THE ENDOCRINE CONTROL OF SEXUAL DEVELOPMENT IN THE MALE GUPPY *POECILIA RETICULATA* PETERS

by

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ABSTRACT

The role of pituitary and gonadal steroids in the development and maintenance of the testis and secondary sex characters of the guppy Poecilia reticulata Peters has been studied by the technique of surgical hypophysectomy and chemical inhibition of gonadotropic action using 'methallibure'.

Hypophysectomy of juvenile or adult guppies completely blocks mitosis in the spermatogonia and their transformation into spermatocytes. However, spermatocytes, spermatids and sperm already present in the adult testis at the time of operation transform into spermatophores. In the absence of the pituitary, the spermatophores rupture after eight weeks and the resulting sperm are phagocytosed within the sperm ducts. Sertoli cells, interstitial cells and the epithelial cells lining the sperm ducts regress in hypophysectomized fish.

Testosterone treatment of the hypophysectomized adult guppy initiates spermatogonial multiplication and the transformation of spermatogonia into spermatocytes; the regressed Sertoli cells, interstitial cells and the epithelial cells lining the sperm ducts resume their normal appearance. Testosterone treatment of hypophysectomized juvenile guppies does not initiate spermatogenesis but the sperm ducts become well differentiated.

Of two particularly well differentiated secondary sex characters of the adult male guppy, the gonopodium (modified anal fin) remains unaffected after hypophysectomy whereas

the lipophores (yellow and red pigments) present on the sides of body become obscure or entirely disappear in the absence of pituitary; the lipophores reappear after testosterone treatment. Secondary sex characters never appear in guppies hypophysectomized as juveniles. When hypophysectomized juveniles are treated with testosterone, secondary sex characters (gonopodium and lipophores) become evident.

The regression of the gametogenetic and the steroidogenetic tissues in the testis of 'methallibure'-treated ($1:10^6$parts) adult guppy is not as complete as in the hypophysectomized fish. This indicates that the release of pituitary gonadotropins is not completely blocked. With the same dose of 'methallibure', however, the gonadotropin release in the juveniles is apparently blocked. In both adult and juvenile guppies 'methallibure' brings about a clear decrease in both the number and mean cell diameter of gonadotrophs. The gonadotropic hormone blocking activity of the compound seems to occur at the level of hormone synthesis.

From these studies it has been concluded that mitotic division of spermatogonia and their transformation into spermatocytes are dependent on pituitary, but the transformation of spermatocytes, spermatids and sperm into spermatophores are pituitary-independent. The release of spermatophores is under the control of pituitary. The regressed Sertoli cells, interstitial cells and the epithelial cells lining the sperm ducts of hypophysectomized fish assume

normal appearance with testosterone treatment. The appearance of secondary sex characters in hypophysectomized juveniles treated with testosterone indicates that secondary sex characters are directly controlled by testosterone. 'Methallibure' completely blocks the synthesis of gonadotropic hormones in the juvenile guppies but not in adults.

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INTRODUCTION

The guppy Poecilia reticulata Peters is a highly specialized monthly breeding ovoviviparous teleost belonging to the family Poeciliidae. Male and female guppies are sexually dimorphic; the female is considerably larger than the male and of a pale greyish green color, while the male is adorned with several bright patches on the sides of the body. The difference in pigmentation and coloring in the males belonging to different races is considerable and depends on genes located in sex chromosomes (Winge 1922; Winge and Ditlevson 1947). The most conspicuous color patches are the lipophores which contain both yellow pigments (Xanthophores) and red pigments (erythrophores).

Another well differentiated secondary sex character found in the male guppy is the highly modified anal fin or gonopodium, with which internal fertilization is effected. As an adaptation to internal fertilization, the male guppy produces sperm-balls or spermatophores which probably prevent the loss of sperm by leakage into the water during transfer to the genital tract of female.

In the guppy, development of the gonad and secondary sex characters has been described in detail. Dildine (1936) and Goodrich et al (1934) discussed the morphogenesis of gonads. Comparable studies of the ovary were reported in detail by Antennius (1959). Vaupel (1929) described spermatogenesis and discussed the method of transfer of the

sperm to the female. The morphogenesis of the gonopodium in the male has been described by Hopper (1949a).

Secondary sex characters have been shown to be involved in complex courtship behavior (Clark and Aronson 1951; Kadow 1954; Baerends et al 1955; Liley 1966). Although courtship behavior has been studied in detail, few analyses are associated with the endocrinology of reproduction (Kadow 1954; Liley 1965, 1968).

Although the influence of exogenous steroids on the gonad and secondary sex characters of the guppy has been tested many times (Eversole 1939, 1941; Regnier 1941; Okada and Yamashita 1944b; Hopper 1949b, 1951), efforts have never been made to eliminate the possible effects of endogenous gonadotropins by the use of hypophysectomized animals. The possibility of a direct action of the pituitary in the development of testis and the differentiation of secondary sex characters of the guppy or any other poeciliid has not been investigated. Similarly, the endocrine control of spermatophore formation, the retention of spermatophores within the testis and their eventual release from the testis have not been studied in any fish.

The technique of hypophysectomy was found to be relatively successful with both the adult and juvenile guppies thus making possible critical experiments concerning pituitary-gonad relations. The role of gonadal steroids in the maintenance or development of the testis and secondary sex characters may be readily analyzed by treating the

hypophysectomized juvenile and adult guppies with testosterone preparations which have been repeatedly shown to be physiologically effective in replacement of natural androgens.

In addition, a technique of chemically inhibiting gonadotropic action has recently been made available. 'Methallibure' (1-a-methylallylthiocarbomoyl-2-methylthiocarbamyl hydrazine; I.C.I. 33,828) blocks the synthesis, release or action of gonadotropic hormones in teleosts (Hoar et al 1967; Wiebe 1968). This agent provides a useful additional technique in the analysis of pituitary-gonad relations in fishes. The site of action of 'methallibure' in blocking gonadotropic action is not known.

With these techniques as a basis for the experimental analysis, the purposes of the present investigation of the male guppy are as follows:

1. To examine the role of the pituitary and the gonadal hormones in spermatogenesis with particular reference to the initiation of spermatocyte formation and the formation of spermatophores.

2. To investigate the possibility of a direct pituitary involvement in the expression of secondary sex characters of adult males.

3. To describe the effects of hypophysectomy and methyl testosterone treatment on the testis of the juvenile guppy prior to its differentiation and before the appearance of secondary sex characters.

4. To compare 'methallibure' effects on the testes and secondary sex characters of the adult and juvenile guppies and to localize the site of action of 'methallibure' in blocking gonadotropic action.

SECTION I

HYPOPHYSECTOMY AND METHYL TESTOSTERONE TREATMENT OF THE ADULT MALES.

INTRODUCTION

The role of the pituitary and the gonadal hormones in the reproductive endocrinology of the adult male guppy has been studied by analyzing the effects of hypophysectomy on the testes and secondary sex characters (gonopodium and lipophores) and subsequently treating such hypophysectomized animals with methyl testosterone.

MATERIALS AND METHODS

Maintenance of fish in breeding aquaria. The initial sample of guppies was purchased from a local aquarium dealer (Northwest-Aquatics, Vancouver, Canada). These were allowed to breed in five glass aquaria of 20- to 60-litre capacity. The bottoms of these aquaria were covered to a depth of 2 cm with coarse sand; water was continuously recirculated and filtered through a pad of glass-wool. Each day feces and uneaten food were removed from the bottom of the aquaria by a fine-meshed net and filters were cleaned at least once a week. About 50% of water in each of the aquaria was renewed once in every two or three weeks.

Aquatic plants (water sprite, Ceratopteris thalictroides) were put on the surface of water in aquaria to provide cover

for the young thus avoiding their being eaten by the adults. There was no special illumination directly above the aquaria but the room was well-lighted with overhead fluorescent tubes and the photoperiod regulated at natural day length by a photocell exposed in a large window facing south. The temperature of all aquaria was maintained at 25 $\pm$ 0.5°C. Fish were fed daily with finely ground commercial trout fish food (J.R. Clark Co.)

Maintenance of hypophysectomized fish. One week prior to the operation brightly colored males (16-22 mm standard length, 90-250 mg in weight) were taken from the breeding aquaria and put in aquaria of 20-litre capacity which contained 16-litre of fish saline [NaCl-5.5 gm, KCl-0.14 gm and $CaCl_2$-0.12 gm in 1 litre of dechlorinated water (Young 1933)]. The males were kept in the saline until the end of the experiment. No plants were put in these experimental aquaria.

Light was provided by 20-watt fluorescent tubes about 15 cm above the aquaria. The photoperiod of 16-hr daily illumination was controlled by Intermatic model T101 clocks. The temperature was maintained at 25 $\pm$ 0.5°C. Fish were fed daily with finely ground commercial trout fish food. Sham-operated adult males which served as controls were also kept in the saline until the end of the experiment.

Procedure of hypophysectomy. Males conditioned to fish-saline for one week, were anesthetized with 1:600 MS 222 (Tricaine Methane Sulphonate-Sandoz). The fish was

then placed ventral side uppermost on a plastic plate fixed at an angle on the surface of wax in a rectangular plastic tray (15x7½x4½ cm). The fish was secured in place by a pair of movable plastic hooks present on the plastic plate. A fine glass tube placed in the buccal cavity of fish served to perfuse the gills with fish-saline. All subsequent procedures were carried out under a binocular microscope (x10).

A small incision was made in the gular region in front of branchiostegal membrane on the right side and extended to the lower lip. The mucous membrane covering the palate was torn away gently with a pair of fine forceps. The pituitary gland could then be seen beneath the parasphenoid bone as a small white body just posterior to the optic chiasma. The parasphenoid bone was now broken by a pair of blunt forceps. The pituitary was picked up with a pair of fine forceps. It was ascertained under the microscope that all three lobes of pituitary were always removed. Sham-operated fish received the same treatment without pituitary removal.

The blood and tissue fragments resulting from the operation were rapidly flushed with fish saline. After the operation, the fish was left in the fish saline accumulated inside the tray during the operation until it started swimming actively again. It was then transferred to an aquarium containing fish-saline. The wound healed in ten to twelve days and no infection was ever noticed. Mortality

was high, amounting to 20-25% during the operation; a further mortality of 25-30% occurred in two weeks following the operation.

Methyl testosterone treatment. Hypophysectomized adult males were transferred eight weeks after the operation to aquaria containing 16-litre of fish saline. To avoid the changes of concentration of testosterone in fish-saline, no sand or filter or plants were used; the aquarium was continuously aerated. A concentration of $1:10^7$ parts (1.6 mgm in 16 litres of fish saline) of methyl testosterone (California Corporation for Biochemical Research, Los Angeles) was added to each aquarium every week (5 applications during 5 weeks). Methyl testosterone was added as a suspension (1.6 mgm in 50 ml. of fish saline) to the aquaria. Four litres of fish saline was renewed in each aquarium every week.

Controls were of two kinds: 1. the hypophysectomized males which did not receive any methyl testosterone treatment and 2. the sham-operated males. The controls were always kept in saline.

Histology. The testes were fixed in Bouins fluid (picric acid-formol-acetic acid) for routine histology. To check on the completeness of pituitary removal, suitable portions of the heads were fixed in formic acid-Bouin fixative and left in the fixative to decalcify for a week. Some of the testes were fixed in Baker's formol-calcium for lipid staining.

The Bouin and Bouin-formic acid fixed testes and head portions were dehydrated in alcohol, cleared in Xylol and embedded in paraffin-wax. Serial longitudinal sections were cut for testes at 5 μ and of head portions at 7 μ and stained with Ehrlich's haematoxylin and eosin.

The testis fixed in formol-calcium was embedded in gelatin (Gurr 1962) and sectioned at 10-15 μ on a freezing microtome. Sections were floated on to slides in iced water. Sections were stained with Sudan black B (0.7% in Propylene glycol), differentiated in 70% propylene glycol and mounted in Hydramount (Chiffelle & Putt 1951).

Measurements. The standard length was measured as the distance between the snout and the last row of scales on the caudal peduncle. The overall body weight before the removal of the gonad was measured to the nearest 1 mg and the gonad weight was measured to the nearest 0.1 mg. The gonad weight was expressed as a percentage of total body weight to give the gonosomatic index (GSI)

$$GSI = \frac{\text{Gonad weight in grams}}{\text{Total body weight in grams}} \times 100$$

To compare the histological picture of the testes of sham-operated, hypophysectomized and testosterone-treated guppies, the total number of acini or cysts containing germ-cells of different stages of spermatogenesis in the median sagittal section were counted and percentage of each cell type calculated (one acinus usually contains germ-cells

in the same stage of maturation). The single section thus selected had cysts containing all stages of spermatogenesis and gave essentially the same percentage composition as was obtained after counting every 20th section of the whole testis.

The epithelial cell heights of efferent ducts (branches of main sperm duct) of testes of sham-operated, hypophysectomized and testosterone-treated fish were measured with an ocular micrometer. Five efferent ducts from one section (just before the efferent ducts join to form the main sperm duct) were selected at random and five measurements of epithelial cell heights were taken from each efferent duct. Thus 25 epithelial cell heights were measured from one section of each testis.

RESULTS

Structure of the adult testis. The testis is a two-lobed body fused in the middle and located in the posterior part of the body-cavity, ventral to the swimbladder. It is covered by a thin, unpigmented peritoneal membrane. The two branched main sperm ducts (one occupying the centre of each lobe of the testis) unite at the posterior and ventral margins of testis to form a short vas deferens which opens almost immediately into the urogenital sinus (Fig. 1a). The main sperm duct and its branches, the efferent ducts are lined by a cuboidal or columnar epithelium (Figs. 4 & 6).

The efferent ducts are surrounded by cysts or acini (the testis is the acinus-type in which no tubules have developed). Each cyst or acinus usually contains germ-cells in the same stage of maturation. Cysts containing spermatogonia and spermatocytes are at the periphery; internal to these are spermatid- and sperm-cysts and finally close to the centre lying adjacent to the efferent ducts are cysts containing 'sperm-balls' or spermatophores (Figs. 1b & 2a,b).

Sertoli cells are arranged around the periphery of the cysts. Sertoli cells lining the cysts of spermatocytes and spermatids are thin and flattened. When the spermatids within the cyst transform into sperm, the Sertoli cells become strikingly enlarged; their nuclei become larger and more rounded; and the sperm heads become attached to the inner margin of Sertoli cells (Fig. 8). Thus a sperm-ball or spermatophore is formed with a compact ring of sperm-heads around the periphery of cyst and the tails in the centre.

According to Vaupel (1929), in the guppy the sperm-heads of spermatophores now withdraw from the cyst-wall, the tails being entwined in the process; a connection of the spermatophore with the main canal is established; the spermatophores then pass into the central canal. He did not describe the fate of Sertoli cells after the spermatophore withdraws from them and reaches the central canal. Medlen (1950) and Chavin & Gordon (1951) were also unable to explain the fate of Sertoli cells after the spermatophores

pass into the lumen of the testicular canal in Gambusia affinis and Xiphophorus maculatus respectively.

In the present investigation it was noted that the wall of the mature cyst containing the spermatophore fuses with the wall of the efferent duct; the spermatophore passes into the lumen of the efferent duct and the Sertoli cells become a part of the epithelial lining of the efferent duct (Fig. 10). Both Sertoli cells and the epithelial cells of the efferent ducts have large oval nuclei with distinct nucleoli. It may be suggested that the spermatophores within the germinal portion of the testis are surrounded by Sertoli cells; and after the spermatophores pass into the efferent duct, the Sertoli cells become continuous with the epithelial lining of the efferent duct. Wiebe (1967) has referred to epithelial cells lining the efferent ducts as Sertoli cells in the Shiner seaperch (Cymatogaster aggregata).

The spermatophores congregate in groups in the efferent ducts and the main sperm duct. A colloidal material is noticed around and in the spaces between the spermatophores (Fig. 6). The source of colloidal material is presumed to be epithelial cells lining the efferent ducts and main sperm duct, because these appear cuboidal and depleted where spermatophores are present, while columnar and filled with secretory droplets where spermatophores are absent. Medlen (1950) has suggested that the colloidal material contains a nutritive substance for the maintenance of sperm and perhaps a mucilaginous substance to cause the spermatophores to adhere to one another.

There is no prominent connective-tissue core in the testis. The testis consists of a tenuous, diffuse, connective tissue stroma with closely packed cysts in the interstices. The interstitial cells (Leydig cells) with rounded nuclei are found in the central part of the testis dispersed in the space between the branches of the efferent ducts (Fig. 12).

Secondary sex characters of the male guppy. The adult guppy has two particularly well-differentiated secondary sex characters: (a) several patches of bright colors on the sides of the body and (b) the gonopodium. The most conspicuous color patches are the lipophores. The brightness of the lipophores may be quantified by assigning + signs -- (++++) very bright; (+++) bright; (++) dull and (+) trace. The validity of this method is open to question, since it is not known whether the arbitrary signs are in linear relation to the content of yellow and red pigments in the area under observation. Since all adult males usually have bright lipophores, they may be assigned ++++ or +++ signs.

The morphological details of the transformation of the male anal fin into the gonopodium have been described by Hopper (1949a). There are 10 rays present in the anal fin of the guppy; in the male, rays 3, 4 and 5 become elongated. Ray 3 becomes thick (bone deposition) and develops a hood and ventral spines. Ray 4 also develops spines but ray 5 has only a hook at its tip and no spines (Fig. 31).

Structure of the testis and secondary sex characters of sham-operated control guppy. Sham-operation does not bring about any changes in the structure of the adult testis. The spermatogonial cysts are at the periphery and the spermatophores are in the centre; the area between the two is filled with the cysts containing the various developmental stages of spermatocytes, spermatids and sperm (Figs. 2a, 2b, 20 and tables II and V). The efferent ducts and the main sperm duct are filled with intact spermatophores (Figs. 4 & 6). The epithelial cells lining the efferent ducts are tall and columnar (Fig. 4 and Table III) and contain lipid droplets (Fig. 14). The interstitial cells have oval nuclei (Fig. 12) and give a positive test for lipids (Fig. 14). The secondary sex characters are unchanged in the sham-operated males.

Structure of the testis of hypophysectomized guppy. Adult male guppies were hypophysectomized and two to three fish were killed at weekly intervals up to eight weeks in one experiment. In another experiment the hypophysectomized fish were killed after 16 weeks. In both the experiments there is a considerable decrease in the gonosomatic indices of the operated fish (Tables I and IV).

After eight weeks the testis of a hypophysectomized guppy is completely packed with spermatophores except for a few spermatogonial cysts at the periphery (Figs. 3a, 3b, 20 and Table II). It seems that the cysts containing spermatocytes, spermatids and sperm present at the time of operation transform

TABLE I G.S.I. of hypophysectomized and sham-operated guppies after 8 weeks.

Fish No	G. S. I.	
	Hypophysectomized	Sham-operated
1	0.84	3.53
2	0.86	3.80
3	0.93	2.75
4	0.91	2.94
5	0.97	4.39
Mean[1] $\pm S_{\bar{x}}$	0.90 ± 0.02	$3.48 \pm 0.30^{***}$

[1]Standard Error

***$P < 0.001$

TABLE II Number and percentage of different stages of spermatogenesis in hypophysectomized and sham-operated guppies after 8 weeks.

		Stages of spermatogenesis									
		SPG		SPC		SPD		SPM		SPR	
		No	%	No	%	No	%	No	%	No	%
Hypophysectomized	Mean[1]	1.6	1.82	0		0		0		97.6	98.18
	$\pm S_{\bar{x}}$	±0.6	±0.48							±16.3	±0.75
Sham-operated	Mean[1]	2.6	1.11	23.0	10.04	16.0	6.84	23.8	11.07	185.2	70.93
	$\pm S_{\bar{x}}$	±0.3	±0.13	±3.5	±2.10	±1.5	±0.77	±3.5	±2.65	±42.6	±±5.47

[1]Mean of 5 observations

$P < 0.001$, contingency table (row x column)

SPG-Spermatogonia; SPC - spermaocytes; SPD - spermatids, SPM-sperm; SPR-spermatophores.

TABLE III Epithelial cell heights of the efferent ducts of hypophysectomized and sham-operated guppies after 8 weeks.

Fish No	Mean epithelial cell height[1] (u)	
	Hypophysectomized	Sham-operated
1	4.3	33.2
2	3.3	20.5
3	4.0	27.1
4	4.2	13.1
5	4.6	22.6
Grand Mean	4.1	23.3

[1]Mean of 25 counts (5 epithelial cell heights in each of 5 efferent ducts in one section of each testis.)

$P < 0.005$ - Analysis of variance based on 5 median counts, 1 in each of 5 efferent ducts of each testis.

TABLE IV G.S.I. of hypophysectomized and sham-operated guppies after 16 weeks.

Fish No	G. S. I. Hypophysectomized	G. S. I. Sham-operated
1	0.63	3.61
2	0.38	4.12
3	0.49	3.73
4	0.46	3.41
5	0.31	3.84
Mean $\pm S_{\bar{x}}$	0.45 ± 0.05	3.74 ± 0.12***

*** $P < 0.001$

TABLE V Number and percentage of different stages of spermatogenesis in hypophysectomized and sham-operated guppies after 16 weeks.

		Stages of spermatogenesis									
		SPG		SPC		SPD		SPM		SPR	
		No	%	No	%	No	%	No	%	No	%
Hypophysectomized	Mean[1]	2.2	4.68	0		0		0		47.4	95.32
	$\pm S\bar{x}$	±0.8	±0.92							±13.6	±0.92
Sham-operated	Mean[1]	3.4	1.88	23.6	12.45	14.6	7.53	22.2	10.73	137.4	67.41
	$\pm S\bar{x}$	±0.4	±0.29	±3.7	±3.02	±2.1	±1.25	±3.9	±0.27	±22.9	±4.95

[1]Mean of 5 observations

$P < 0.001$, contingency table (row x column)

into spermatophores. During the second week after the operation few spermatocytes, many spermatids and sperm are evident in the testis but by the end of the third week only sperm-cysts are noticed (Fig. 11). From the fourth week onwards after the operation the testis contains only spermatogonia and spermatophores. This indicates that spermatogonia are not transformed into spermatocytes in the absence of the pituitary but spermatocytes, spermatids and sperm are transformed into spermatophores during the first three weeks. The presence of spermatophores at the periphery of the testis of a hypophysectomized guppy (Figs. 3a,b) with no trace of disintegrating cysts containing earlier stages of spermatogenesis suggests that spermatocytes, spermatids and sperm do not disintegrate but transform into spermatophores.

In the hypophysectomized guppy no mitotic division of spermatogonia is evident; many spermatophores are found ruptured in the efferent ducts and the main sperm duct (Figs. 5 & 7). The epithelial cells lining the efferent ducts decrease in height to become squamous (Fig. 5 and Table III). The epithelial cells of the efferent ducts do not contain any lipid droplets. Sertoli cells also regress in the hypophysectomized animal; their nuclei change from rounded condition to flattened shape (Fig. 9). There is an apparent increase in the number of fibroblasts throughout the testis (Fig. 5). The interstitial cells are regressed with shrunken nuclei (Fig. 13) and no lipid droplets were evident in frozen sections stained with Sudan black B.

The structure of the testis of a hypophysectomized guppy after 16 weeks is similar to the testis after eight weeks except for the fact that most of the spermatophores are ruptured (Table V and VI). The differences in percentage compositions of spermatogonia and spermatophores in the testes of hypophysectomized guppies after eight and 16 weeks are not statistically significant (Table VI). Thus the trend of deteriorating spermatophores which is noticeable after eight weeks of hypophysectomy is much more pronounced after 16 weeks. There is a strong indication that the sperm resulting from ruptured spermatophores are being phagocytosed within the efferent ducts and the main sperm duct (Fig. 15). The entire testis appears as a mass of fibrous connective tissue (Fig. 15) because of rupture and disappearance of spermatophores and pronounced increase in the number of fibroblasts.

Secondary sex characters of hypophysectomized guppy. In both the experiments (eight and 16 weeks after hypophysectomy) the structure of the gonopodium is unaltered. The lipophores become faint or entirely disappear both on the sides of the body and on the tail and were rated as 0 to + in the arbitrary scale described earlier.

Structure of the testis and secondary sex characters of hypophysectomized control guppy (testosterone experiment). The testis has few spermatogonial cysts at the periphery; the rest of the testis is filled with spermatophores, many of which are ruptured (Figs. 3, 21 and Table VII). Sertoli

TABLE VI Number and percentage of spermatogonia and spermatophores in hypophysectomized guppies after 8 weeks and 16 weeks

		Stages of spermatogenesis									
		SPG		SPC		SPD		SPM		SPR	
		No	%	No	%	No	%	No	%	No	%
8 weeks after hypophysectomy	Mean[1]	1.6	1.82	0		0		0		97.6	98.18
	$\pm S\bar{x}$	$\pm$0.6	$\pm$0.48							$\pm$16.3	$\pm$0.75
16 weeks after Hypophysectomy	Mean[1]	2.2	4.68	0		0		0		47.4	95.32
	$\pm S\bar{x}$	$\pm$0.8	$\pm$0.92							$\pm$13.6	$\pm$0.92

[1]Mean of 5 observations

$P < 0.5$, contingency table (row x column)

TABLE VII Number and percentage of different stages of spermatogenesis in hypophysectomized, testosterone-treated and sham-operated guppies.

