Soon after the sperms are shed intensive spermatogenesis starts. After Stage V, the testis of *Tilapia* never returns to Stage I (i.e. V–I). In rare cases, usually after the end of a season of several spawnings, the testis might return to Stage V–II. Otherwise, soon after spawning, the testis returns to Stage V–III.

Discussion

A number of contributions exist on various aspects of spermatogenesis in fish. Latest reports on these aspects are given by Rai (1965); Stanley, Chieffi & Botte, (1965); Ahsan, (1966); and Rastogi (1968). In view of the small percentage of teleostean species studied, comparatively less is known about the exact nature of spermatogenesis within and among different species and groups of fish during the reproductive cycle.

Two views exist regarding the origin of the germ cells in fish. According to Hann (1927), Bennington (1936), Bullough (1939), Jones (1940), Weisel (1943), Stenger (1959), Henderson (1962) and Rai (1965), the reserve stock of dormant germ cells, present throughout the year, give rise to the next crop of spermatogonia, without migration. But Turner (1919), Foley (1926), Lofts & Marshall (1957), and Rastogi (1966) observed that migratory cells, originating from some part of the testis, produce a new crop of germ cells. Rastogi (1968) has observed the formation of germ cells from the interstitial cells in the freshwater Spiny eel, (*Macrognathus aculeatum*). In *Tilapia aurea* Eckstein & Spira (1965) have observed the migration of gonocytes from the splanchnopleura along the lateral part of the somatopleura into the germinal tissue. While the origin of the germ cell in *T. mossambica* is not the main objective of the present investigation, there is, however, evidence in support of the view of the latter workers. The second and subsequent crop of spermatogonia is developed from "resting" spermatogonia in *T. mossambica* by mitotic divisions.

There are periods of increased spermatogenetic activity—the period of spermatogonial proliferation in young, virgin males, and the period when the males have newly spent testes. No quiescent period as is characteristic of other species (see Craig-Bennet, 1931; Lofts & Marshall, 1957; Rai, 1965; Ahsan, 1966; and Rastogi, 1966) has been observed in *T. mossambica.*

There are few descriptions of the stages of maturation in fish. Fish of major commercial importance, like the herring, have received adequate attention in this respect (e.g. Hjort, 1910; Ehrenbaum, 1930; Bowers & Holliday, 1961). The international scale of maturity stages of the gonad (Hjort, 1910) is generally quite adequate for obtaining an overall picture of the maturity range of a given sample especially of marine fish with long life span. With regard to tropical freshwater fish such as *Tilapia mossambica* which mature very early (three to four months), this international scale of maturity stages cannot be successfully applied. The stages of maturation described in the present report for *Tilapia* is therefore a modification of the existing one. Hjort (1910) divided the scale of maturity into seven stages. In the present investigation, it has been found convenient to divide it into five stages only.

Under the environmental conditions of the present experiment, a positive significant correlation ($r = 0{\cdot}953$) was found between length and stage of maturation, and a highly significant correlation ($r = 0{\cdot}968$ between weight and stage of maturation (Table I).

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