		Stages of spermatogenesis									
		SPG		SPC		SPD		SPM		SPR	
		No	%	No	%	No	%	No	%	No	%
(A) Hypophysectomized	Mean[1]	1.8	3.52	0		0		0		55.8	96.48
	$\pm S\bar{x}$	±0.4	±0.99							±11.0	±0.99
(B) Testosterone-treated	Mean	9.8	46.23	4.4	22.16	0		0		6.8	31.58
	$\pm S\bar{x}$	±1.9	±2.16	±0.5	±1.19					±1.5	±1.87
(C) Sham-operated	Mean	3.8	1.70	23.4	11.08	14.4	6.94	20.2	9.70	152.4	70.57
	$\pm S\bar{x}$	±1.1	±0.35	±2.2	±1.16	±3.2	±1.59	±6.7	±3.24	±19.4	±5.52

[1]Mean of 5 observations

$P < 0.001$ between A & B; A & C; B & C: Contingency table (row x column)

cells and interstitial cells regress and their nuclei are shrunken (Figs. 9 & 13). The epithelial cells lining the efferent ducts are regressed (Fig. 5 and Table VIII). The lipophores become faint or entirely disappear.

Structure of the testis and secondary sex characters of sham-operated control guppy (testosterone experiment). The structure of the testis of sham-operated guppy is exactly similar to the adult testis previously described. All stages of spermatogenesis are present (Figs. 2 & 21 and Table VII). The efferent ducts and the main sperm duct are filled with intact spermatophores (Figs. 4 & 6). The epithelial cells lining the efferent ducts are tall and columnar (Fig. 4 and Table VIII). The secondary sex characters are unchanged in sham-operated males.

Effect of methyl testosterone on the adult testis of hypophysectomized guppy. Hypophysectomized male guppies were treated with methyl testosterone for five weeks. During this treatment two to three fish were killed at weekly intervals; the remaining (five) were killed at the end of the fifth week. There is a significant increase in the gonosomatic index of hypophysectomized guppy following methyl testosterone treatment (Table IX) associated with a rapid multiplication of both spermatogonial and spermatocyte-cysts (Figs. 16, 21 and Table VII). Cysts containing spermatocytes are evident by the end of second week of testosterone treatment but no later stages of spermatogenesis appear during the next three

TABLE VIII Epithelial cell heights of the efferent ducts of hypophysectomized, testosterone-treated and sham-operated guppies.

Fish No	Mean epithelial cell height[1] (u) (A) Hypophysectomized	(B) Testosterone-treated	(C) Sham-operated
1	4.4	34.0	25.5
2	4.8	31.5	21.2
3	3.8	30.2	18.6
4	4.3	38.9	22.4
5	3.5	39.4	20.1
Grand Mean	4.2	34.8	21.6

[1]Mean of 25 counts (5 epithelial cell heights in each of 5 efferent ducts in one section of each testis).

$P < 0.01$ between A & B; A & C; B & C.

Tukey's test - $W_{.01} = 5.05$. Test based on 5 median counts, 1 in each of 5 efferent ducts of each testis.

TABLE IX G.S.I. of hypophysectomized, testosterone-treated and sham-operated guppies.

Fish No	G. S. I. (A) Hypophysectomized	(B) Testosterone-treated	(C) Sham-operated
1	0.69	1.37	3.04
2	0.67	1.39	3.10
3	0.48	1.18	2.76
4	0.77	1.34	3.41
5	0.71	1.12	3.27
Mean $\pm S_{\bar{x}}$	0.66 ± 0.04	1.28 ± 0.05	3.12 ± 0.11**

** $P < 0.01$ between A & B; A & C; B & C.
Tukey's test - $W_{.01} = 0.40$

weeks. Thus testosterone treatment does not lead to complete restoration of spermatogenesis in hypophysectomized guppies.

Occasional mitotic division in the spermatogonial cysts can be noticed (Fig. 17). The resorption of sperm, resulting from the rupture of spermatophores, is complete within the efferent ducts and the main sperm duct during the first two weeks of testosterone treatment (Fig. 18). This indicates that the exogenous testosterone enhances the rupture of spermatophores and the phagocytosis (resorption) of sperm. Only few spermatophores are left intact at the periphery (Fig. 16). Methyl testosterone treatment stimulates the epithelial cells of both efferent ducts and main sperm duct so that they assume the tall columnar appearance of the normal animal (Fig. 19 and Table VIII). Sertoli cells and interstitial cells are also restored to normal and their nuclei become spherical in shape with conspicuous nucleoli (Fig. 17). The epithelial cells lining the sperm ducts, Sertoli cells and interstitial cells respond to the testosterone treatment in a similar way. They regress in the absence of the pituitary and attain their normal appearance following testosterone treatment. Except for the cysts containing spermatogonia, spermatocytes and spermatophores, the whole testis is filled with fibrous connective tissue (Fig. 16).

Effect of methyl testosterone on secondary sex characters of hypophysectomized guppy. The gonopodium which was unaffected by hypophysectomy remains unchanged when the animals are treated with methyl testosterone. There is moderate recovery in the content of lipophores present on the sides of body and the tail. The lipophores were rated as ++ in the arbitrary quantitative rating. This suggests that the lipophores are directly controlled by the androgens.

Figure 1a: Section of whole testis of adult guppy.

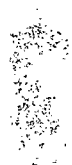


Figure 1b: Detail of a portion of testis indicated by arrow in 1a.

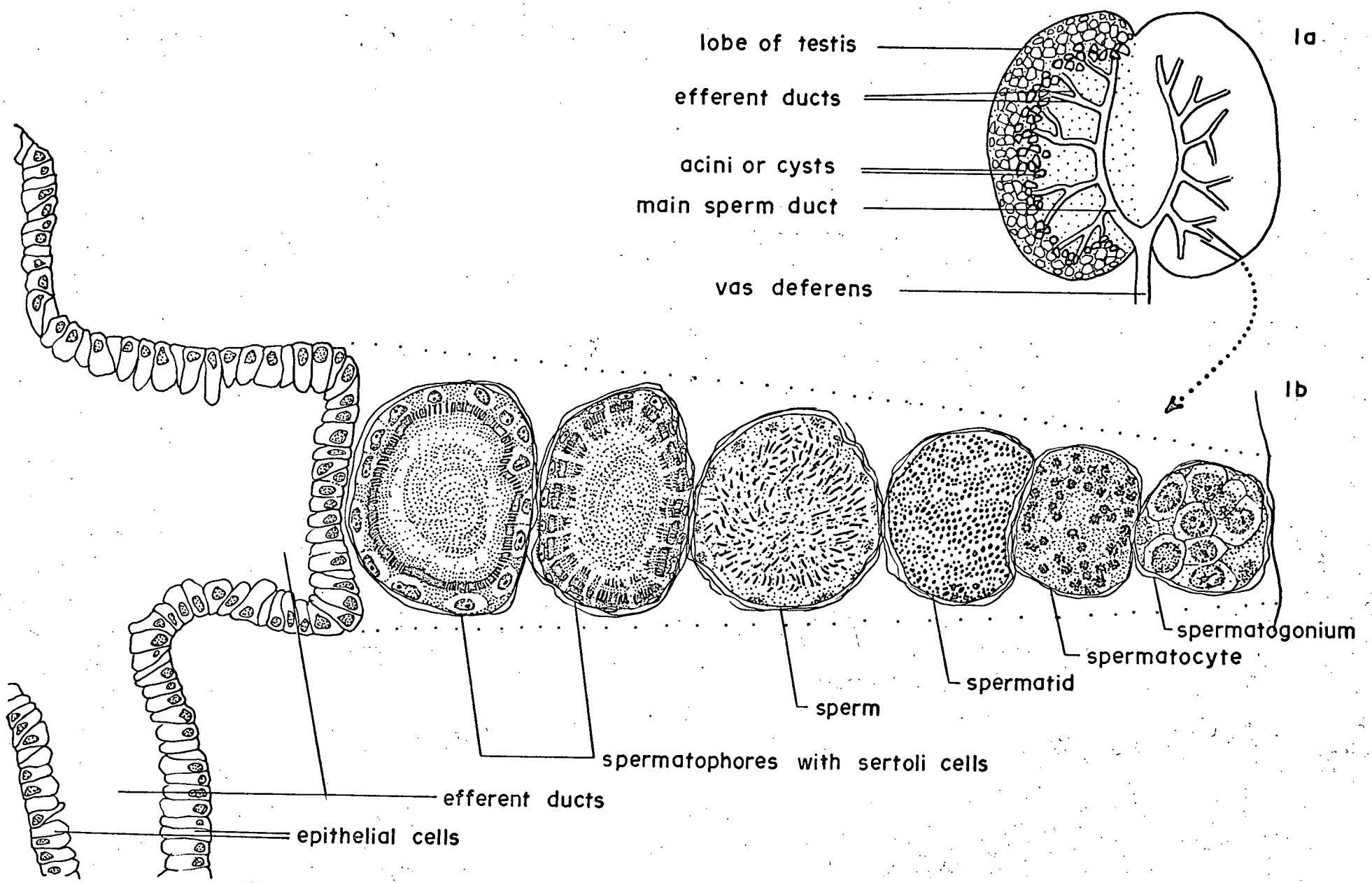

lobe of testis
efferent ducts
acini or cysts
main sperm duct
vas deferens
1a
1b
spermatogonium
spermatocyte
spermatid
sperm
spermatophores with sertoli cells
efferent ducts
epithelial cells

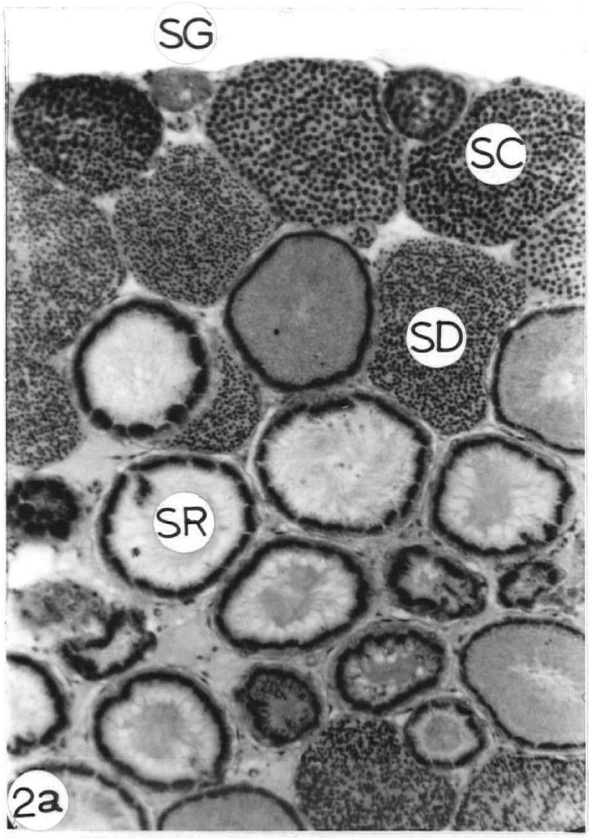
Figure 2a: Sagittal section of adult testis showing different stages of spermatogenesis (x 200)

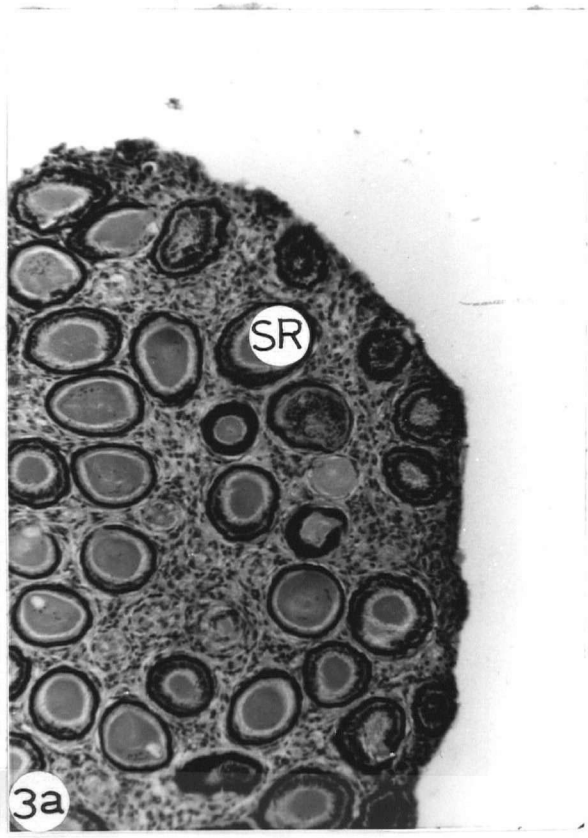
Figure 2b: Magnified view of a portion of 2a (x 900)

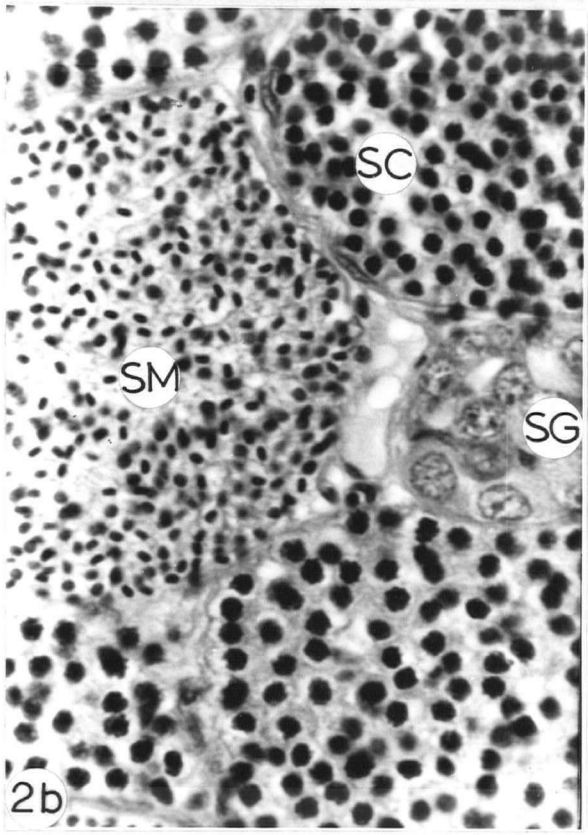
Figure 3a: Sagittal section of testis eight weeks after hypophysectomy showing only spermatophores (x 200)

Figure 3b: Magnified view of a portion of 3a (x 900)

SG - Spermatogia; SC - Spermatocytes;
SD - Spermatids; SM - Sperm; SR - Spermatophores


SG
SC
SD
SR
2a


SR
3a


SC
SM
SG
2b

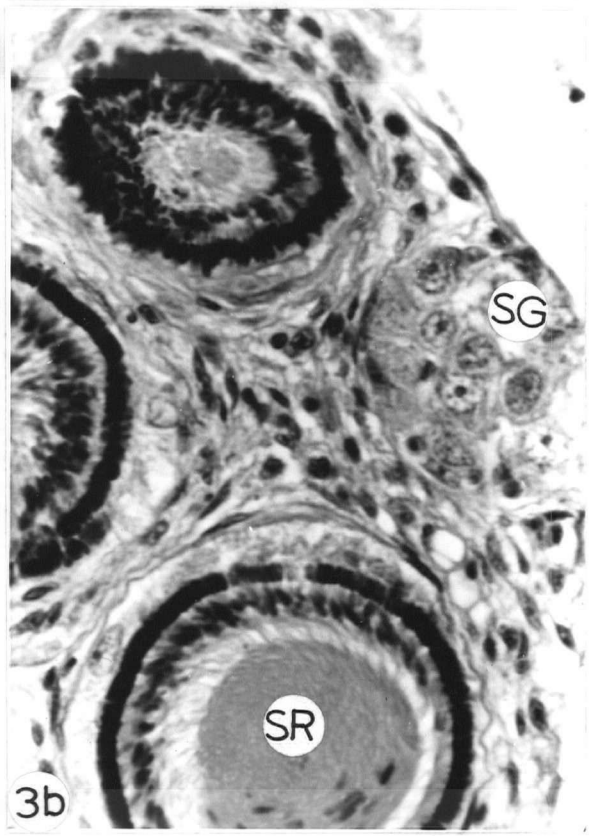

SG
SR
3b

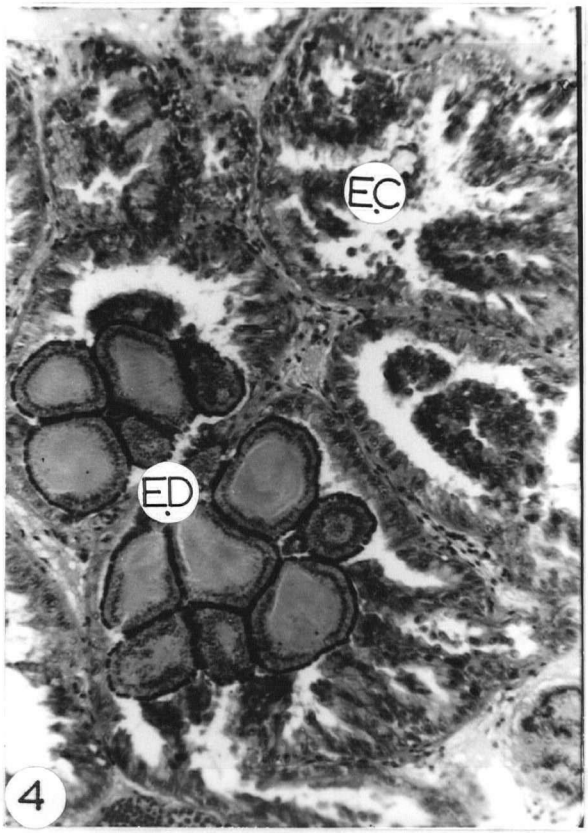
Figure 4: Efferent ducts of adult testis containing spermatophores (x 200).

Figure 5: Ruptured spermatophores in the efferent ducts of testis eight weeks after hypophysectomy (x 200)

Figure 6: Main sperm duct of adult testis containing spermatophores (x 200).
Note the colloidal material in main sperm duct.

Figure 7: Ruptured spermatophores in the main sperm duct of testis eight weeks after hypophysectomy (x 200)

E.D. - Efferent ducts; M.D.- Main sperm duct;
E.C. - Epithelial cells; C.L. - Colloidal material.



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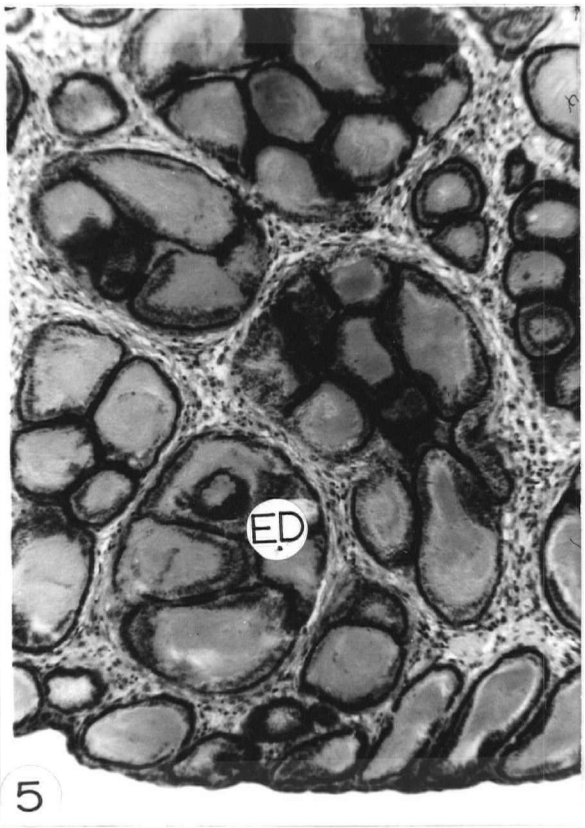

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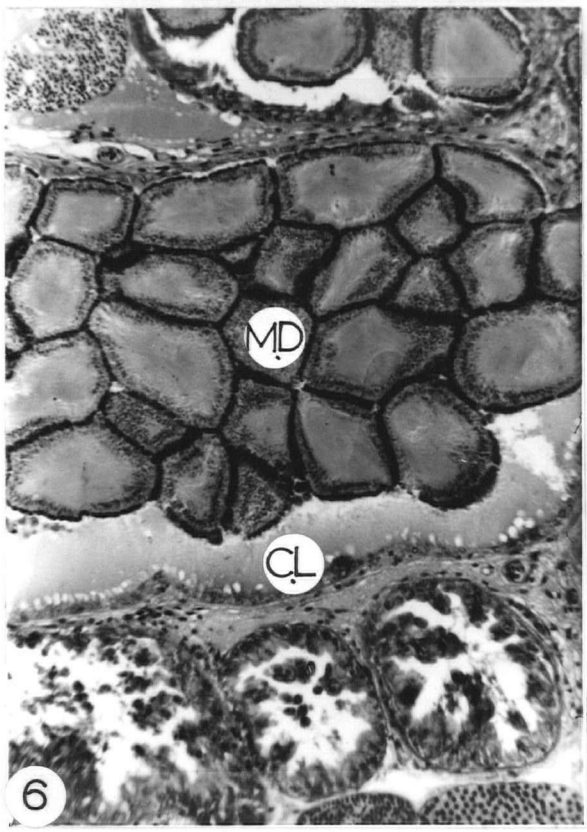

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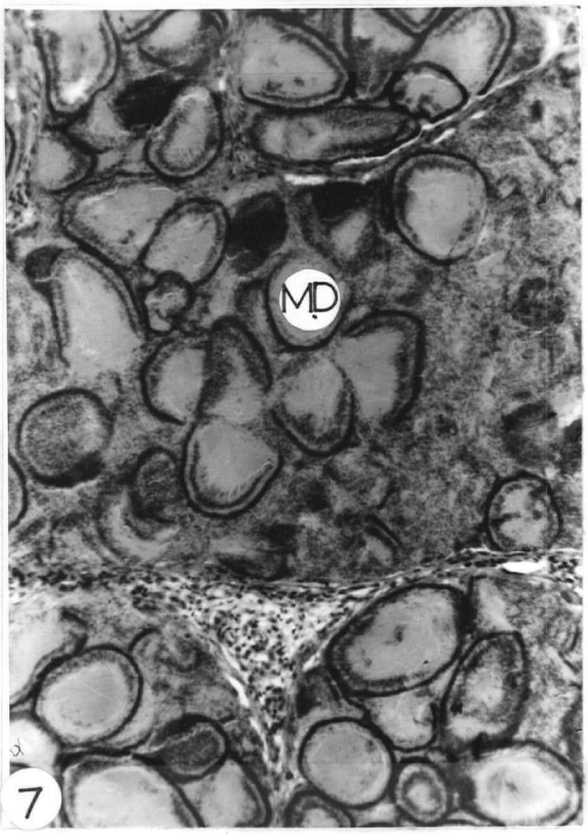


7

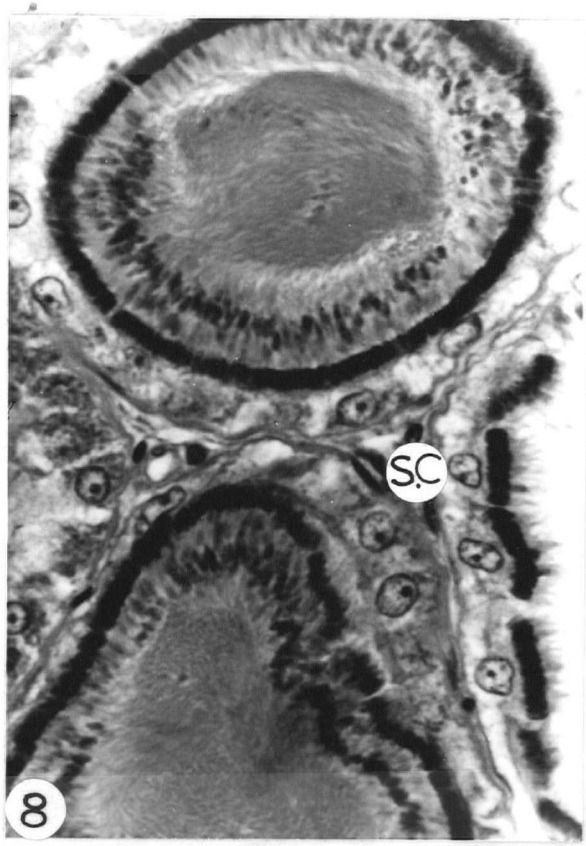
Figure 8: Sertoli cells of adult testis with sperm heads attached to them (x 900).

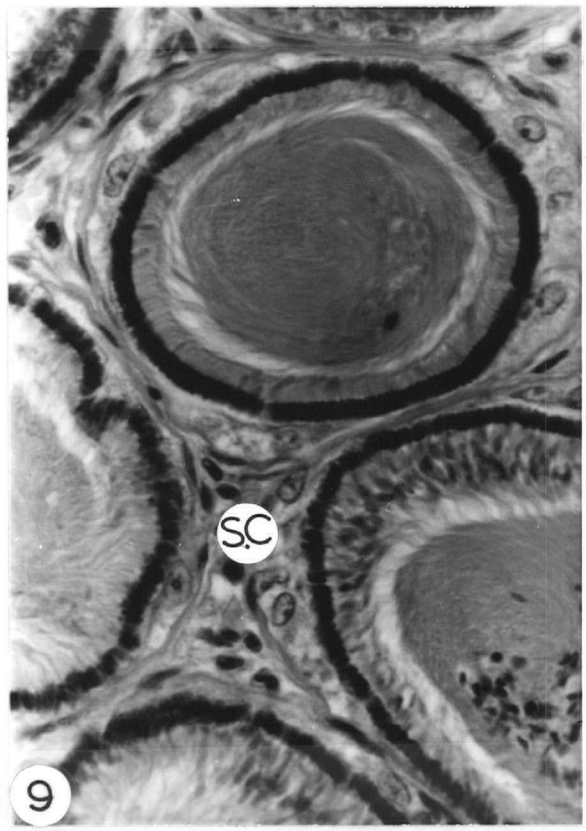
Figure 9: Regressed Sertoli cells of testis eight weeks after hypophysectomy (x 900).

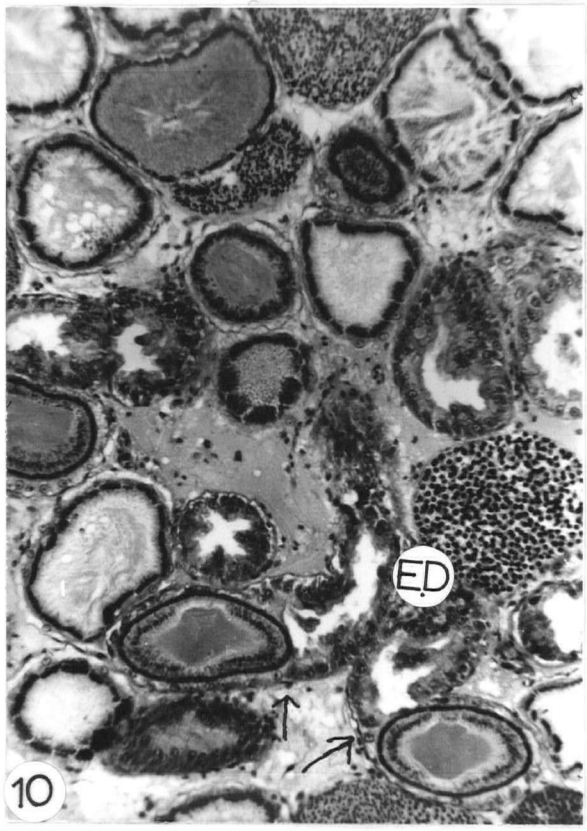
Figure 10: Spermatophores with surrounding Sertoli cells of adult testis opening into efferent ducts (indicated by arrows) (x 200).

Figure 11: Testis at the end of third week after hypophysectomy containing only sperm-cysts and spermatophores (x 200).

S.C. - Sertoli cells.


S.C
8


S.C
9


E.D
10

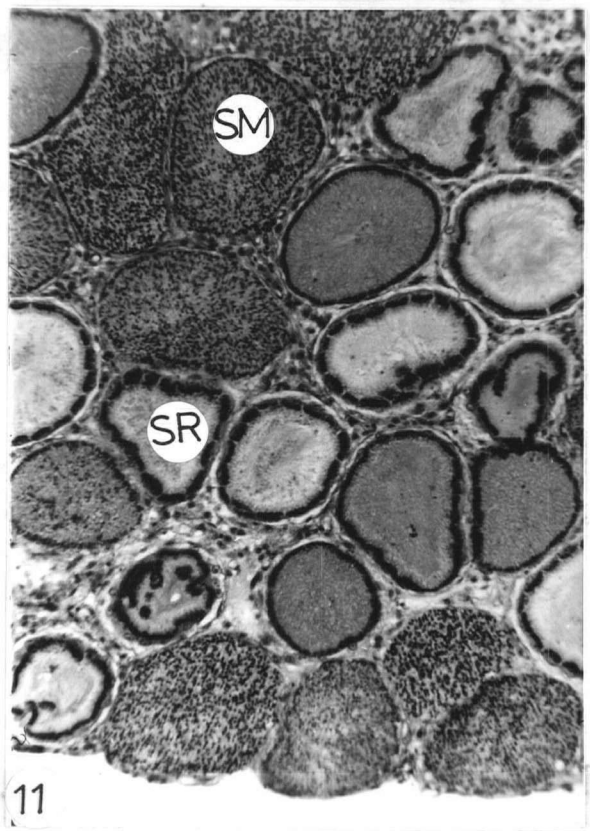

SM
SR
11

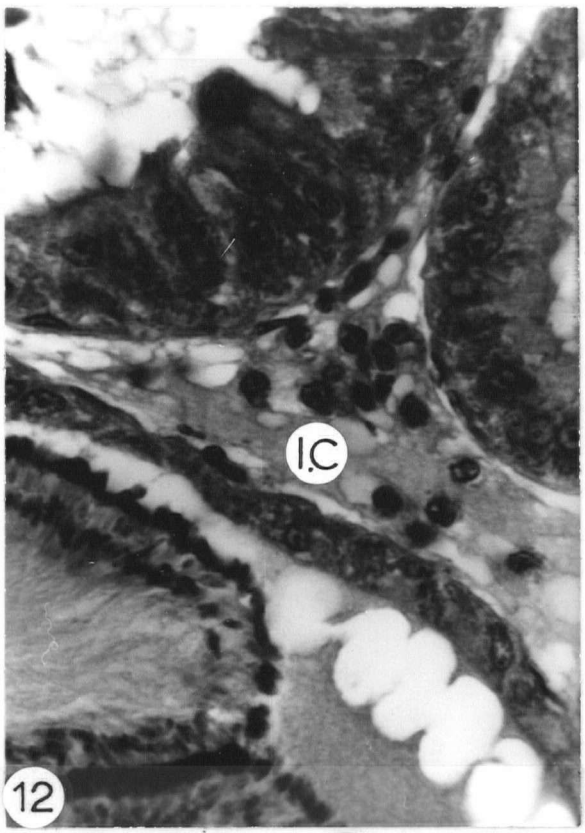
Figure 12: Interstitial cells of adult testis (x 900).

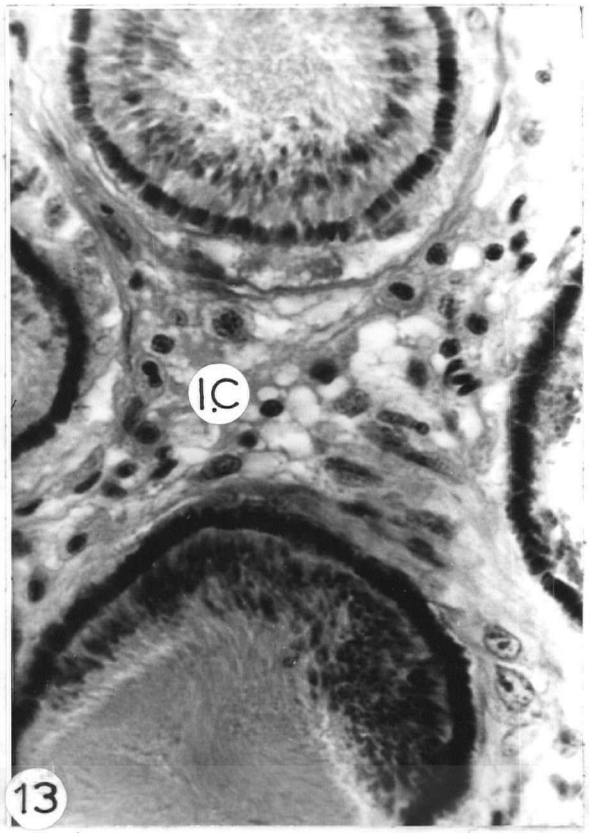
Figure 13: Regressed interstitial cells of testis eight weeks after hypophysectomy (x 900).

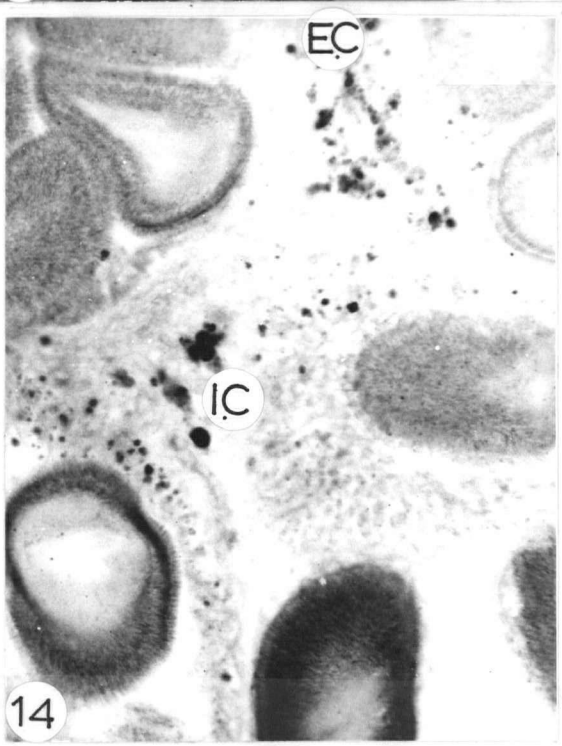
Figure 14: Interstitial cells and epithelial cells lining the efferent ducts of adult testis stained with Sudan black B (x 350).

Figure 15: Testis showing remnant sperm being phagocytosed in efferent ducts 16 weeks after hypophysectomy (x 200).

I.C - Interstitial cells; R.S - Remnant sperm


I.C
12


I.C
13


E.C
I.C
14

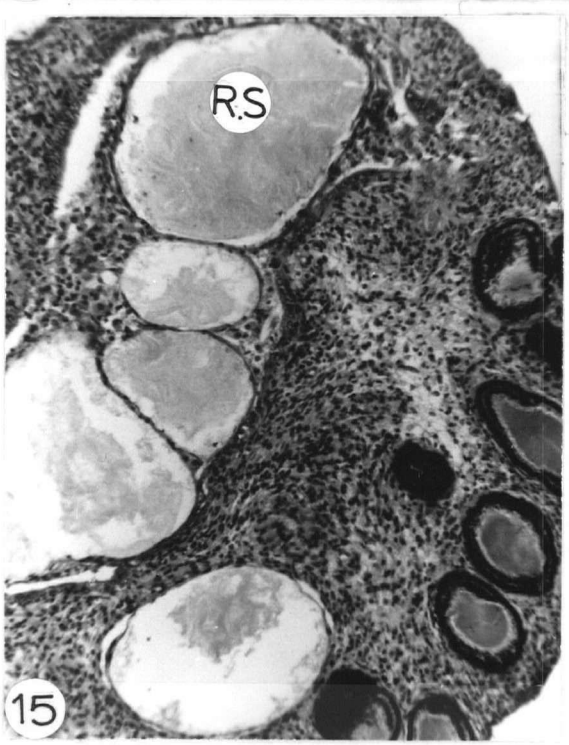
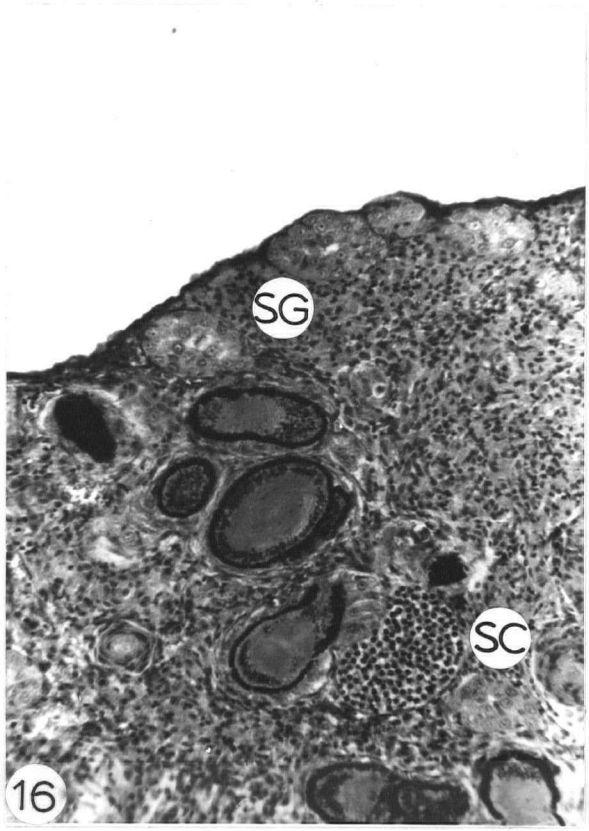

R.S
15

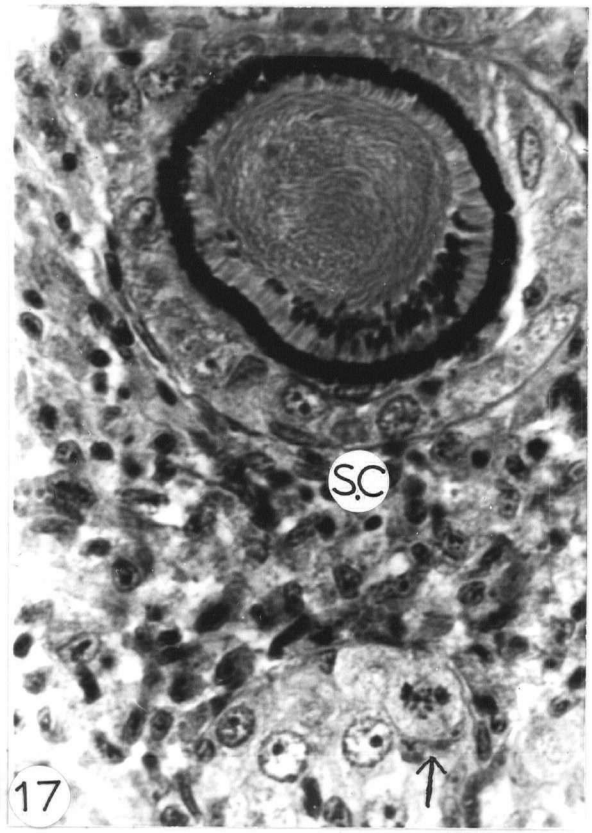
Figure 16: Testis of hypophysectomized guppy treated with testosterone for five weeks (x 200).

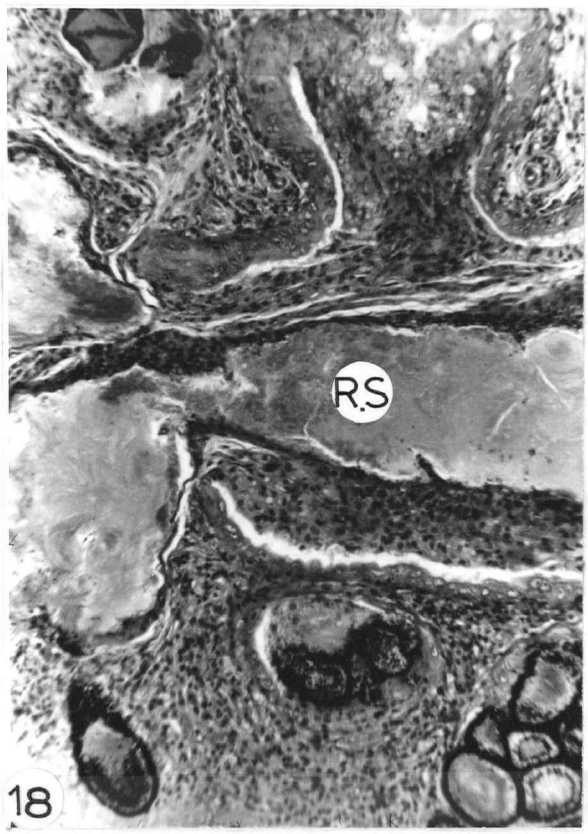
Figure 17: Magnified view of a portion of figure 16 (x 900). Arrow indicates mitotic division in spermatogonia. Sertoli cells assume normal appearance.

Figure 18: Remnant sperm in main sperm duct of hypophysectomized guppy after two weeks of testosterone treatment (x 200). Note regressed epithelial cells lining main sperm duct.

Figure 19: Tall epithelial cells reappear in sperm duct of hypophysectomized guppy after five weeks of testosterone treatment (x 200).


SG
SC
16


S.C
17


R.S
18

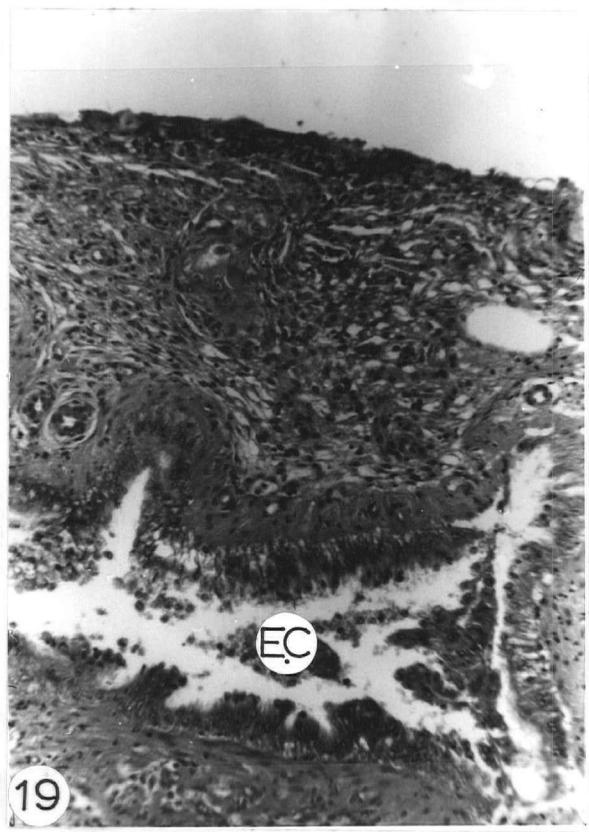

E.C
19

Figure 20: Percentage of different stages of spermatogenesis in hypophysectomized and sham-operated adult guppies after eight weeks. The vertical line on top of each bar represents Standard Error.

SPG - Spermatogonia; SPC - Spermatocytes;
SPD - Spermatids; SPM - Sperm;
SPR - Spermatophores.

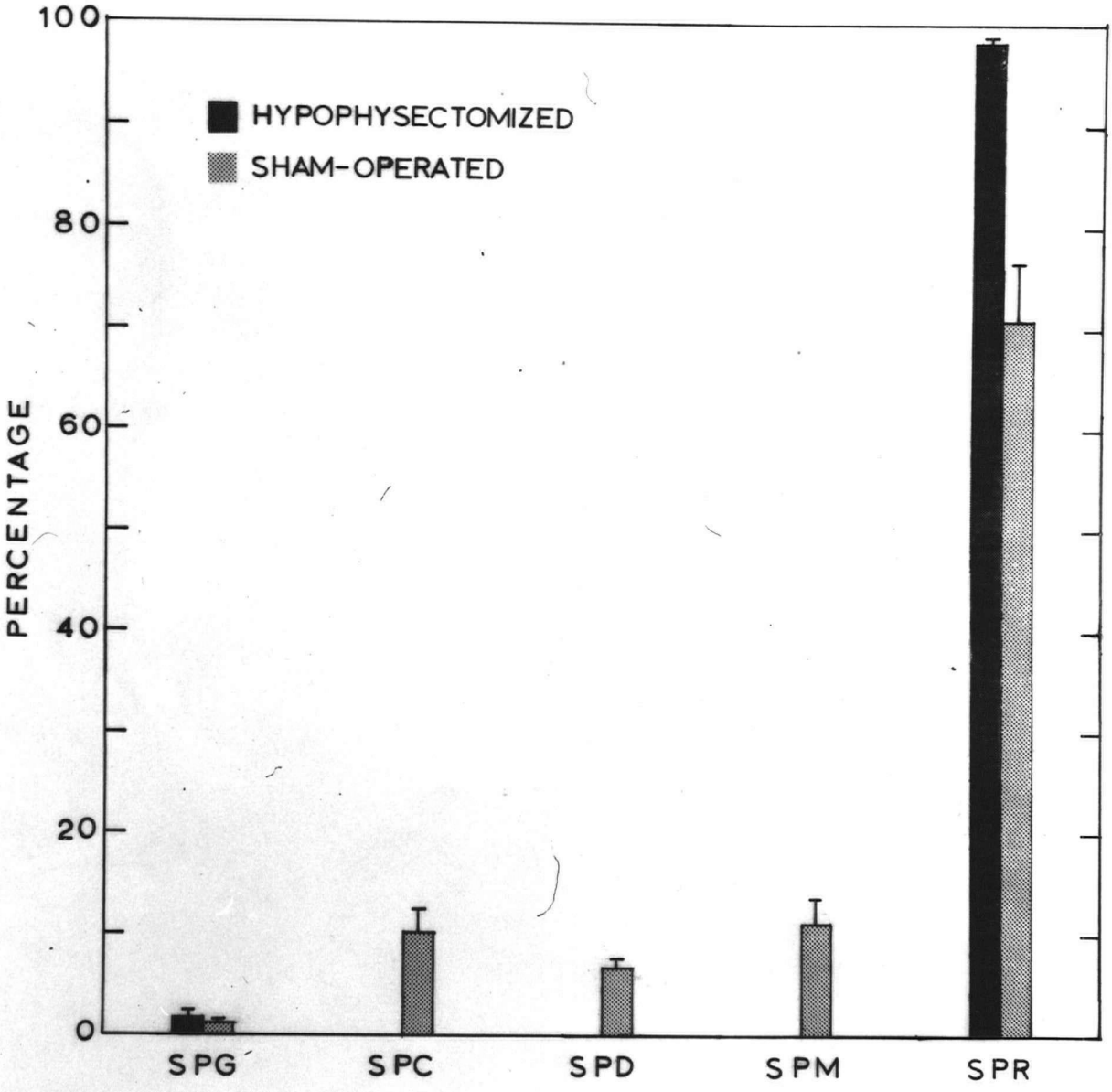
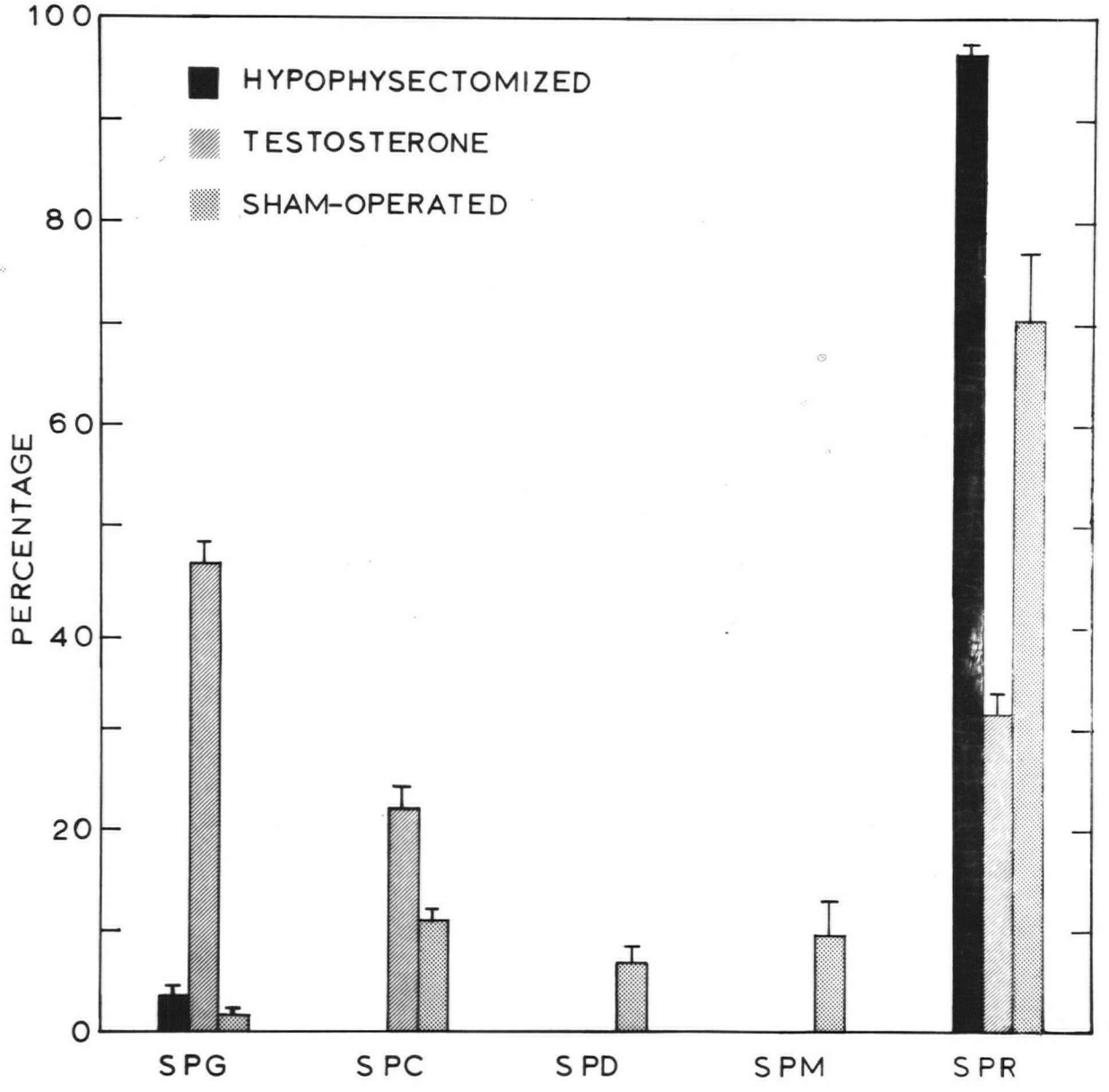

100
80
60
40
20
0
PERCENTAGE
HYPOPHYSECTOMIZED
SHAM-OPERATED
SPG
SPC
SPD
SPM
SPR

Figure 21: Percentage of different stages of spermatogenesis in hypophysectomized; testosterone-treated and sham-operated adult guppies.


100
80
60
40
20
0
PERCENTAGE
HYPOPHYSECTOMIZED
TESTOSTERONE
SHAM-OPERATED
SPG
SPC
SPD
SPM
SPR

SECTION II

HYPOPHYSECTOMY AND METHYL TESTOSTERONE TREATMENT OF THE JUVENILE GUPPIES.

INTRODUCTION

Although gonadal differentiation occurs shortly before birth, the two sexes are externally indistinguishable in the newly born young. In male embryos, the germ cells are relatively uniformly distributed among stroma cells but at birth the spermatogonia tend to migrate to the periphery. Later (21 days after birth) the sperm ducts appear as narrow slits surrounded by stroma cells (Goodrich et al 1934). In this section, the effects of hypophysectomy on such juvenile males without secondary sex characters and with gonads containing only spermatogonia, are described. The results of treating such hypophysectomized juvenile males with methyl testosterone should indicate whether secondary sex characters are directly controlled by pituitary hormones or secondarily by the androgens.

MATERIALS AND METHODS

Maintenance of hypophysectomized juvenile fish. One week prior to the operation juvenile guppies (9-11 mm standard length; 14-30 mg in weight) including both males and females (since sex is indistinguishable at this stage)

were taken from the breeding aquaria and put in aquaria with 16-litre of fish saline. The juveniles (both hypophysectomized and sham-operated) were kept in this medium until the end of the experiment. A group of juveniles was killed as initial controls immediately after removal from the breeding aquaria. The maintenance of juveniles was similar to that described earlier for hypophysectomized adult males.

Procedure of hypophysectomy. Juvenile guppies conditioned to fish saline for one week were anesthetized with 1:800 MS 222 (Tricaine Methane Sulphonate-Sandoz). The fish was placed ventral side uppermost on a (6 x 3 x ½ cm) piece of soft plastic sponge fixed in the middle of a wax-filled rectangular plastic tray (16 x 8 x 3½ cm). The fish was secured in place by a thin strip of soft plastic sponge stretched across the ventral surface just posterior to the gills and was immersed in fish saline during the operation.

The operation of the juvenile guppy for the removal of the pituitary was similar to that previously described for the adult male. Mortality was high, amounting to 30-35% during the operation; a further mortality of 25-30% occurred in two weeks following the operation.

Methyl testosterone treatment: The aquaria with 16-litre of fish saline were used for testosterone experiment. Two different concentrations of methyl testosterone were used; $1:2 \times 10^6$ (8 mgm of methyl testosterone in 16 litres of fish saline per week) and $1:10^7$ (1.6 mgm of methyl testosterone in 16 litres of fish saline every week). The treatment of hypophysectomized juvenile guppies with methyl

testosterone was begun one week after the operation. The treatments continued for eight weeks. The maintenance of juveniles was similar to that described earlier for methyl testosterone treated hypophysectomized adult males. Hypophysectomized juvenile guppies which did not receive any testosterone treatment and sham-operated juveniles were the two types of controls and were also kept in fish saline.

Histology. Because of the small size, the entire body of the juvenile guppy except for its caudal peduncle and the tail was fixed in formic acid-Bouin fixative and left for seven days in fixative to decalcify before dehydration in ethyl alcohol and embedding in paraffin wax. Serial longitudinal sections were cut at 5 u and stained with Ehrlich's haematoxylin and eosin.

Measurements. The percentage of different stages of spermatogenesis present in the testes of the juvenile or the adult guppies (since sham-operated juveniles became adults during the experimental period) was calculated in the same way as explained previously for the adult males. The total number of efferent ducts in the median sagittal section was counted.

The width and lumen of five randomly chosen efferent ducts (before they join to form main sperm duct) were measured with an ocular micrometer in one section of each testis of hypophysectomized, testosterone treated or sham-operated guppies. The height of epithelial cells and

width of lumen (space between epithelial cell linings) of main sperm duct in the median sagittal section of each testis were measured at five different places which covered the entire length of main sperm duct.

RESULTS

Structure of the juvenile testis (initial control).

The juvenile testis consists of two lobes connected by a bridge of mesodermal or stroma cells. It is surrounded by a thin, squamous, peritoneal membrane. The testis is situated in the posterior part of the body cavity, ventral to the airbladder and dorsal to the pancreas and the coils of intestine.

No other stages of spermatogenesis except spermatogonia are found in the testis. Spermatogonia are present in small cysts at the periphery of testis (Figs. 22, 27 and Table X); the centre is filled with stroma cells (Fig. 23). Mitotic division is not evident in the spermatogonial cysts. Spermatogonia are oval with distinct cellular membranes and homogeneous cytoplasm except for the presence of occasional fine basophilia. Their nuclei are almost round with evenly distributed fine chromatin granules. There is usually a single nucleolus. The stroma cells, on the other hand, have no distinct cellular membranes. Their nuclei are elliptical and each contains an irregular and coarse reticulum. The Sertoli cells and interstitial cells are not evident in the testis.

TABLE X Number and percentage of different stages of spermatogenesis in juvenile control; juvenile hypophysectomized and juvenile sham-operated guppies.

		Stages of spermatogenesis									
		SPG		SPC		SPD		SPM		SPR	
		No	%	No	%	No	%	No	%	No	%
(A) Juvenile Control	Mean[1]	13.4	100	0		0		0		0	
	$\pm S_{\bar{x}}$	±1.28									
(B) Juvenile Hypophysectomized	Mean[1]	12.4	100	0		0		0		0	
	$\pm S_{\bar{x}}$	±0.68									
(C) Juvenile Sham-operated	Mean[1]	3.8	2.17	34.8	20.03	23.0	13.07	36.4	20.92	78	43.81
	$\pm S_{\bar{x}}$	±0.8	±0.45	±4.6	±2.83	±1.1	±0.29	±2.6	±1.96	±10.9	±4.31

[1]Mean of 5 observations.

$P < 0.001$ - A & C; B & C.
Contingency table (row x column)

During development the stroma cells in the centre of the testis are rearranged to form small efferent ducts, although the lumen in the efferent duct is hardly distinct (Fig. 23 and Table XI). The efferent ducts from the two lobes of the testis open at the posterior end into a main sperm duct with a narrow lumen (Fig. 25 and Table XII). The epithelial cells lining the main sperm duct are squamous and are derived from stroma cells (the main sperm duct appears as a result of rearrangement of stroma cells). The main sperm duct opens into the urogenital sinus.

Structure of the testis of hypophysectomized juvenile. Since the control sham-operated juveniles differentiated into adults in six weeks, the hypophysectomized juvenile guppies were also killed after six weeks. No changes take place in the structure of juvenile testis in the absence of the pituitary except for an increase in the number of connective tissue cells. The spermatogonial cysts are present at the periphery but show no mitotic division; consequently there is no increase in their number (Figs. 22, 27 and Table X). The Sertoli cells are not evident.

The stroma cells are not differentiated into the interstitial cells (stroma cells have elliptical nuclei and contain an irregular reticulum, whereas interstitial cells have round nuclei with a distinct nucleolus). There is no increase in the total number, width and the size of lumina of the efferent ducts (Fig. 23 and Table XI). The epithelial

TABLE XI Total number, width and lumen of efferent ducts of juvenile control, juvenile hypophysectomized and juvenile sham-operated guppies.

Fish No	Efferent ducts (A) Juvenile control			(B) Juvenile Hypophysectomized			(C) Juvenile Sham-operated		
	No	Width(u) Mean[1] ±$S_{\bar{x}}$	Lumen(u) Mean[1] ±$S_{\bar{x}}$	No	Width(u) Mean[1] ±$S_{\bar{x}}$	Lumen(u) Mean[1] ±$S_{\bar{x}}$	No	Width(u) Mean[1] ±$S_{\bar{x}}$	Lumen(u) Mean[1] ±$S_{\bar{x}}$
1	8	15.66 ±1.90	1.08 ±0.18	7	13.50 ±1.30	1.08 ±0.18	19	122.18 ±7.38	76.26 ±6.30
2	9	16.38 ±1.52	1.26 ±0.22	7	14.22 ±0.77	1.44 ±0.22	17	114.80 ±8.60	63.96 ±7.95
3	7	18.18 ±1.12	1.26 ±0.22	8	10.98 ±0.52	1.62 ±0.34	19	127.10 ±15.0	71.34 ±14.87
4	6	10.62 ±0.34	1.08 ±0.18	6	10.80 ±0.80	1.26 ±0.22	18	121.76 ±14.36	77.08 ±12.12
5	9	12.96 ±0.46	1.44 ±0.36	8	16.56 ±1.67	1.26 ±0.22	21	118.90 ±9.44	64.78 ±7.03
Mean[1] ±$S_{\bar{x}}$	7.2 ±0.58			7.2 ±0.37			18.8 ±0.66		

[1]Mean of 5 observations

$P < 0.01$ - A & C; B & C (for no., width and lumen)

Not significant - A & B (for no., width and lumen)

Tukey's test - $W_{.01}$ for no. - 2.77
$W_{.01}$ for width - 11.98
$W_{.01}$ for lumen - 10.67

TABLE XII Epithelial cell heights and lumen of main sperm duct of juvenile control; juvenile hypophysectomized and juvenile sham-operated guppies.

Fish No	Main sperm duct (u; Mean[1] ± $S\bar{x}$)					
	(A) Juvenile Control		(B) Juvenile Hypophysectomized		(C) Juvenile Sham-operated	
	Epithelial cells	Lumen	Epithelial cells	Lumen	Epithelial cells	Lumen
1	3.24 ±0.22	2.16 ±0.46	2.88 ±0.18	1.18 ±0.28	12.34 ±1.27	159.80 ±8.30
2	2.88 ±0.18	1.62 ±0.34	2.70 ±0.28	1.62 ±0.44	13.12 ±0.82	82.82 ±4.38
3	2.52 ±0.18	1.98 ±0.18	2.52 ±0.34	1.98 ±0.34	17.22 ±2.39	179.58 ±12.53
4	1.98 ±0.18	1.26 ±0.22	2.34 ±0.22	1.80 ±0.28	13.12 ±0.82	182.04 ±7.09
5	2.88 ±0.52	1.26 ±0.36	2.16 ±0.22	1.62 ±0.18	13.94 ±1.00	86.10 ±2.90

[1]Mean of 5 observations

$P < 0.01$ - A & C; B & C (for epithelial cells and lumen)

Not significant - A & B (for epithelial cells and lumen)

Tukey's test - $W_{.01}$ for epithelial cells - 1.71

$W_{.01}$ for lumen - 23.59

cells lining the main sperm duct remain squamous and the width of the lumen of the main sperm duct is not increased (Fig. 25 and Table XII).

Thus it is apparent that further development of the juvenile testis including the differentiation of the interstitial cells and Sertoli cells is stopped in the absence of the pituitary.

No secondary sex characters are present in the juveniles at the beginning of the experiment (Fig. 29). Secondary sex characters do not develop in animals hypophysectomized as juveniles. Both lipophore pigments and the anal fin remain in the sexually indifferent condition (Fig. 30). Thus it is evident that the presence of the pituitary is essential for the differentiation of secondary sex characters in the juvenile guppy.

Structure of the testis and secondary sex characters of the sham-operated juvenile. By the end of the experiment (six weeks) as was to be expected, the sham-operated juveniles were differentiated into adult males and females. The testis contains all stages of spermatogenesis (Fig. 2 and Table X). Spermatophores are also present in the efferent ducts and the main sperm duct (Figs 4 & 6). There is a considerable increase in the total number, width and the size of lumina of the efferent ducts (Fig. 4 and Table XI). The width of lumen of the main sperm duct also increases considerably and the epithelial cells lining it are columnar (Fig. 6 and Table XII).

Secondary sex characters are well differentiated in the males. The anal fin is transformed into the gonopodium (Fig. 31) while bright patches of lipophores appear on the sides of the body and on the tail.

Structure of the testis of hypophysectomized juvenile (control of testosterone experiment). The testis contains only spermatogonial cysts at the periphery and stroma cells in the centre (Figs. 22, 28 and Table XIII). The efferent ducts with narrow lumina are present in the centre of testis (Fig. 23 and Table XIV). The main sperm duct has narrow lumen and is lined by squamous epithelial cells (Fig. 25 and Table XV). No secondary sex characters develop.

Structure of the testis and secondary sex characters of the sham-operated juvenile. (Control of testosterone experiment). As might be expected, the sham-operated juveniles were differentiated into adult males and females by the end of the experiment (eight weeks). The testis contains all stages of spermatogeneis (Figs 2, 28 and Table XIII). The efferent ducts are well differentiated and are filled with spermatophores (Fig. 4 and Table XIV). The main sperm duct is also filled with spermatophores and has columnar epithelial cells (Fig. 6 and Table XV).

The males have distinct secondary sex characters. The anal fin is fully transformed into a typical gonopodium (Fig. 31), while bright patches of lipophores appear on the sides of the body; the lipophore index for these fish was +++ and ++++.

TABLE XIII Number and percentage of different stages of spermatogenesis in juvenile hypophysectomized; juvenile hypophysectomized and testosterone treated; juvenile sham-operated guppies.

		Stages of spermatogenesis									
		SPG		SPC		SPD		SPM		SPR	
		No	%	No	%	No	%	No	%	No	%
(A) Juvenile hypophysectomized	Mean[1]	12.8	100	0		0		0		0	
	$\pm S\bar{x}$	±0.4									
(B) Juvenile hypophysectomized & testosterone treated	Mean[1]	14.2	100	0		0		0		0	
	$\pm S\bar{x}$	±2.1									
(C) Juvenile sham-operated	Mean[1]	4.2	2.63	34.2	21.32	22.4	14.20	27.6	17.43	71.4	44.42
	$\pm S\bar{x}$	±1.0	±0.65	±5.1	±5.1	±1.8	±1.40	±4.5	±2.85	±7.2	±2.96

[1]Mean of 5 observations

$P < 0.001$ - A & C; B & C , contingency table (row x column)

TABLE XIV Total number, width and lumen of efferent ducts of juvenile hypophysectomized; juvenile hypophysectomized and testosterone treated; juvenile sham-operated guppies

Fish No	Efferent ducts								
	(A) Juvenile Hypophysectomized			(B) Juvenile Hypox. & Testo. treated			(C) Juvenile Sham-operated		
	No	Width (u) Mean[1] ±$S\bar{x}$	Lumen (u) Mean[1] ±$S\bar{x}$	No	Width (u) Mean[1] ±$S\bar{x}$	Lumen (u) Mean[1] ±$S\bar{x}$	No	Width (u) Mean[1] ±$S\bar{x}$	Lumen (u) Mean[1] ±$S\bar{x}$
1	9	18.36 ±0.67	1.08 ±0.18	39	33.44 ±5.38	14.76 ±3.99	12	132.84 ±15.43	75.44 ±12.67
2	9	17.10 ±1.03	1.44 ±0.36	41	32.58 ±3.42	11.70 ±2.55	17	108.22 ±8.06	59.04 ±8.36
3	8	12.06 ±0.73	1.62 ±0.18	31	28.44 ±2.95	9.36 ±1.38	19	117.26 ±7.05	63.14 ±8.16
4	7	12.24 ±1.26	1.62 ±0.34	28	27.36 ±2.44	8.28 ±1.04	18	111.52 ±8.44	72.16 ±4.78
5	7	11.16 ±0.61	1.44 ±0.36	26	30.06 ±2.63	8.28 ±1.25	20	102.5 ±6.08	63.82 ±3.23
Mean[1] ±$S\bar{x}$	8 ±0.1			33 ±3.0			19 ±0.71		

[1]Mean of 5 observations

$P < 0.01$ - A & B (for number and width only)

$P < 0.01$ - A & C; B & C; (for no, width and lumen)

Tukey's test - $W_{.01}$ for no - 9.07

$W_{.01}$ for width - 11.75

$W_{.01}$ for lumen - 9.16

$P < 0.05$ - A & B (for lumen only)

Tukey's test - $W_{.05}$ for lumen - 7.28

TABLE XV Epithelial cell heights and lumen of main sperm duct of juvenile hypophysectomized; juvenile hypophysectomized and testosterone-treated; juvenile sham-operated guppies.

Fish No	Main sperm duct (u; Mean[1] ±SE)					
	(A) Juvenile Hypophysectomized		(B) Juvenile Hypox. & Testesterone-treated		(C) Juvenile sham-operated	
	Epithelial cells	Lumen	Epithelial cells	Lumen	Epithelial cells	Lumen
1	3.60 ±0.28	1.08 ±0.18	16.20 ±2.36	26.28 ±3.26	10.74 ±0.95	136.94 ±11.12
2	3.42 ±0.34	1.26 ±0.22	9.18 ±1.00	21.96 ±4.08	13.94 ±4.02	94.30 ±10.37
3	2.52 ±0.34	2.16 ±0.36	11.88 ±0.96	19.80 ±3.14	11.48 ±1.53	113.16 ±13.57
4	2.88 ±0.34	1.62 ±0.18	8.46 ±0.73	16.56 ±3.06	14.72 ±2.78	113.16 ±14.53
5	2.16 ±0.22	1.80 ±0.28	7.02 ±0.60	10.62 ±1.74	13.12 ±2.01	130.38 ±18.89

[1]Mean of 5 observations

$P < 0.01$ - A & B; A & C; (for epithelial cells and lumen)

$P < 0.01$ - B & C (only lumen)

Tukey's test - $W_{.01}$ for epithelial cells - 3.39
$W_{.01}$ for lumen - 16.57

Not significant - B & C (epithelial cells)

Effect of methyl testosterone on the testis of hypophysectomized juvenile. No differences were evident in animals exposed to the two different concentrations of methyl testosterone (1:2 x 10^6 and $1:10^7$). Methyl testosterone treatment of fish hypophysectomized as juveniles does not seem to stimulate mitotic division in the spermatogonial cysts, nor is there any increase in the number of the cysts (Figs. 22, 28 and Table XIII). Spermatocytes or any other stages of spermatogenesis do not appear in the testis. In contrast, the methyl testosterone treatment of the hypophysectomized adult guppy stimulates mitotic divisions in the spermatogonial cysts and spermatocytes also appear.

This indicates that exogenous testosterone cannot directly act on the juvenile testis and initiate spermatogenesis. In the juvenile guppy the pituitary was removed before the testis received any gonadotropin stimulation. It seems that spermatogenesis in the juvenile testis can be triggered only by gonadotropins.

The stroma cells present in the centre of the testis of the hypophysectomized juvenile guppy do not become differentiated into interstitial cells following testosterone treatment. The Sertoli cells are not evident. Perhaps, gonadotropins regulate the differentiation of interstitial cells and Sertoli cells in the juvenile testis.

The most significant change in the structure of the testis of testosterone treated juvenile is the differentiation

of the efferent ducts and the main sperm duct. The total number, width and the size of lumina of the efferent ducts increase significantly (Fig. 24 and Table XIV). The lumen of the main sperm duct becomes very wide and the epithelial cells lining sperm duct become tall and columnar and even exceed in size the epithelial cells of the sperm duct of sham-operated juveniles (Fig. 26 and Table XV). This suggest that the differentiation of the efferent ducts and the main sperm duct is under the control of the androgens and gonodotropins are not directly involved. Methyl testosterone treatment also causes an increase in the number of fibroblasts in the juvenile testis.

Effect of methyl testosterone on secondary sex characters of hypophysectomized juvenile. All changes required to transform an anal fin into a gonopodium are initiated after testosterone treatment of hypophysectomized juveniles but the development of the fin is not complete. The ray 3 becomes thick (bone deposition) and develops a small hood which does not extend beyond the ray 3 (as in a normal gonopodium). The segments of rays 3 and 4 do not develop spines, which are well developed in a normal gonopodium. There is a small hook developed at the tip of ray 5 but it is smaller when compared to the hook of a normal gonopodium (Fig. 32).

The lipophores appear as two narrow bands, one on the upper margin and the other on the lower margin of the caudal fin. These two bands join at the posterior margin of

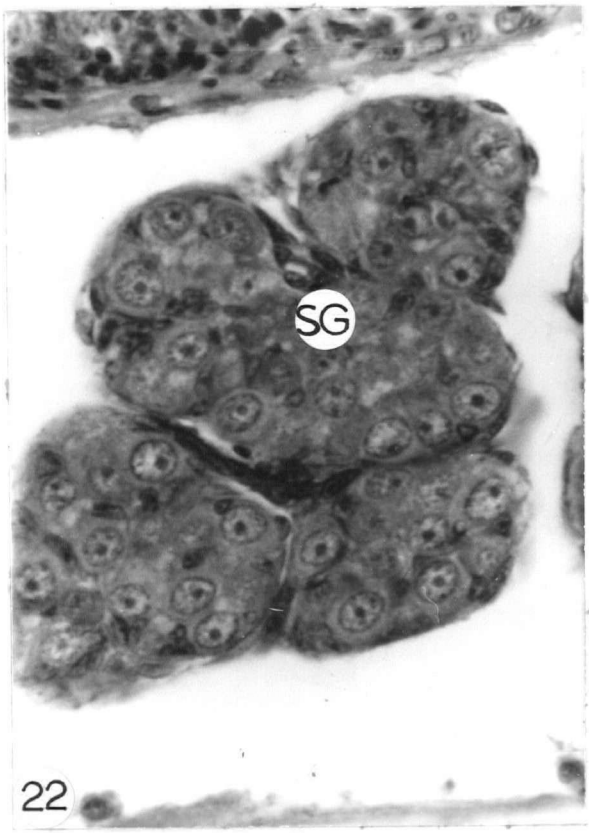
the caudal peduncle and extend onto the caudal peduncle as a single band. It is not evident beyond the area of the caudal peduncle (as is found in the adult males). Lipophores are also found dispersed on the anal fin. The brightness of lipophores is less than in normal adult males and was assigned ++ in the arbitrary scale described earlier.

The secondary sex characters which developed following testosterone treatment are similar in the hypophysectomized male and female juveniles. It seems that the secondary sex characters in the guppy are directly under the control of androgens. The incomplete differentiation of the secondary sex characters may be due to degree of dissimilarity between the synthetic exogenous androgen and the naturally occuring endogenous androgen or that the exogenous androgen cannot lead to complete differentiation of secondary sex characters in the absence of the pituitary.

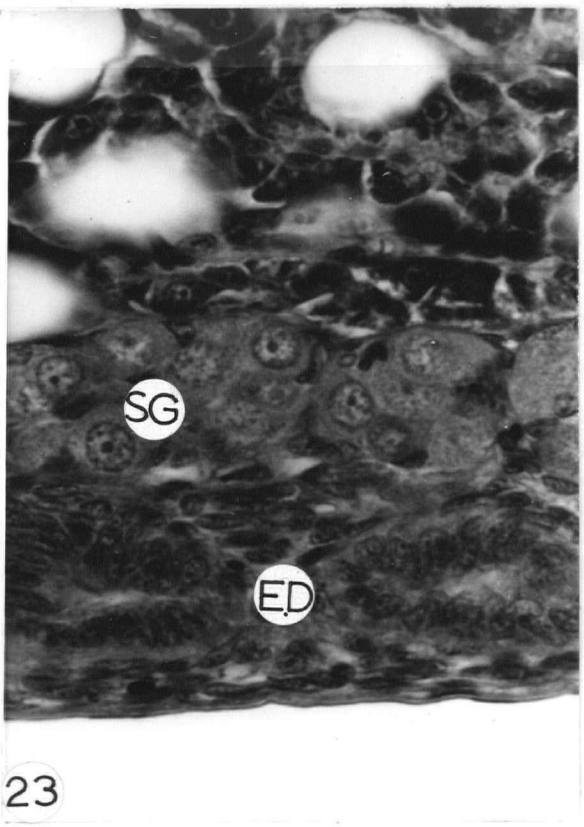
Figure 22: Sagittal section of testis of juvenile guppy showing only spermatogonial cysts (x 900).

Figure 23: Sagittal section of testis of juvenile guppy showing spermatogonia and efferent ducts (x 900).

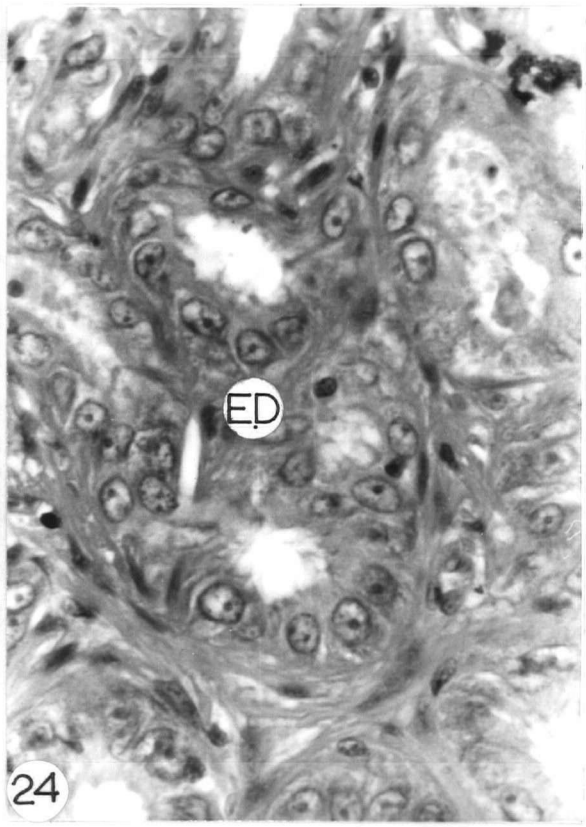
Figure 24: Efferent ducts of hypophysectomized juvenile treated with testosterone (x 900).



22



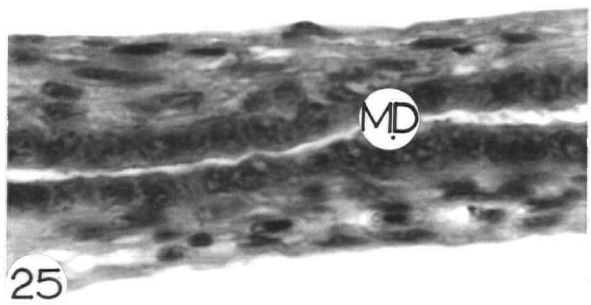
23



24

Figure 25: Sagittal section of testis of juvenile guppy showing main sperm duct lined by squamous epithelial cells (x 900).

Figure 26: Main sperm duct of testis of hypophysectomized juvenile treated with testosterone (x 900). Note the columnar epithelial cells lining main sperm duct.



25

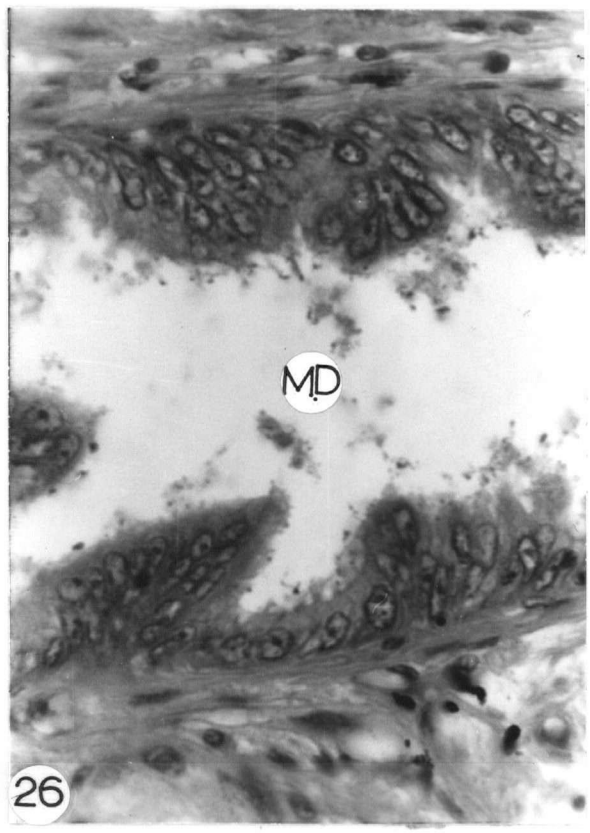


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Figure 27: Percentage of different stages of spermatogenesis in juvenile control; juvenile hypophysectomized and juvenile sham-operated guppies.

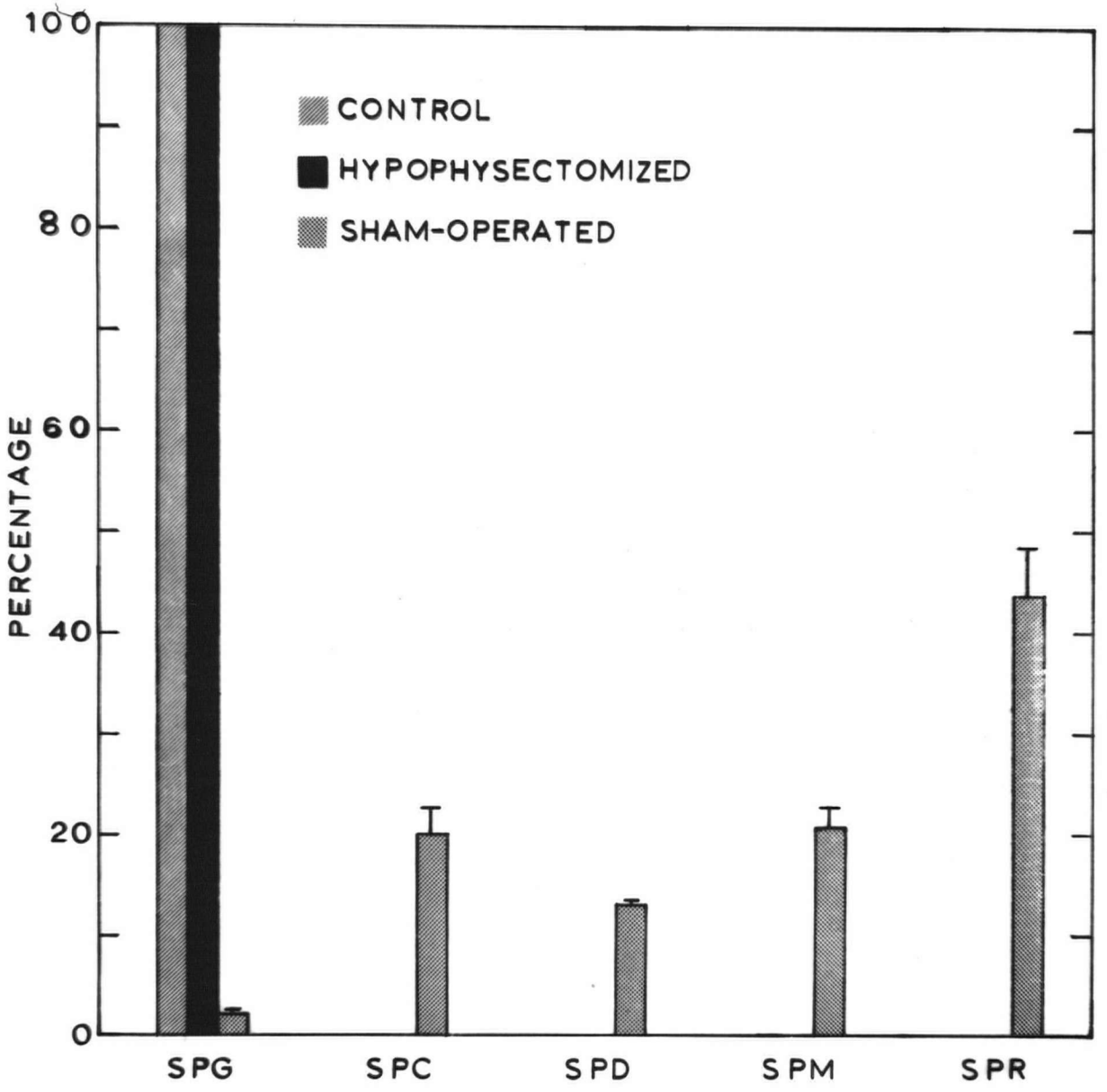

100
80
60
40
20
0
PERCENTAGE
CONTROL
HYPOPHYSECTOMIZED
SHAM-OPERATED
SPG
SPC
SPD
SPM
SPR

Figure 28: Percentage of different stages of spermatogenesis in juvenile hypophysectomized; juvenile hypophysectomized and testosterone-treated; juvenile sham-operated guppies.

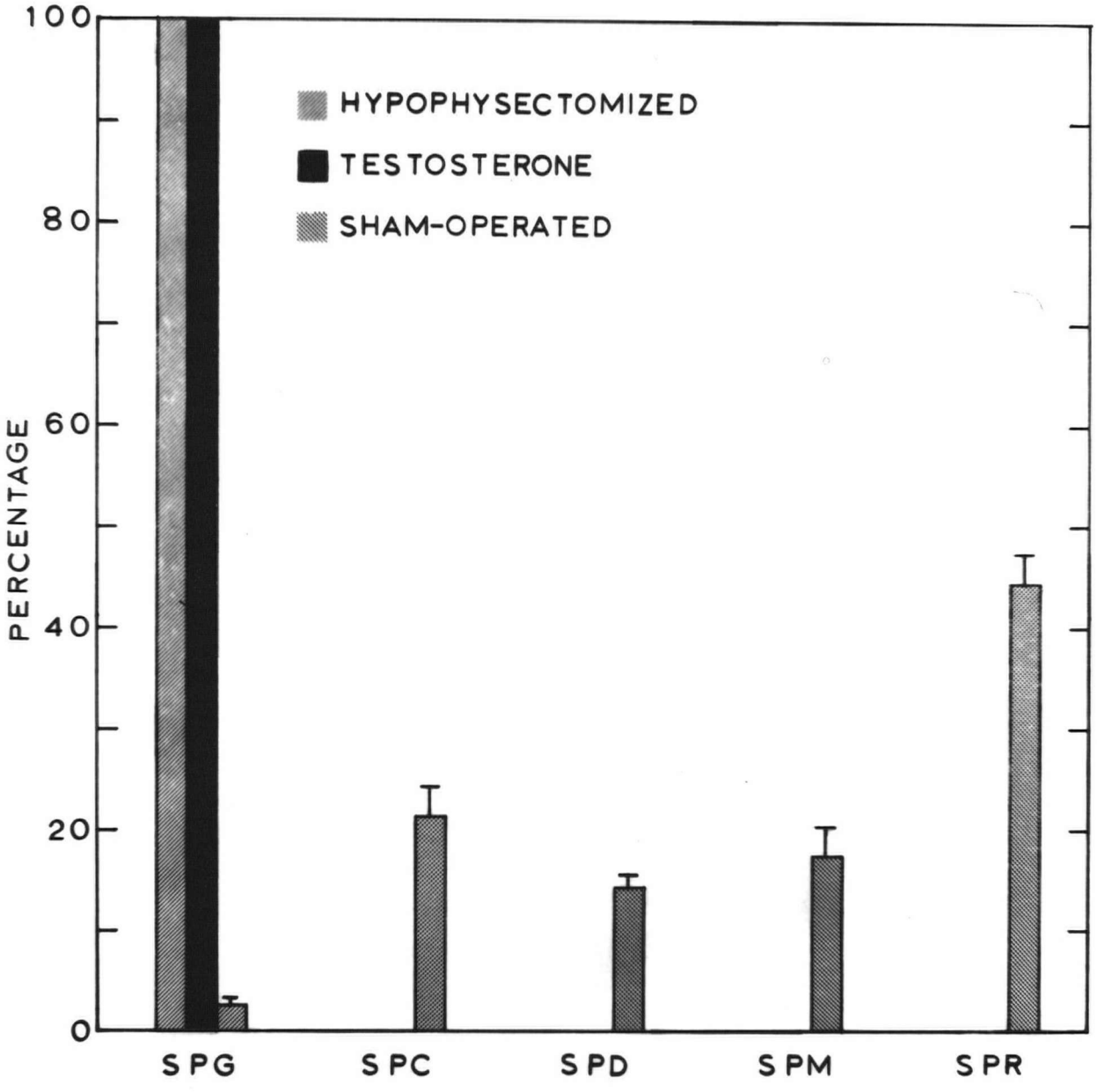
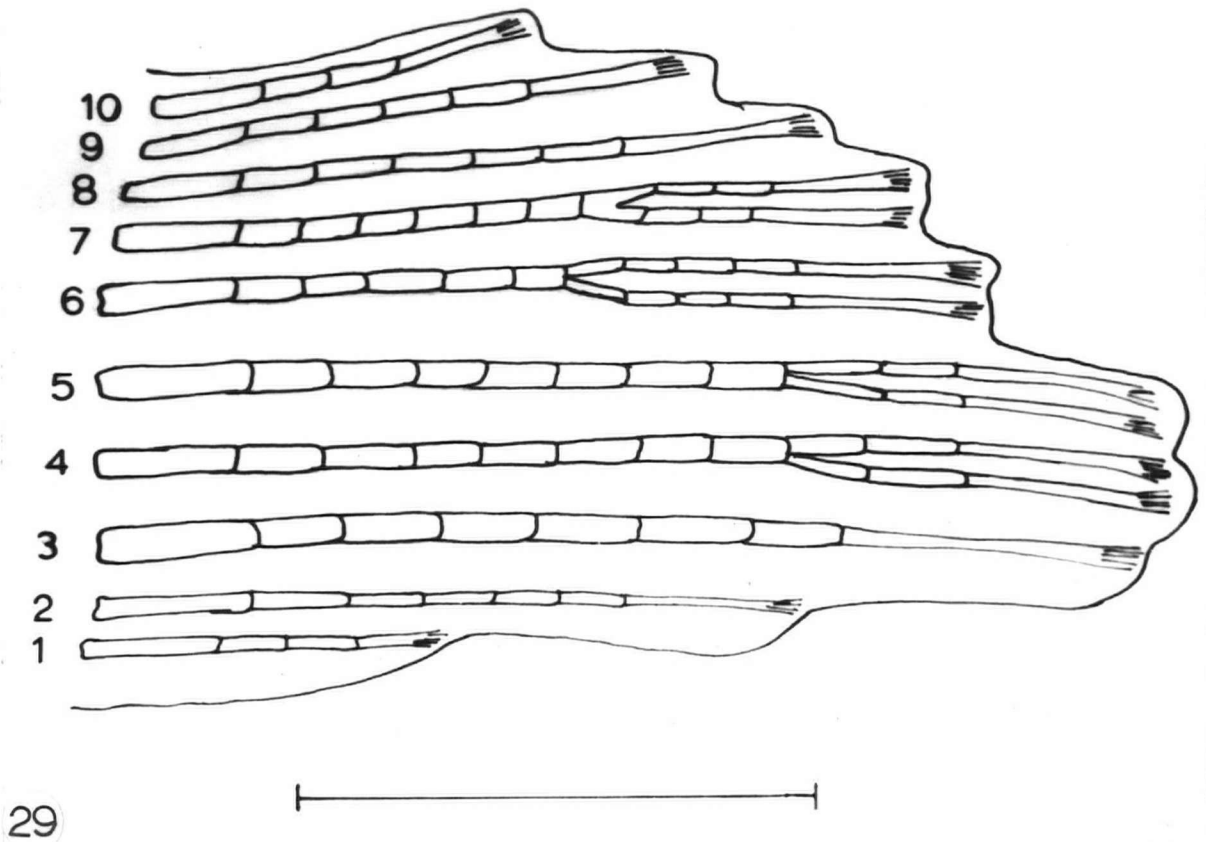

100
80
60
40
20
0
PERCENTAGE
HYPOPHYSECTOMIZED
TESTOSTERONE
SHAM-OPERATED
SPG
SPC
SPD
SPM
SPR

Figure 29: Anal fin of juvenile guppy. Fin-rays are numberd from cranial (ventral) to caudal (dorsal) border of fin. The line below the diagram represents 1 mm.

Figure 30: Anal fin of hypophysectomized juvenile.



29

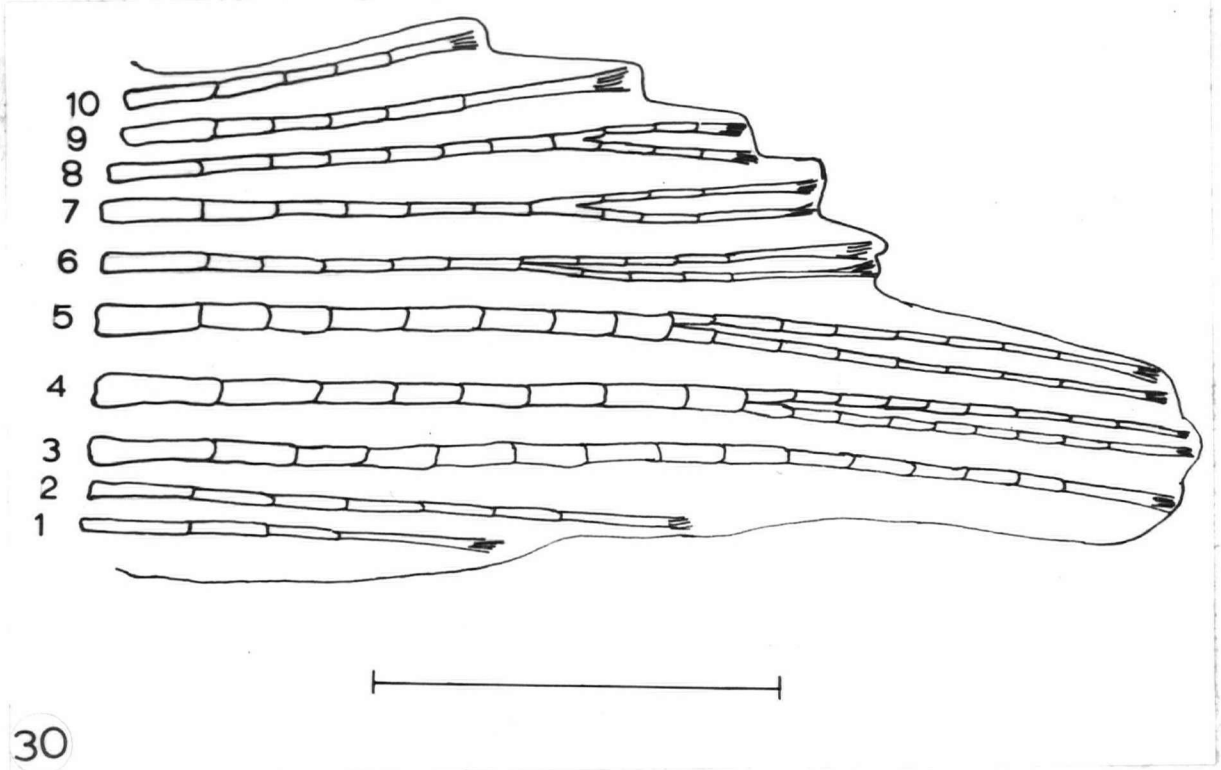


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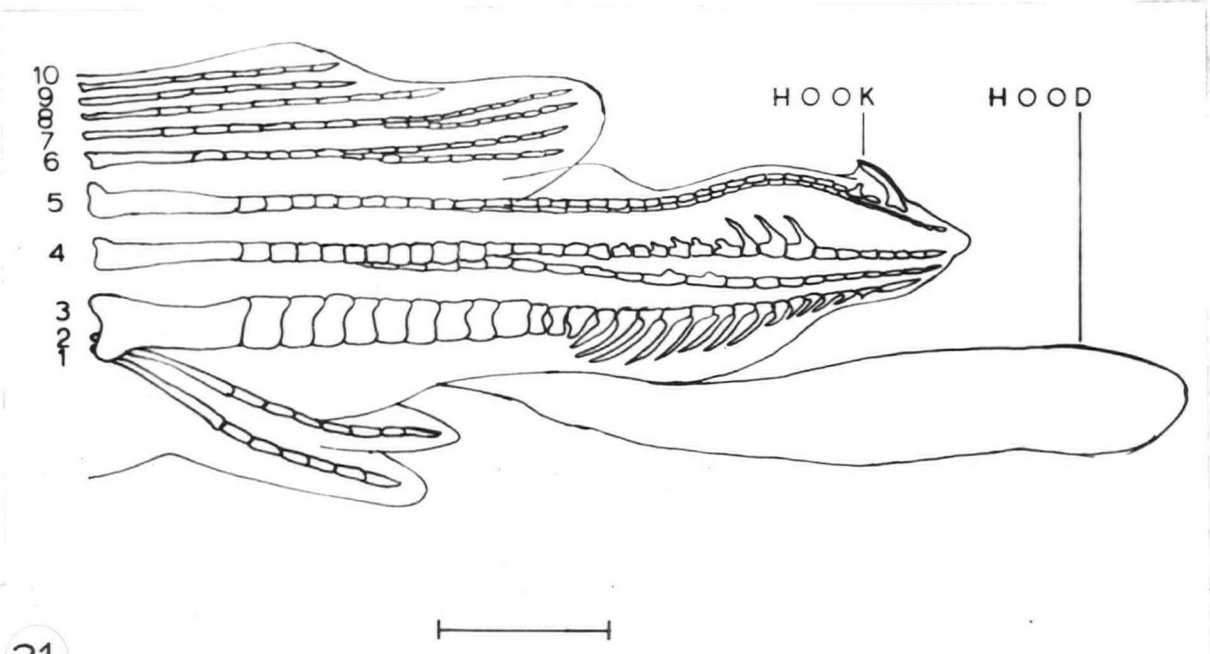
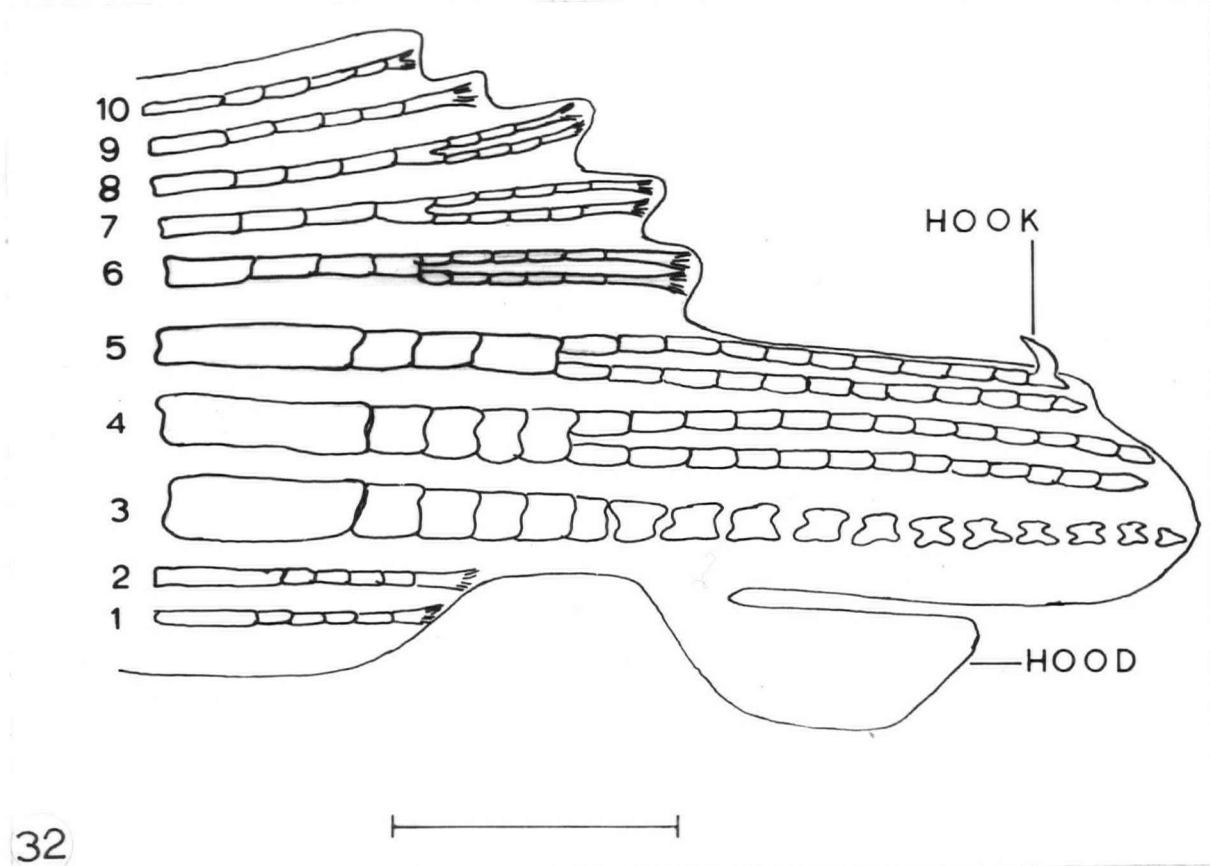
Figure 31: Gonopodium (modified anal fin) of adult male.

Figure 32: Anal fin of hypophysectomized juvenile treated with testosterone.



31



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SECTION III

'METHALLIBURE' TREATMENT OF THE JUVENILE AND ADULT MALES

INTRODUCTION

Hoar et al (1967) and Wiebe (1968) concluded that 'methallibure' effectively blocks the pituitary gonadotropic action. This point may be further substantiated by comparing the effects of 'methallibure' treatment with that of hypophysectomy. In this section, an attempt is made to compare the effects of 'methallibure' treatment (pharmacological hypophysectomy) on the testes and secondary sex characters of the adult and juvenile guppies with that of surgical hypophysectomy described in earlier sections. The site of action of 'methallibure' in eliminating gonadotropic hormone activity is not known. An action at the level of the pituitary would be reflected in the cytology of the gland. The effects of 'methallibure' on the appearance of the pituitary complex, in particular of the mesoadenohypophysis are described.

MATERIALS AND METHODS

Maintenance of 'methallibure' treated fish. Mature adult males (17-22 mm standard length; 100-230 mg in weight) and immature juvenile guppies (8-12 mm standard length; 8-28 mg in weight) were taken from the breeding aquaria and put in four aquaria (two for adults and two for juveniles), each of which contained 16 litres of dechlorinated tap water.

A group of juvenile fish was killed immediately after removal from the breeding aquaria to serve as initial controls. The maintenance was similar to that previously described for methyl testosterone treated hypophysectomized adult and juvenile guppies.

'Methallibure' treatment was initiated after the fish were acclimated for one week. Since two aquaria contained adults and two contained juveniles, 'methallibure' treatment was limited to one aquarium in each case; the other served as control. A concentration of $1:10^6$ parts (16 mgm in 16 litres of water) of 'methallibure' was added to the aquaria every alternate day (28 applications during eight weeks).

Histology. The fixation and staining of the testes of the adult and juvenile guppies were similar to that described earlier. The heads were fixed in formic acid-Bouins after removal of the roof of the skull and left for seven days in the fixative to decalcify. After dehydration in ethyl alcohol and embedding in paraffin, the heads were sectioned longitudinally or transversely at 5 u, the sections were stained with combinations of alcian blue-PAS-orange G (AB - PAS - OG), aldehyde thionine - PAS - napthol yellow (AT - PAS - NY), Gabe's aldehyde fuchsin (AF) or Cleveland-Wolfe trichrome (Herlant 1956).

Measurements. Measurements of the testes of 'methallibure' treated adult and juvenile guppies were similar to the testis of hypophysectomized adult and juvenile guppies described earlier. By means of an ocular micrometer,

the diameters of ten cells of each of gonadotrophs, somatotrophs and thyrotrophs from the sagittal sections of five pituitary glands of both adult and juvenile guppies were measured (a total of 50 cells of each type in each group).

RESULTS

Structure of the testis and secondary sex characters of adult control. The structure of the testis of the adult guppy has been described in detail in the earlier sections. In short, all stages of spermatogenesis are present (Figs. 2, 36 and Table XVII). The efferent ducts and the main sperm duct are filled with intact spermatophores (Figs. 4 & 6). The epithelial cells lining the efferent ducts are tall and columnar (Fig. 4 and Table XVIII). The secondary sex characters have also been described earlier. The lipophore index was rated +++ or ++++.

Effect of 'methallibure' on the testis and secondary sex characters of the adult guppy. The 'methallibure' treatment was continued for eight weeks. The gonosomatic index of 'methallibure' treated fish is significantly decreased (Table XVI). The testis contains a few cysts of spermatogonia, spermatocytes, spermatids and sperm but spermatophores are present in abundance (Figs. 33, 36 and Table XVII). The spermatophores present in the efferent ducts and the main sperm duct are usually intact (Fig. 34). The epithelial cells lining the efferent ducts become considerably reduced (Fig. 34 and Table XVIII) but Sertoli cells and interstitial cells do not seem to be regressed.

TABLE XVI Gonosomatic index (G.S.I.) of 'methallibure' treated and control guppies after 8 weeks.

Fish No	G. S. I. 'Methallibure' treated	Control
1	1.30	2.92
2	1.89	3.53
3	1.91	3.40
4	1.37	3.28
5	1.18	3.63
Mean $\pm S_{\bar{x}}$	1.53 ± 0.12	$3.35 \pm 0.12^{***}$

*** $P < 0.001$

TABLE XVII Number and percentage of different stages of spermatogenesis in 'methallibure' treated and control guppies.

		Stages of spermatogenesis									
		SPG		SPC		SPD		SPM		SPR	
		No	%	No	%	No	%	No	%	No	%
'Methallibure' treated	Mean[1]	2.4	1.39	3.4	2.02	6.6	3.91	7.4	4.43	159	88.26
	$\pm S_{\bar{x}}$	$\pm$0.6	$\pm$0.38	$\pm$0.8	$\pm$0.54	$\pm$2.7	$\pm$1.53	$\pm$2.9	$\pm$1.82	$\pm$19.1	$\pm$3.66
Control	Mean[1]	2.6	1.18	33.6	14.74	14.2	6.99	18.4	8.79	159.8	68.29
	$\pm S_{\bar{x}}$	$\pm$0.7	$\pm$0.37	$\pm$5.9	$\pm$2.47	$\pm$3.6	$\pm$2.00	$\pm$3.7	$\pm$2.20	$\pm$26.9	$\pm$5.34

[1]Mean of 5 observations

$P < 0.001$, contingency table (row x column)

TABLE XVIII Epithelial cell heights of efferent ducts of 'methallibure' treated and control guppies.

Fish No	Mean epithelial cell height (u)[1] "Methallibure' treated	Control
1	7.3	17.7
2	9.9	22.9
3	6.9	16.4
4	6.8	15.1
5	7.9	14.1
Grand Mean	7.8	17.2

[1]Mean of 25 counts (5 epithelial cell heights in each of 5 efferent ducts in one section of each testis)

$P < 0.025$ - analysis of variance based on 5 median counts, 1 in each of 5 efferent ducts in 1 section of each testis.

No changes are evident in the structure of the gonopodium but there is a marked decrease in the lipophore pigmentation both on the sides of the body and on the tail. The lipophore index was rated + or ++.

Comparison of the effects of hypophysectomy and 'methallibure' treatment on the adult testes and secondary sex characters. The gonosomatic index in both cases is significantly decreased. The effects of hypophysectomy on spermatogenetic stages of testis are more apparent than in 'methallibure' treatment. The testis of a hypophysectomized guppy contains only spermatogonial cysts and spermatophores whereas the testis of 'methallibure' treated guppy contains all stages of spermatogenesis, although the earlier stages are few (Figs. 3, 33 and Table XIX). Since hypophysectomy completely blocks the transformation of spermatogonia into spermatocytes the presence of few spermatocytes in the 'methallibure' treated testis suggests that a complete blockage of gonadotropin secretion was not attained (Table XIX). After hypophysectomy many spermatophores are found ruptured in the efferent ducts and the main sperm duct; by contrast, following 'methallibure' treatment the spermatophores are usually intact. The epithelial cell heights of the efferent ducts are more reduced following hypophysectomy than 'methallibure' treatment. Hypophysectomy brings about the regression of Sertoli cells and interstitial cells but 'methallibure' treatment does not. Perhaps, even a small release of gonadotropins prevents the regression of Sertoli cells and interstitial cells.

TABLE XIX Number and percentage of different stages of spermatogenesis in hypophysectomized and 'methallibure' treated guppies after 8 weeks.

		Stages of spermatogenesis									
		SPG		SPC		SPD		SPM		SPR	
		No	%	No	%	No	%	No	%	No	%
Hypophy-sectomized	Mean[1]	1.6	1.82	0		0		0		97.6	98.18
	$\pm S_{\bar{x}}$	$\pm$0.6	$\pm$0.48							$\pm$16.3	$\pm$0.75
'Methallibure' treated	Mean[1]	2.4	1.39	3.4	2.02	6.6	3.91	7.4	4.43	159	88.26
	$\pm S_{\bar{x}}$	$\pm$0.6	$\pm$0.38	$\pm$0.8	$\pm$0.54	$\pm$2.7	$\pm$1.53	$\pm$2.9	$\pm$1.82	$\pm$19.1	$\pm$3.66

[1]Mean of 5 observations

$P < 0.001$, contingency table (row x column) for all stages of spermatogenesis

$P < 0.005$, contingency table (row x column) for only 2 stages, spermatogonia and spermatocytes of hypophysectomized and 'methallibure'-treated guppies.

No changes are evident in the structure of the gonopodium of an adult fish either after hypophysectomy or 'methallibure' treatment. The decrease in lipophore pigmentation is more pronounced after hypophysectomy than 'methallibure' treatment.

Structure of the juvenile testis (Initial control) The structure of the juvenile testis has been already described in Section II. Spermatogonial cysts are present at the periphery (Figs. 22, 37 and Table XX); efferent ducts with narrow lumina are present in the centre (Fig. 23 and Table XXI). The main sperm duct has narrow lumen and squamous epithelial cells (Fig. 25 and Table XXII).

Structure of the testis and secondary sex characters of the juvenile guppy not treated with 'methallibure'. As might be expected, the juveniles not treated with 'methallibure', were differentiated into adult males and females by the end of the experiment (eight weeks). All stages of spermatogenesis are present in the testis (Figs. 2, 37 and Table XX). Although the efferent ducts (Fig. 35 and Table XXI) and the main sperm duct (Fig. 35 and Table XXII) are well differentiated, they do not contain spermatophores.

Secondary sex characters are very distinct in the males.

Effect of 'methallibure' on the testis of the juvenile fish. The juvenile fish were treated with 'methallibure' for eight weeks. No developmental changes occurred in the structure of the juvenile testis following 'methallibure' treatment. Mitotic division in spermatogonial cysts was never evident and the number of spermatogonial cysts remains

TABLE XX Number and percentage of different stages of spermatogenesis in juvenile control, juvenile 'methallibure' treated and juvenile non-'methallibure' treated guppies.

		Stages of spermatogenesis									
		SPG		SPC		SPD		SPM		SPR	
		No	%	No	%	No	%	No	%	No	%
(A) Juvenile Control	Mean[1]	15.8	100	0		0		0		0	
	$\pm S\bar{x}$	±1.3									
(B) Juvenile Meth. treated	Mean[1]	17.8	100	0		0		0		0	
	$\pm S\bar{x}$	±0.6									
(C) Juvenile non-meth. treated	Mean[1]	1.8	1.38	35.2	26.14	24.0	18.13	43.6	31.37	29.6	22.98
	$\pm S\bar{x}$	±0.2	±0.19	±6.3	±3.42	±2.5	±1.85	±10.9	±5.04	±2.3	±3.31

[1]Mean of 5 observations

$P < 0.001$ - A & C; B & C: Econtingency table (row x column)

TABLE XXI Total number, width and lumen of efferent ducts of juvenile control, juvenile 'methallibure' treated and juvenile non-'methallibure' treated guppies

Fish No	Efferent ducts								
	Juvenile Control			Juvenile 'Methallibure' treated (B)			Juvenile Non-'Methallibure' treated (C)		
	No	Width(u) Mean[1] ±S$\bar{x}$	Lumen(u) Mean[1] ±S$\bar{x}$	No	Width(u) Mean[1] ±S$\bar{x}$	Lumen(u) Mean[1] ±S$\bar{x}$	No	Width(u) Mean[1] ±S$\bar{x}$	Lumen(u) Mean[1] ±S$\bar{x}$
1	18	19.80 ±1.51	1.44 ±0.36	21	19.08 ±0.77	1.08 ±0.18	41	64.78 ±7.27	33.62 ±8.14
2	16	15.66 ±0.54	1.26 ±0.22	20	19.44 ±0.46	1.62 ±0.18	29	61.50 ±4.67	28.70 ±3.43
3	14	20.16 ±0.93	1.80 ±0.28	15	19.62 ±1.98	1.62 ±0.34	28	71.34 ±5.28	38.54 ±6.34
4	18	18.0 ±0.57	1.44 ±0.22	22	17.82 ±0.72	1.80 ±0.28	29	74.62 ±8.54	35.26 ±6.16
5	16	17.28 ±0.52	1.62 ±0.34	18	17.64 ±0.73	1.80 ±0.28	31	68.06 ±4.60	36.90 ±5.50
Mean[1] ±S$\bar{x}$	16.4 ±0.7			19.2 ±1.2			31.6 ±2.4		

[1]Mean of 5 observations

$P < 0.01$ - A & C, B & C (for total no., width and lumen)

Not significant - A & B (for total no., width and lumen)

Tukey's test - $W_{.01}$ for no. - 8.16
$W_{.01}$ for width - 6.96
$W_{.01}$ for lumen - 6.42

TABLE XXII Epithelial cell heights and lumen of main sperm duct of juvenile control, juvenile 'methallibure' treated, and juvenile non-'methallibure treated guppies.

Fish No	Main sperm duct (u; Mean[1] ±$S\bar{x}$)					
	(A) Juvenile Control		(B) Juvenile 'Methallibure' treated		(C) Juvenile non-'Methallibure' treated	
	Epithelial cells	Lumen	Epithelial cells	Lumen	Epithelial cells	Lumen
1	3.06 ±0.22	1.62 ±0.34	3.60 ±0.40	1.44 ±0.36	15.98 ±1.53	54.12 ±5.98
2	2.88 ±0.34	1.80 ±0.28	3.42 ±0.34	2.34 ±0.46	15.98 ±2.39	40.18 ±2.91
3	3.42 ±0.34	2.70 ±0.40	3.06 ±0.61	1.98 ±0.18	9.84 ±1.00	85.90 ±5.27
4	3.78 ±0.34	3.22 ±0.36	3.42 ±0.44	2.34 ±0.36	10.66 ±1.00	86.10 ±9.52
5	3.60 ±0.40	2.52 ±0.66	3.78 ±0.18	3.06 ±0.46	10.66 ±1.64	76.26 ±6.81

[1]Mean of 5 observations

$P < 0.01$ - A & C; B & C (for epithelial cells and lumen)

Not significant - A & B (for epithelial cells and lumen)
Tukey's test - $W_{.01}$ for epithelial cells - 2.14
$W_{.01}$ for lumen - 11.34

the same (Figs. 22, 37 and Table XX). There is no increase in the total number, width and the size of lumina of the efferent ducts (Fig. 23) and Table XXI). The epithelial cells lining main sperm duct remain squamous and the width of lumen of main sperm duct is not increased (Fig. 25 and Table XXII). Thus it seems that the further development of the testis is stopped when the juveniles are treated with 'methallibure'.

Secondary sex characters are absent in the juveniles. They do not develop in juveniles treated with 'methallibure'. Thus it is apparent that 'methallibure' prevents the appearance of secondary sex characters in juveniles.

Comparison of the effects of hypophysectomy and 'methallibure' treatment on the testes of the juveniles. In the juvenile guppy, treatment with 'methallibure' produced identical effects to hypophysectomy. The testis contains only peripheral spermatogonial cysts (Table XXIII). Both the treatments inhibit the appearance of secondary sex characters in the juvenile fish. These points indicate that gonadotropin secretion is completely stopped when the juveniles are treated with 'methallibure'.

Morphology of the pituitary complex. The structure of the adenohypophysis (AH) of the guppy has been described in part by Sokol (1961); a brief description of the pituitary complex with suffice here.

The pituitary complex in the guppy is divided, as in most teleosts, into 4 regions, the pro-, meso-, and meta-AH, and the neurohypophysis (NH) (Fig. 38a).

TABLE XXIII Number and percentage of different stages of spermatogenesis in hypophysectomized juvenile guppy after 6 weeks and 'methallibure' treated juvenile guppy after 8 weeks.

		Stages of spermatogenesis									
		SPG		SPC		SPD		SPM		SPR	
		No	%	No	%	No	%	No	%	No	%
Hypophy-sectomized	Mean[1]	12.4	100	0		0		0		0	
	$\pm S_{\bar{x}}$	$\pm$0.68									
'Methallibure' treated	Mean[1]	17.8	100	0		0		0		0	
	$\pm S_{\bar{x}}$	$\pm$0.6									

[1]Mean of 5 observations.

The pro-AH, (Figs. 38a, 39a & 40-42) which composes 30-40% of the pituitary gland is largely formed of closely packed acidophils of diameter 4.7 ± 0.5 u; these are probably homologous with the cells in the pro-AH of Fundulus heteroclitus which are capable of binding anti-ovine prolactin serum (Emmart, Pickford & Wilhelmi 1966), and are thought to be 'prolactin cells'. Bordering the ramifications of the NH with the pro-AH is found a further cell type, less organe G positive (OG +ve) than the 'prolactin cells', of diameter 4.0 ± 0.3 u which probably represent the adrenocorticotrophs described by Olivereau (1964).

The meso-AH is composed of 3 cell types recognizable by their staining characteristics. One is acidophilic, more or less rounded, of diameter ranging from 3.9 to 5.2 u; the cells are scattered throughout the central zone of the meso-AH (Figs. 38b & 39b) and are considered to have a somatotrophic function (Olivereau & Ridgeway 1962; Levenstein 1939; Fontaine & Olivereau 1949). The two remaining cell types are basophils which, in the guppy, are arranged in two distinct regions. One region is in the ventral half of the meso-AH (Fig. 38a); in mature adults these cells are of angular outline with mean diameter 4.8 to 5.9 u; and stain readily with AB, AT, AF, and PAS and are commonly distributed around large sinuses or capillaries which are prevalent in this ventral region of the gland (Figs. 38 & 39). Sokol (1961) considered these basophils to have a gonadotrophic function because of their changing appearance

during sexual maturation, although she was unable to separate them tinctorially from the second group of meso-AH basophils found close to the ramifications of the NH into the meso-AH. The cells of this second group (Figs. 38b & 39b) are oval in section and generally larger (6.7 to 8.3 u) with the cytoplasm more PAS +ve than the gonadotrophs; after staining with AT-PAS-NY, the cytoplasm appears grey in colour while that of the gonadotrophs is dark blue. They are thought to have a thyrotrophic activity (Sokol 1961; Olivereau 1963).

Two cell types are readily recognised in the meta-AH (Fig. 38a) one of which is readily stainable with PAS (cell diameter 6.9 $\pm$ 0.2 u). The functions of these two cell types are not known; one is thought to produce melanophore stimulating hormone.

The NH is formed of nerve fibres which have two origins. One type of fibre originates in the pre-optic nucleus (PON) and contains material readily stainable with the so-called 'neurosecretory' stains such as AB, AF, and AT+NY (Leatherland, Budtz & Dodd 1966)(Figs. 38a,b). The stainable material in P. reticulata is found in greatest amounts in the interdigitations of the NH with the meta-AH, and less so in the ramifications into the meso- and pro-AH. In these regions the stainable material appears finely granulated, whereas in the dorsal region of the NH it takes the form of large extracellular accumulations (Figs. 41 & 42b). The second type of nerve fibre terminating in the NH originates in the

nucleus lateralis tuberis (NLT)(Fig. 38b) and is not stainable with these 'neurosecretory' stains. The nucleus is composed of neurones of diameter 6.0 to 7.0 u and is found in the hypothalamus immediately dorso-lateral to the pituitary gland, as well as in the dorsal region of the NH itself. Neither the neurones nor the axons of the NLT stain with the combinations used here, although the AT-PAS-NY combination stains the cytoplasm slightly grey and the nucleus yellow.

In the experimental procedures described below, no changes are found in either the size or appearance of the intrinsic endocrine cells of the pro- or meta-AH, nor of the neurones of NLT or PON; although there was considerable variation in the amount of stainable material in the NH, it was not consistent with experimental conditions. Only the cell types of the meso-AH show changes and will be considered below.

Effect of 'methallibure' on the meso-adenohypophysis of the adult male guppy. There appears to be more gonadotroph pituitary cells in the ventral meso-AH of the control fish (group I)(Figs. 38a,b) compared with the 'methallibure' treated fish (group II)(Figs. 39a,b). The mean diameter of these cells is similarly significantly higher ($p < 0.001$) and the amount of AF+ve material in their cytoplasm is noticeably greater in the control fish (Table XXIV). As already noted, the ventral part of the meso-AH is often richly supplied with

TABLE XXIV Effect of 'methallibure' (ICI 33.828) on the mean cell diameter of the mesoadenohypophysial gonadotrophs, somatotrophs, and thyrotrophs of the adult and juvenile guppy, Poecilia reticulata, Peters.

Experimental Condition	No. of Fish	Weight range (mg) Initial	Final	No. of glands measured	Mean diameter of cells (u) ± Standard Error Gonadotroph	Somatotroph	Thyrotroph
Group I Adult Controls	13	100-230	120-275	5	5.5±0.2	4.3±0.1	7.4±0.2
Group II Adult 'Methallibure'	12	100-190	130-200	5	4.2±0.1	4.3±0.1*	8.5±0.1
Group III Juvenile Controls	12	-	8-20	5	4.5±0.1+	4.5±0.2**	5.5±0.1+++
Group IV Juvenile Controls	10	8-20	90-180	5	5.7±0.1	4.6±0.1	8.9±0.3
Group V Juvenile 'Methallibure'	16	9-28	11-55	5	3.7±0.2++	-***	7.5±0.2

+ gonadotrophs not recognised in 1 case; ++ only recognised in 1 case; +++ thyrotrophs only recognised in 2 cases. * somatotrophs not recognised in 2 cases; ** only recognised in 2 cases; *** not recognised in any case.

$p < 0.001$ between mean diameter of gonadotrophs of group IV and V, I and II, III and IV and $p = 0.01$-0.02 between groups III and V; $p < 0.001$ between mean diameter of thyrotrophs of groups IV and V, I and II, I and IV, II and III, I and III, III and V and III and IV. Differences between mean diameters of gonadotrophs of groups I and IV, II and III, I and III, and of somatotrophs of groups I and II, I and IV, II and III, I and III, III and IV not signficant.

blood sinuses or capillaries which appear to be intimately connected with the gonadotroph cells; a larger number of the 'methallibure' treated fish are found to have these large sinuses; in control fish they tend to be less numerous.

Few somatotrophs are evident in either the control fish, (in which the cells were absent or not recognisable in 2 cases) or the experimental fish; no differences are found between the mean cell diameter of the two groups (Table XXIV).

Few thyrotrophs are found in the meso-AH of the fish in group I compared with the experimental group II (Figs. 38b & 39b) in which the cell diameter is also significantly ($p < 0.001$) larger (8.5 ± 0.1 u in the 'methallibure' treated fish compared with 7.4 ± 0.2 u in the control group). The thyrotrophs of the 'methallibure' treated fish are also more PAS+ve than those of the control fish.

Effect of 'methallibure' on the meso-adenohypophysis of the juvenile guppy. The meso-AH of the young fish killed at the beginning of the experiment (group III) is only partially differentiated (Fig. 40). The gonadotrophs are very significantly smaller ($p < 0.001$) in diameter and fewer in number than in the adult controls (group I), and have very little AF+ve cytoplasm. Blood sinuses are evident in only one of the pituitary glands examined. The thyrotrophs and somatotrophs are not clearly differentiated in this group; they were recognised in only two cases. The somatotrophs are similar in size and appearance to those of the adult, but

the thyrotrophs are very significantly ($p < 0.001$) smaller than those of either of the adult groups (I or II).

The control fish killed 8 weeks after commencement of the experiment (group IV) have a well differentiated meso-AH (Fig. 42a) with more numerous gonadotrophs of a significantly larger mean diameter ($p < 0.001$) than those of either of the fish of Group III, or of the 'methallibure' treated adults (group II). Blood sinuses associated with the gonadotrophs are more numerous in this group than in the younger control fish of group III.

Thyrotrophs in this group (IV) are fairly numerous (Fig. 42b) and of significantly greater diameter ($p < 0.001$) than in groups I, III, or V.

Gonadotrophs are evident in only two of the pituitary glands of the juvenile 'methallibure' treated fish (group V) (Fig. 41), and where present they contain little AF+ve stainable material. They are of significantly smaller diameter ($p < 0.001$) than those of all the other groups. Blood sinuses are commonly found but are generally small.

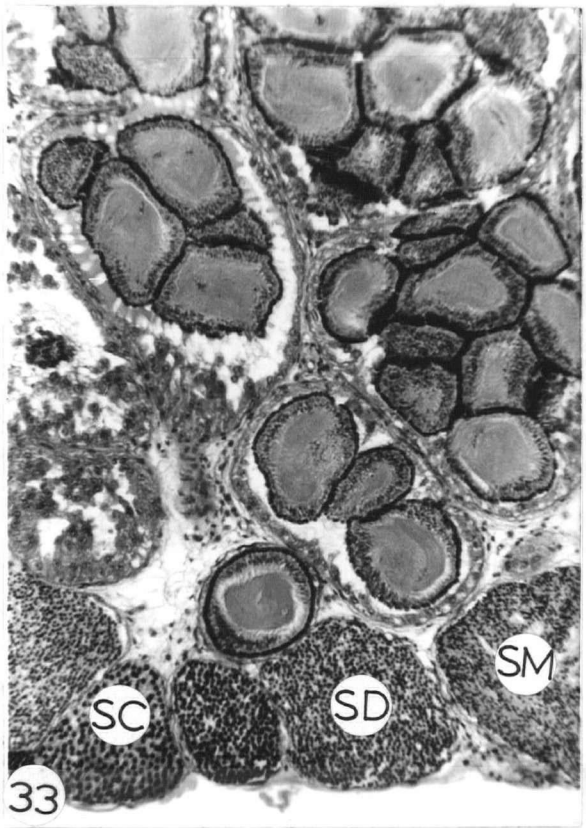
Somatotrophs were not identified in any of the glands examined in this group (V), although this may be because the poor differentiation of the gonadotrophs did not allow positive identification of these cells.

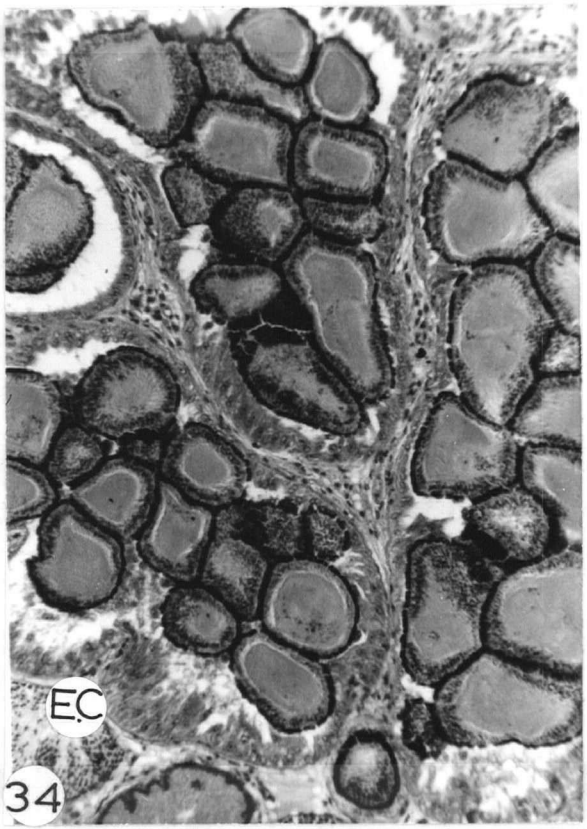
The meso-AH of this group (V) contains numerous thyrotrophs, although not as many as the comparable controls of the same age (group IV); they are, however, significantly larger ($p < 0.001$) than those of the control fish of similar size (group III).

Figure 33: Sagittal section of testis of adult guppy treated with 'methallibure' showing few cysts of earlier stages of spermatogenesis (x 200).

Figure 34: Same as figure 33 showing epithelial cells lining the efferent ducts (x 200).

Figure 35: Sagittal section of testis of juvenile guppy not treated with 'methallibure' (which became adult during the period of experiment). Note the efferent ducts and main sperm duct do not contain spermatophores (x 200).


SC
SD
SM
33


E.C
34

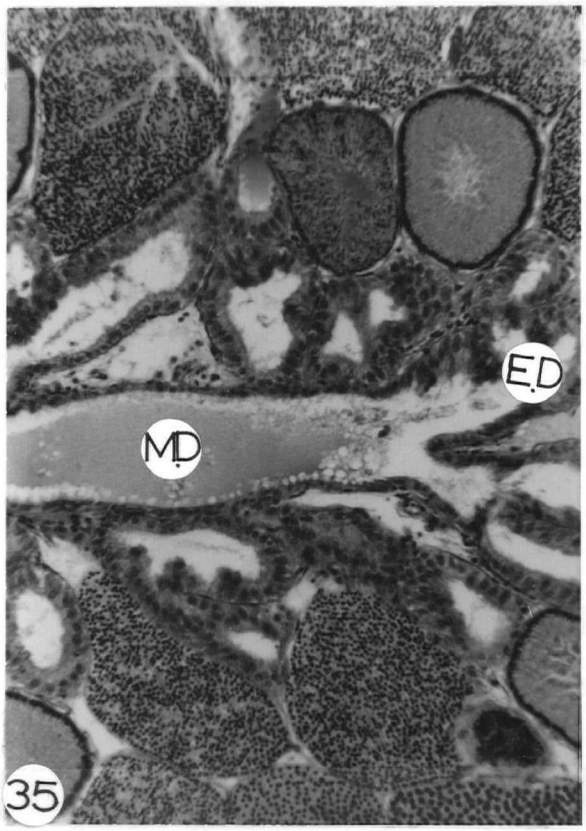

E.D
M.D
35

Figure 36: Percentage of different stages of spermatogenesis in 'methallibure'-treated and control adult guppies after eight weeks.

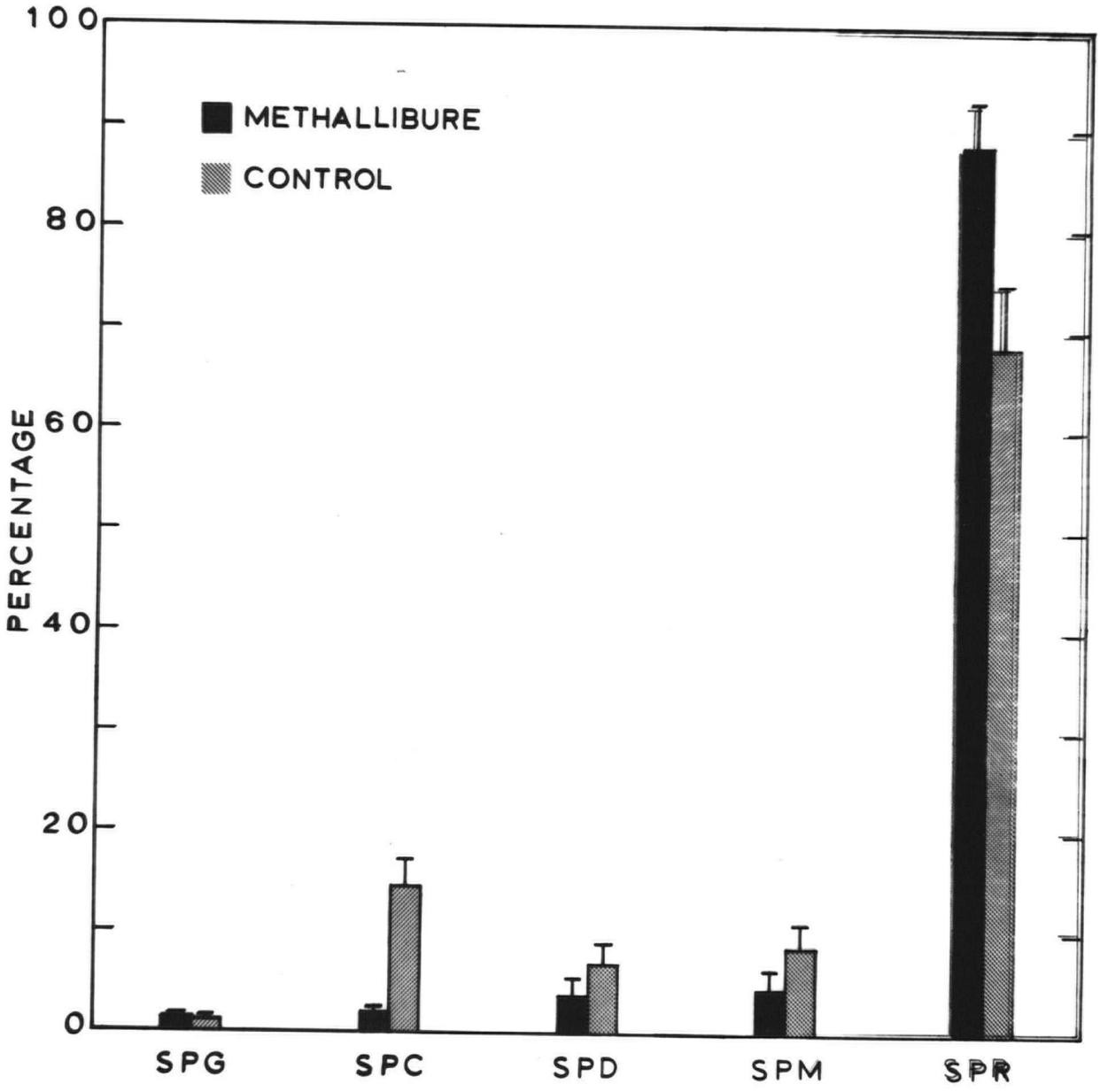

100
80
60
40
20
0
PERCENTAGE
METHALLIBURE
CONTROL
SPG
SPC
SPD
SPM
SPR

Figure 37: Percentage of different stages of spermatogenesis in juvenile control; juvenile 'methallibure'-treated and juvenile non-'methallibure' treated guppies.

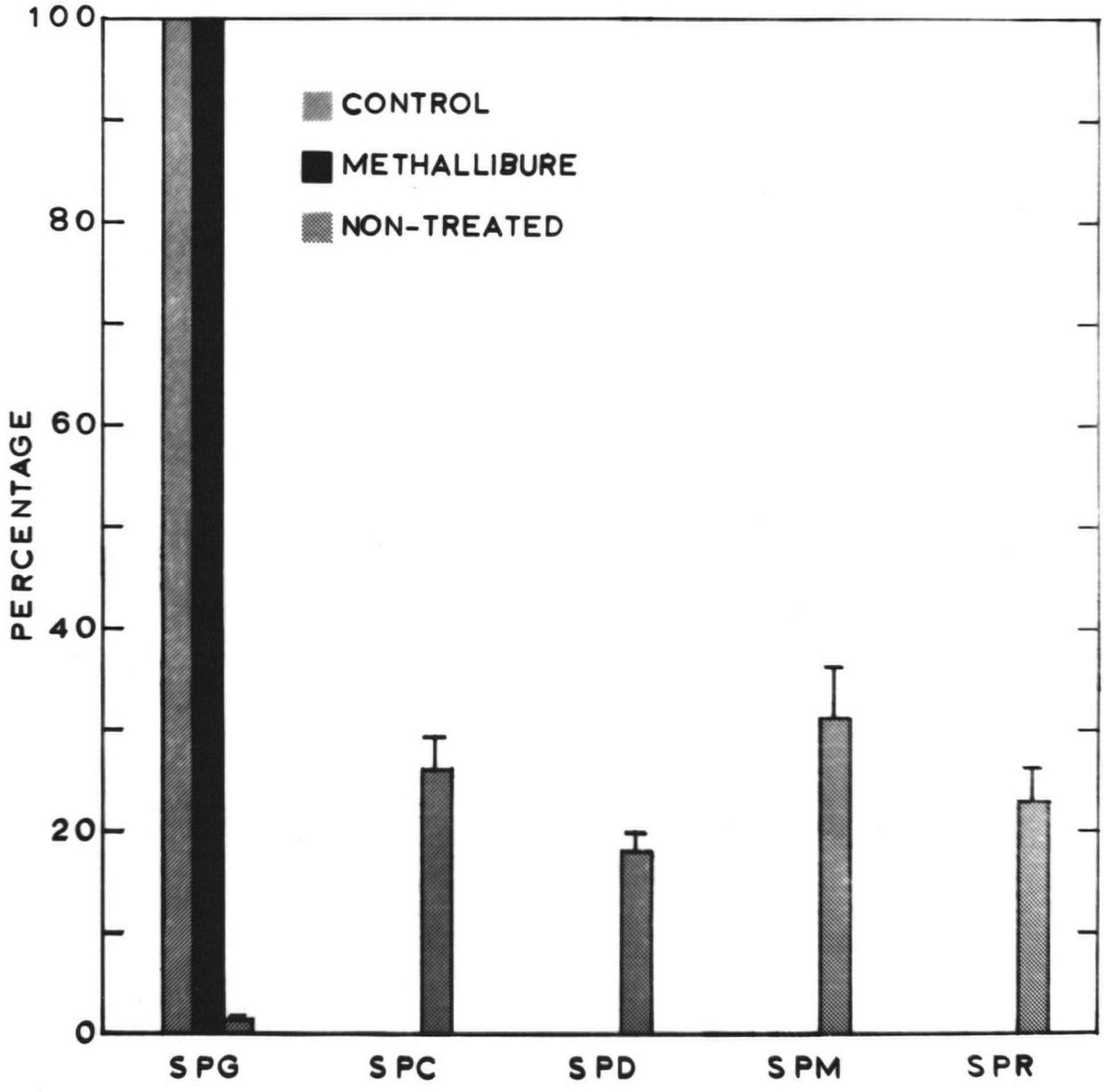
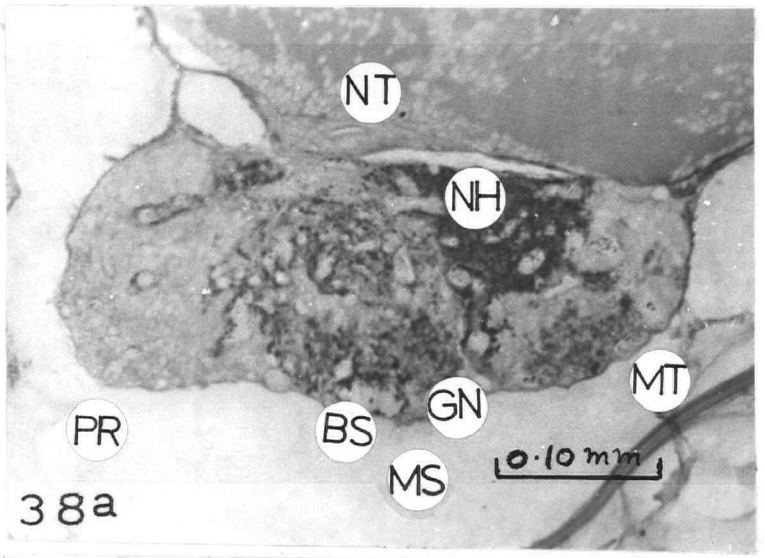

100
80
60
40
20
0
PERCENTAGE
CONTROL
METHALLIBURE
NON-TREATED
SPG
SPC
SPD
SPM
SPR

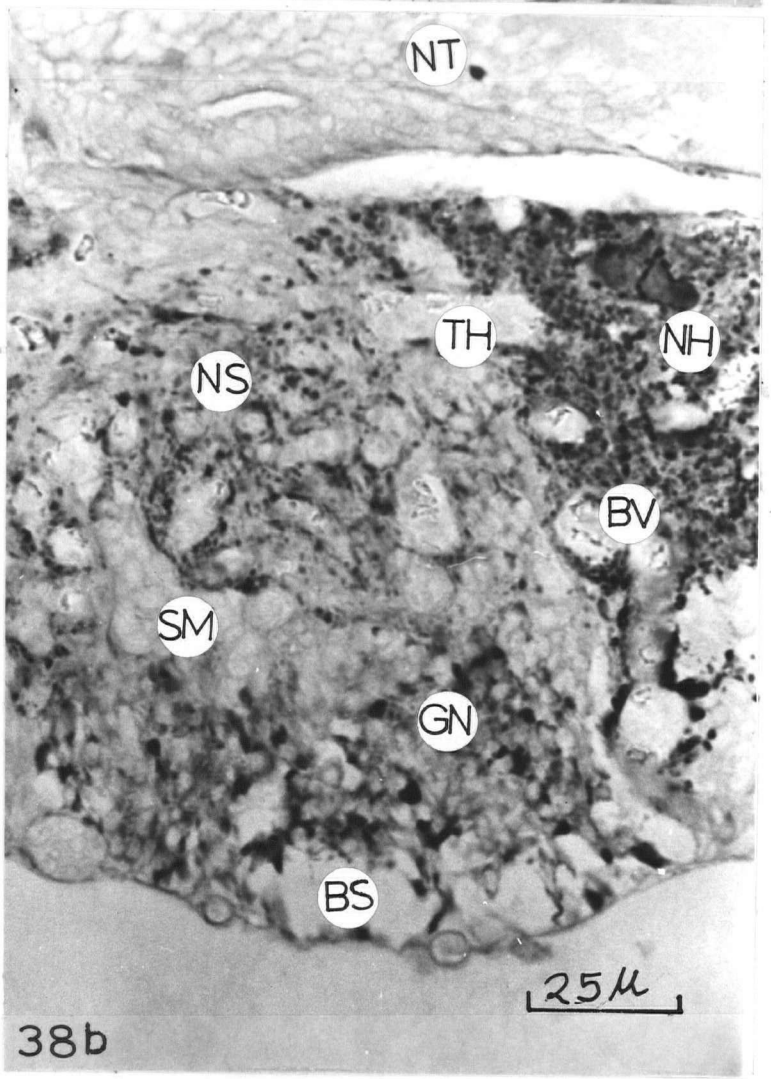
Figure 38a: Sagittal section of pituitary gland of adult control guppy. Note numerous gonadotrophs in ventral region and blood sinuses associated with these cells.

Figure 38b: Magnified view of a portion of 38a.

BS - Blood Sinuses; BV - Blood Vessels;
GN - Gonadotrophs; MS - Meso-adenohypophysis;
MT - Meta-adenohypophysis; NH - Neurohypophysis;
NT - Nucleus lateralis tuberis; NS - Neurosecretion;
PR - Pro-adenohypophysis; SM - Somatotrophs;
TH - Thyrotrophs; TV - Third ventricle.



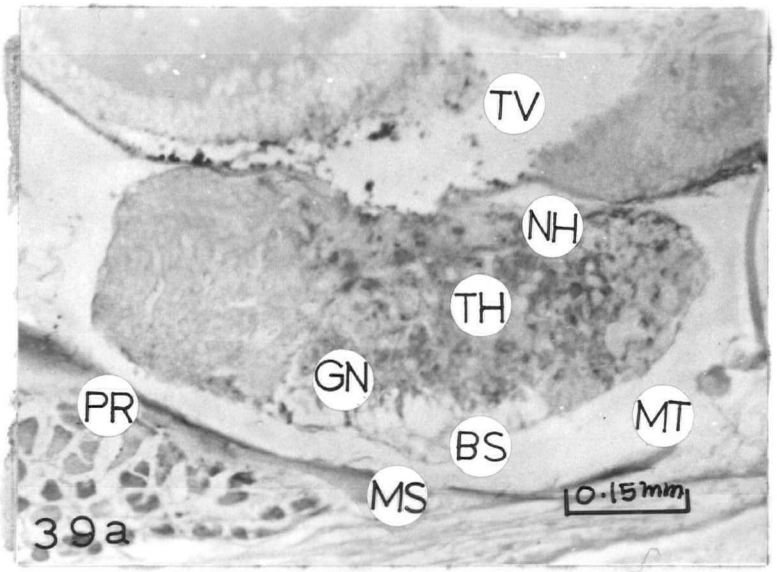
38a



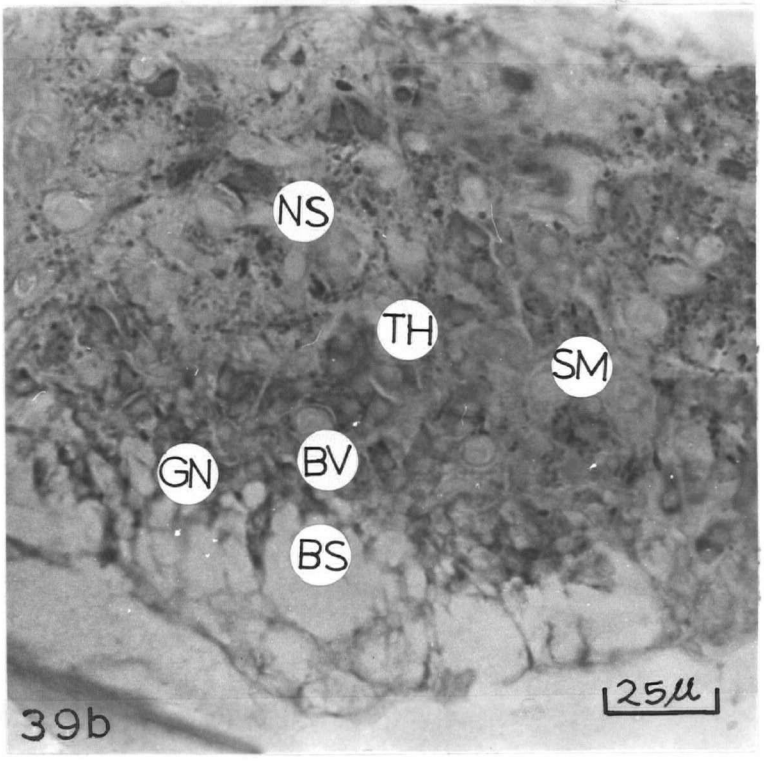
38b

Figure 39a: Sagittal section of pituitary gland of adult 'methallibure'-treated guppy. Note few gonadotrophs around large blood sinuses, large thyrotrophs close to neurohypophysis.

Figure 39b: Magnified view of a portion of 39a.



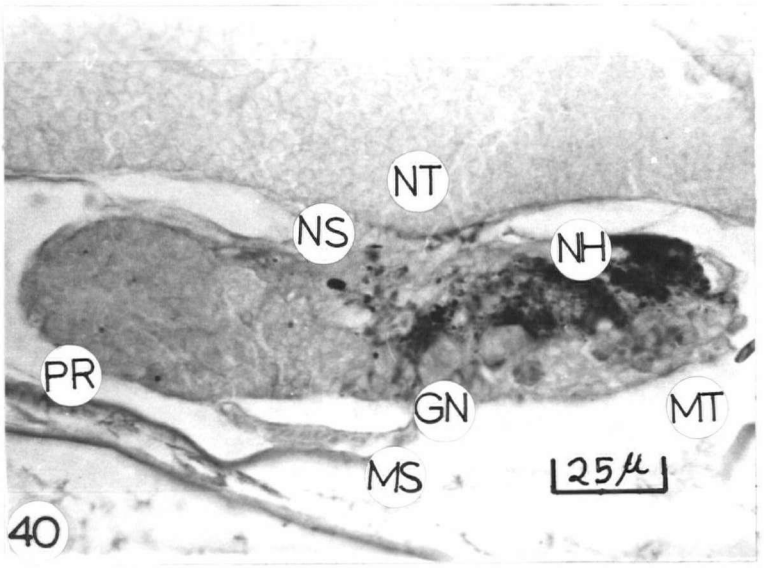
39a



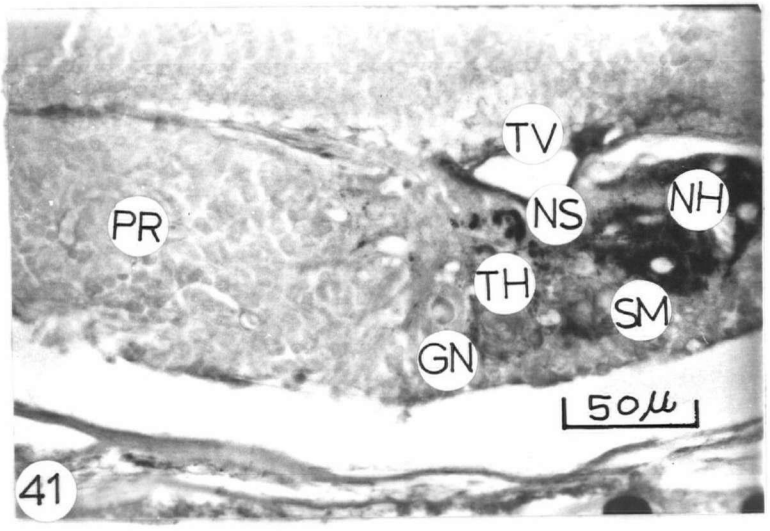
39b

Figure 40: Sagittal section of pituitary gland of juvenile control guppy (group III). Note poor development of meso-adenohypophysis, few gonadotrophs, other cell types not recognisable.

Figure 41: Sagittal section of pituitary gland of juvenile 'methallibure'-treated guppy. Note almost complete absence of gonadotrophs (Similar to fig. 40) and developed thyrotrophs adjacent to neurohypophysis.



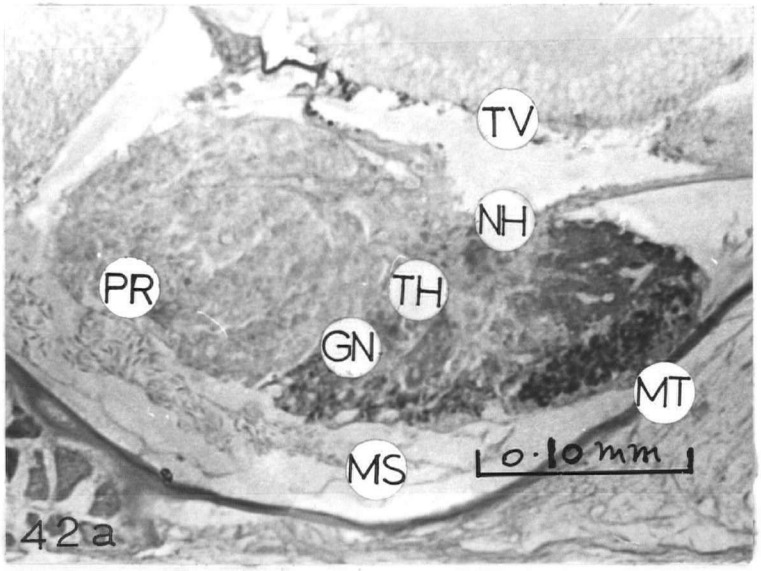
40



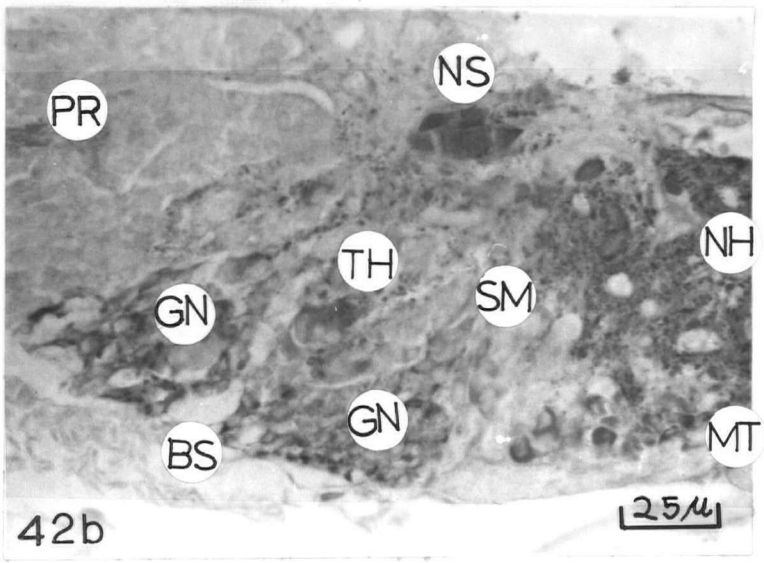
41

Figure 42a: Sagittal section of pituitary gland of juvenile control guppy (group VI) not treated with 'methallibure' (which became adult during the period of experiment). Note numerous gonadotrophs in ventral margin and thyrotrophs adjacent to neurohypophysis.

Figure 42b: Magnified view of a portion of 42a.



42a



42b

GENERAL DISCUSSION

The investigation was initiated with the premise that a study of the hypophysectomized male guppy would further the understanding of pituitary-gonad relationships in fishes, clarify some of the controversial issues concerning the details of gonadotropic and androgenic action on the testis and male secondary sex characters and provide specific information on physiological regulation of endocrine controls in the male of a live-bearing cyprinodont. The study has differed from that of previous workers in this field not only in its orientation toward a male ovoviviparous species but also in the comparative study of adults with juveniles hypophysectomized prior to the differentiation of the gonad and secondary sex characters. Vivien (1941) was the first and only investigator to demonstrate that hypophysectomy prevents the development of the gonad in a juvenile teleost (Gobius paganellus). Vivien's investigation, however, was a general one and provided no details of the effects on the cytology of the gonad. An added feature in the present analysis of pituitary-gonad relations has been the use of the gonadotropic blocking agent 'methallibure'.

The initial premise has been justified and pertinent additional data have been obtained with respect to (a) the locus of pituitary regulation in spermatogenesis (b) the pituitary involvement in spermiation (c) the role of androgens in spermatogenesis and in the control of

secondary sex characters and (d) the physiology and hormonal regulation of the Sertoli cells and the epithelial cells lining the sperm ducts. In addition, several areas of gonadal physiology have been investigated for the first time in fishes with significant data pertaining to (a) the development and endocrine control of spermatophore formation (b) the hormonal involvement in differentiation of the gonad and secondary sex characters of juvenile fishes and (c) the comparative study of 'methallibure' effects on juveniles and adults.

The following topics are considered significant contributions of this thesis and have been selected for discussion:

a. The role of the pituitary and the androgens in the control of spermatogenesis including spermatophores.
b. The endocrine control of the release of spermatophores, the development and maintenance of interstitial cells, epithelial cells lining the sperm ducts and Sertoli cells.
c The role of the pituitary and the androgens in the control of secondary sex characters.
d. The blocking action of 'methallibure' on the effects of the gonadotropins and the site of action of 'methallibure'.

a. The role of the pituitary and the androgens in the control of spermatogenesis including spermatophores.

Role of the pituitary

While it is well established that tht testes of teleosts show a suppression of spermatogenesis after hypophysectomy, there are comparatively few detailed studies of the cytological changes. The older literature includes the work of Vivien (1938, 1941) on Gobius paganellus, and that of Matthews (1939) and Burger (1941) on Fundulus heteroclitus. More recent investigations are those of Barr (1963) on Pleuronectes platessa, Roy (1964) and Belsare (1965) on Ophicephalus punctatus, Ahsan (1966) on Couesius plumbeus, Lofts et al (1966) on Fundulus heteroclitus, Donaldson and McBride (1967) on Salmo gairdnerii, Sundararaj and Nayyar (1967) on Heteropneustes fossilis, and Yamazaki and Donaldson (1968) on Carassius auratus. From a review of this literature, it is evident that the effect of hypophysectomy on the testes varies considerably at different times of the year (Matthews 1939; Vivien 1941; Barr 1963).

There is considerable disagreement in the literature concerning the precise stage in spermatogenesis which is affected by hypophysectomy. In Fundulus heteroclitus, spermatogonial divisions continue but the later stages of spermatogenesis are suppressed (Matthews 1939; Burger 1941; Pickford 1953; Lofts et al 1966). Likewise, Sundararaj and Nayyar (1967) using Heteropneustes fossilis found that spermatogonial division continues and the only cell types

present in the testis of hypophysectomized fish are the spermatogonia and the sperm. On the contrary, however, Barr (1963) working on plaice, Pleuronectes platessa and Ahsan (1966) on lake chub, Couesius plumbeus described the suppression of spermatogonial divisions in the absence of the pituitary; the conversion of spermatogonia into spermatocytes ceases but spermatogenesis, if well underway, continues and sperm are formed. Dodd et al (1960) in their studies on Scyliorhinus caniculus, likewise, found that hypophysectomy stops the transformation of a spermatogonium into a spermatocyte, but spermatocytes and all succeeding stages of spermatogenesis already established at the time of operation apparently develop in normal fashion into sperm. Dodd et al (1960) did not observe mitosis in spermatogonia. Yamazaki and Donaldson (1968) noted that in Carassius auratus, the mitotic division of spermatogonia is completely suppressed by hypophysectomy and spermatocytes, spermatids and sperm disappear.

The teleosts studied in all these previous investigations are oviparous, seasonal breeders. The guppy Poecilia reticulata differs from the teleosts previously studied in being an ovoviviparous, monthly breeder. As an adaptation to internal fertilization the male guppy produces sperm-balls or spermatophores. The histology of spermatophore formation has been described in a number of poeciliids: Phalloceros caudo-maculatus and Cnesterodon decem-maculatus (Philippi 1908); Poecilia reticulata (Vaupel 1929; Goodrich

et al 1934); Gambusia affinis (Self 1940; Medlen 1950); Xiphophorus helleri (Essenberg 1924; Vallowe 1957) and Xiphophorus maculatus (Wolf 1931; Chavin and Gordon 1951). However, the possible endocrine control of spermatophore formation, the physiology of the spermatophores while in the testis and their eventual discharge have not been previously described.

In the testis of a control guppy, the spermatogonial cysts are peripherally located while the spermatophores are in the centre; the area between the two is filled with cysts containing the various developmental stages of spermatocytes, spermatids and sperm (Fig. 2). By contrast, the testis of a hypophysectomized guppy is completely packed with spermatophores except for a few spermatogonial cysts near the periphery (Fig. 3).

This suggests that hypophysectomy (presumably the lack of gonadotropin) blocks the transformation of spermatogonia into spermatocytes, but does not prevent the transformation of spermatocytes, spermatids, and sperm into spermatophores. This finding is similar to that described in Pleuronectes platessa (Barr 1963), Couesius plumbeus (Ahsan 1966) and Scyliorhinus caniculus (Dodd et al 1960). A similar situation has been noted in the Amphibia; van Oordt (1956) concluded from study of the frog, Rana temporaria that the later stages of spermatogenesis beyond spermatogonia are not under the pituitary control.

The presence of spermatophores at the periphery of the testis of a hypophysectomized guppy with no trace of

disintegrating cysts containing earlier stages of spermatogenesis indicates that spermatocytes, spermatids and sperm do not degenerate but transform into spermatophores. This observation is in contradiction to the findings in oviparous teleosts such as Gobius paganellus (Vivien 1941), Fundulus heteroclitus (Matthews 1939; Burger 1941; Pickford 1953; Lofts et al 1966), Pleuronectes platessa (Barr 1963), Couesius plumbeus (Ahsan 1966), Heteropneustes fossilis (Sundararaj and Nayyar 1967) and Carassius auratus (Yamazaki and Donaldson 1968). Investigators who have studied these oviparous species state that the spermatocytes and spermatids degenerate or disappear in the absence of the pituitary. This difference is difficult to explain but may be due to the fact that the guppy is an ovoviviparous teleost and produces relatively few spermatophores, thus conservation of the earlier stages is essential; whereas the rest are oviparous and produce enormous number of sperm.

Since mitotic division was never noted in the spermatogonial cysts of the hypophysectomized guppy, it seems likely that spermatogonial multiplication ceases in the absence of the pituitary. This is further evidenced by the fact that percentage composition of spermatogonial cysts of control and experimental guppies has not changed during the eight-week experimental period (Table II). This finding is in accord with that of Yamazaki and Donaldson (1968) on goldfish, Carassius auratus and that of van Oordt (1960) on frog, Rana temporaria.

Testes of a newly born guppy contain only spermatogonial cysts. The primary spermatogonia begin to divide to form nests of cells (beginning of spermatogenesis) at about 36 days after birth (Goodrich et al 1934). Sokol (1961) found that gonadotrophs in the ventral region of meso-adenohypophysis become granulated (initiation of gonadotropin secretion) during the fifth week after birth. It is thus evident that there is a direct correlation between the secretion of gonadotropins and the initiation of spermatogenesis. The role of the pituitary in the development of testis has been analyzed by removing the pituitary of the juvenile guppy before the gonadotrophs were differentiated.

No changes take place in the structure of the juvenile testis following hypophysectomy. Mitoses were never observed and there was no evidence of an increase in the number of spermatogonial cysts (Table X). The later stages of spermatogenesis do not appear. During the course of the above experiment sham-operated juveniles became adults and their testes contained all stages of spermatogenesis. It indicates that hypophysectomy prevents the mitotic division of spermatogonia and their transformation into spermatocytes. This finding is similar to that of Chang and Witschi (1955) on urodeles (Triturus) and anurans (Rana, Bufo). They noted that even after a prolonged period, the testes of hypophysectomized larval amphibians contain only spermatogonia.

It is concluded that hypophysectomy of both adult and juvenile guppies prevents mitosis in the spermatogonia and blocks their transformation into spermatocytes. The hypophysectomy of the adult guppy does not prevent the transformation of spermatocytes, spermatids and sperm into spermatophores. This indicates that the later stages of spermatogenesis are pituitary-independent.

Role of the androgens

In a study of effects of various steroids in sexual development of intact guppy, Eversole (1939, 1941) suggested that testosterone propionate hastens germ cell maturation. There have been several other reports of stimulation of testes of intact teleost fish by androgens (Pickford and Atz 1957; Dodd 1960).

The role of androgens in spermatogenesis may be readily analyzed by treating hypophysectomized fish with androgens. There are, however, very few observations on the effects of androgens on the testes of hypophysectomized fish (Burger 1942; Lofts et al 1966; Sundararaj and Nayyar 1967). Lofts et al (1966) working on Fundulus heteroclitus and Sundararaj and Nayyar (1967) on Heteropneustes fossilis demonstrated that spermatogenesis is completely restored with testosterone treatment. On the contrary, however, Burger (1942) also working with the hypophysectomized Fundulus stated that spermatogenesis is poorly maintained by androgens.

Methyl testosterone treatment of hypophysectomized adult guppies significantly increases their gonosomatic indices.

This finding is similar to that described in Fundulus (Lofts et al 1966) and Heteropneustes (Sundararaj and Nayyar 1967). Exogenous methyl testosterone appears to have a direct spermatokinetic effect on the testis of the hypophysectomized guppy. In contrast to the hypophysectomized controls, active mitotic divisions are evident within spermatogonial cysts; the number of spermatogonial cysts increases and cysts containing spermatocytes are differentiated (Table VII). Testicular stimulation reaches only the spermatocytal stage. This observation is in contrast to the findings of Lofts et al (1966) in Fundulus and Sundararaj and Nayyar (1967) in Heteropneustes. They reported complete restoration of spermatogenesis with testosterone. The differences in activation of the regressed testis is difficult to explain but may be attributed to the fact that Poecilia is an ovoviviparous, monthly breeder whereas Fundulus and Heteropneustes are oviparous, seasonal breeders.

In higher vertebrates the effect of testosterone on the regressed testis varies. Testosterone is known to exert a stimulatory effect on spermatogenesis in hypophysectomized mammals (Boccabella 1963; Clermont and Harvey 1966). In contrast to the findings noted above, Basu and Nandi (1965) reported that treatment of hypophysectomized frogs with testosterone results in greater suppression of spermatogenesis than that observed after hypophysectomy alone.

There is no published account of the effects of testosterone treatment on hypophysectomized juvenile fish. Unlike the effect found in adults, methyl testosterone treatment of hypophysectomized juvenile guppies does not stimulate mitotic divisions in the spermatogonial cysts; later stages of spermatogenesis do not appear.

The differences in activation of the testes of hypophysectomized adult and juvenile guppies by methyl testosterone may be explained in the following way: the adult testis had well differentiated interstitial cells which became regressed in the absence of pituitary and stopped producing androgen. The methyl testosterone treatment of the hypophysectomized adult compensates for this loss in androgen production and that is why the wave of spermatogenesis in initiated. It seems likely that the production of androgen by interstitial cells in adult testis has synergistic action with gonadotropins in spermatogenesis.

On the other hand in the juveniles, the interstitial cells were not differentiated and the pituitary was removed before the testis had ever received any gonadotropin stimulation. It may be suggested that exogenous testosterone cannot act directly on the juvenile testis and bring about spermatogenesis and differentiation of interstitial cells until the tissue has been primed or triggered by gonadotropins.

b. The endocrine control of the release of spermatophores, the development and maintenance of interstitial cells, epithelial cells lining the sperm ducts and Sertoli cells.

Release of spermatophores

There is considerable disagreement in literature concerning the endocrine control of spermiation in oviparous teleosts. This control evidently varies in the same fish at different times of the year. When the pituitary of Gobius paganellus was removed during winter, sperm disappeared from the testis, whereas 70 percent of operated fish retained sperm when the pituitary was removed shortly before the natural reproductive climax (Vivien 1938, 1941). Similar results were obtained in Fundulus heteroclitus during the fall and spring (Matthews 1939). Pickford (1953) found no sperm in Fundulus five months after the operation. Lofts et al (1966) also working with Fundulus described empty lobules without sperm in the testis after hypophysectomy. In Pleuronectes platessa, sperm are shed normally in the absence of the pituitary (Barr 1963). Ahsan (1966) noted that Couesius plumbeus spermiates normally in the absence of the pituitary in both the prespawning and spawning phases of its annual cycle. Likewise, Yamazaki and Donaldson (1968) found that sperm disappear from the testis of hypophysectomized Carassius auratus. On the contrary, however, Sundararaj and Nayyar (1967) working on Heteropneustes fossilis noted that sperm persist as long as 337 days after hypophysectomy.

The endocrine control of the release of spermatophores from the testis has not been previously studied. Eight weeks after hypophysectomy, many spermatophores in the testis of the guppy are found ruptured but the sperm are not released. The rupture of the spermatophores may be due to the fact that the epithelial cells of the sperm ducts surrounding the spermatophores cease to produce the nutritive or colloidal material in the absence of the pituitary. There is a strong indication that the sperm resulting from ruptured spermatophores are being phagocytosed within the efferent ducts and the main sperm duct (Fig. 15). The rupture of the spermatophores and the resorption of the sperm within the sperm ducts become accelerated when the hypophysectomized guppy is treated with testosterone. No sperm are noticed in the sperm ducts after two weeks of testosterone treatment (Fig 18), whereas remnant sperm are present in the hypophysectomized control. It may be suggested that the exogenous testosterone accelerates the phagocytosis of sperm.

Interstitial cells.

Buser-Lahaye (1953), Lofts et al (1966), Sundararaj and Nayyar (1967) and Yamazaki and Donaldson (1968) found signs of atrophy in the interstitial cells of different teleosts following hypophysectomy. Ahsan (1966) noted that pituitary removal suppresses the secretory activity of lobule-boundary cells in Couesius plumbeus. Likewise, hypophysectomy causes the regression of the interstitial

cells in the adult guppy. The interstitial cells do not contain any lipid droplets and their nuclei become shrunken.

Regressed interstitial cells of hypophysectomized guppy are stimulated following testosterone administration. Their nuclei assume a more rounded shape with a distinct nucleolus. This finding is in accord with that of Lofts et al (1966) in Fundulus. On the contrary, however, Sundararaj and Nayyar (1967) found that the interstitial cells remained atrophied in Heteropneustes.

The stroma cells of the testis of hypophysectomized juvenile guppy are not differentiated into interstitial cells. Testosterone treatment of the hypophysectomized juvenile does not cause the differentiation of interstitial cells either. It may be concluded from the data that the differentiation of interstitial cells from the stroma cells requires priming or triggering by gonadotropins as in spermatogenesis. The testosterone can stimulate the regressed interstitial cells of adult testis which produced androgens before hypophysectomy but cannot lead to the proliferation of the interstitial cells in the juveniles.

Epithelial cells lining the sperm ducts.

The epithelial cells lining the efferent ducts of an intact adult guppy are of the tall columnar type and contain lipid droplets. The presence of lipid droplets indicates that these epithelial cells may be responsible for secreting androgens. Moser (1967), likewise, noted that sudanophilic droplets are prevalent in the cells of the efferent ducts

of the rockfish, Sebastodes paucinspinis. Wiebe (1967) also reported the presence of steroid dehydrogenases in epithelial cells lining the efferent ducts of the seaperch, Cymatogaster aggregata.

The spermatophores in an efferent duct of an intact guppy are surrounded by a colloidal material which seems to be secreted by the epithelial cells. The colloidal material might contain a nutritive substance as has been suggested by Medlen (1950) in his study on Gambusia affinis.

The epithelial lining of the efferent ducts becomes reduced and does not contain any lipid droplets after hypophysectomy. No colloidal material is secreted by the epithelial cells of the efferent ducts in the absence of the pituitary and it is thought that the rupture of spermatophores in the efferent ducts and the main sperm duct might be due to the fact that the epithelial cells cease to produce androgens and the colloidal material.

When the hypophysectomized guppy is treated with testosterone, the regressed epithelial cells of the efferent ducts and the main sperm duct hypertrophy and assume the tall columnar appearance of the normal animal. This indicates that the epithelial lining of the efferent ducts is under the steroid control. Likewise, Burger (1942) and Lofts et al (1966) noted a hypertrophy of the efferent ducts in Fundulus heteroclitus.

There is no increase in the number, width and the size of the lumina of the efferent ducts in the testis of juvenile

guppy following hypophysectomy. Methyl testosterone treatment of the hypophysectomized juveniles brings about complete differentiation of the efferent ducts and the main sperm duct. The total number, width and the size of lumina of the efferent ducts increase significantly. The lumen of the main sperm duct becomes very wide and the lining epithelial cells attain tall columnar appearance of the adult guppy.

It is concluded that the differentiation and maintenance of the efferent ducts and the main sperm duct of both the juvenile and adult testes depend on androgens and gonadotropin secretion is not directly involved. Unlike the gametogenetic and endocrine tissues of the testis, an initial priming with gonadotropin is evidently unnecessary for the action of androgen.

Sertoli cells

Among the fishes, Sertoli cells have been reported in Gasterosteus (Craig-Bennet 1931), Poecilia (Follenius 1953, Fundulus (Lofts et al 1966) and in Elasmobranchs (Matthews 1950; Stanley 1962), but these workers do not describe the function of Sertoli cells. In the testis of an intact guppy, Sertoli cells play an important part in the formation of spermatophores. These cells become strikingly enlarged as the sperm become more mature and the sperm heads become attached to their inner margin. Considering the enormous change in the size of Sertoli cells and sperm heads becoming attached to them, it may be suggested that Sertoli cells serve as nutrient cells as they are believed to be in higher

vertebrates. Sertoli cells in mammals contain considerable glycogen in their cytoplasm and this is considered evidence that they serve as nutrient cells for the germinal epithalium (Paulsen 1968). Burgos (1955) reported that glycogen is present in the Sertoli cells of frog, Rana pipiens. Tinctorial response of carbohydrates is also detectable in Sertoli cells of Carassius auratus (Yamamoto and Yamazaki 1967). Moser (1967) reported that the tubule boundary cells (corresponding to Sertoli cells) of Sebastodes paucispinis contain carbohydrate granules and act as a substrate for the nutrition of gametes. However, this may not be the only function of these cells. According to Vaupel (1929) the Sertoli cells of the guppy are phagocytic and ingest the cytoplasm (containing the Golgi remnant) discarded when spermatids are transformed into sperm. Similar function has been ascribed to Sertoli cells in mammals (Lacy 1968).

There is no published account of the effect of hypophysectomy on Sertoli cells in any teleost. In the present study, it was evident that the Sertoli cells of the hypophysectomized guppy regress and their nuclei change from rounded condition to flattened shape (Fig. 9). Burgos (1955) found that Sertoli cells of Rana pipiens atrophy and their glycogen content disappears following hypophysectomy. Regressed Sertoli cells of the hypophysectomized guppy are restored to normal following testosterone administration (Fig. 17). Their nuclei become rounded in shape once more with a conspicuous nucleolus. This indicates that Sertoli

cells are under steroid control. The Sertoli cells are not differentiated in the juvenile testis after hypophysectomy. Even testosterone treatment of hypophysectomized juveniles does not bring about the proliferation of Sertoli cells. Perhaps Sertoli cells can be differentiated only after being primed with gonadotropins.

In the guppy, not only Sertoli cells regress after hypophysectomy and attain their normal appearance with testosterone treatment; both the interstitial cells and the epithelial cells lining the sperm ducts behave in a similar fashion. The interstitial cells and the epithelial cells lining the sperm ducts contain lipid droplets and produce androgens. It may be suggested that Sertoli cells also produce androgens as they are believed to do in mammals (Teilum 1950).

c. The role of the pituitary and the androgens in the control of secondary sex characters.

All secondary sex characters in teleosts are under testicular control as shown by treatment with exogenous sex steroids and castration experiments (Pickford and Atz 1957; Dodd 1960; Hoar 1965, 1966). The pituitary directly regulates the androgen production of the testis and thus indirectly controls the secondary sex characters. In Gobius paganellus (Vivien 1938, 1941) and Fundulus heteroclitus (Matthews 1939; Burger 1941; Pickford 1953; Lofts et al 1966) the nuptial coloration or breeding dress (the most

apparent secondary sex character) disappears in the absence of the pituitary. Likewise, the bright lipophores present on the sides of the body of adult guppy become very faint or entirely disappear following hypophysectomy.

In contrast, no changes are evident in the structure of the gonopodium of the hypophysectomized guppy. Morphologically the gonopodium is a well differentiated structure and it is natural to expect that once morphogenesis is complete, the structure will not regress.

Although the influence of exogenous steroids on the sex accessories and secondary sex characters of the teleosts has been tested many times (Pickford and Atz 1957), efforts have rarely been made to eliminate the possible effects of endogenous gonadotropins by the use of hypophysectomized animals. There are only two published accounts on the effects of androgens on the secondary sex characters of hypophysectomized fish. Burger (1942) and Lofts et al (1966) noted that the nuptial coloration reappeared in hypophysectomized Fundulus following testosterone treatment. Similarly the testosterone treatment of the adult hypophysectomized guppy leads to moderate recovery in the content of lipophores present on the sides of the body. The gonopodium of the guppy, which remains unaffected after hypophysectomy, does not change after testosterone treatment.

The respective role of the pituitary and the androgens in the differentiation of secondary sex characters of the juvenile guppy has been analyzed by first removing the

pituitary and subsequently treating such hypophysectomized juveniles with testosterone. Secondary sex characters including both the lipophore pigments and the gonopodium, are absent in the juvenile guppy; the juvenile appearance is maintained in the hypophysectomized juveniles until the end of experimental period (eight weeks). During this period, the sham-operated juveniles developed into adult males with distinct secondary sex characters. When the hypophysectomized juveniles are treated with testosterone, the secondary sex characters (gonopodium and lipophores) become differentiated but the differentiation is not as complete as adult controls. The incomplete differentiation of secondary sex characters may be due to degree of dissimilarity between the synthetic exogenous androgen and the naturally occurring endogenous androgen or that the exogenous androgen cannot lead to complete differentiation of secondary sex characters in the absence of pituitary gonadotropins.

It might also be suggested that the dose used was inadequate. However, the concentration of testosterone (1.2×10^{6} parts) used in the experiment appears quite sufficient when compared to the dosages successfully used by previous workers for stimulating testis and secondary sex characters of intact fish (Pickford and Atz 1957) and is probably not the main reason for the incomplete differentiation of secondary sex characters.

Data obtained from hypophysectomy and testosterone treatment of both adult and juvenile guppies suggest that

secondary sex characters are directly controlled by the androgens and indirectly by the pituitary gonadotropins.

d. The blocking action of 'methallibure' on the effects of the gonadotropins and the site of action of 'methallibure'

Blocking action of 'methallibure'.

The effect of 'methallibure' on juvenile fish has not been previously reported. No developmental changes occur in the structure of testis of juvenile guppy following 'methallibure' treatment (Table XX). The number of spermatogonial cysts remains the same and these are not transformed into spermatocytes. The further differentiation of the efferent ducts and the main sperm duct is checked. Secondary sex characters do not appear. All developmental changes of the juvenile testis are stopped in the same fashion as is evident after hypophysectomy.

Since 'methallibure' treatment produces identical effects to hypophysectomy it is concluded that gonadotropin secretion of the juvenile guppy is entirely blocked with 'methallibure'. In the juveniles the gonadotropin secretion was completely blocked because the 'methallibure' treatment was begun before the gonadotrophs were differentiated, since according to Sokol (1961) it is not until the fifth week after the birth that lightly granulated basophils appear in the ventral region of meso-adenohypophysis. Thus chemical hypophysectomy ('methallibure' treatment) of juveniles is as

effective as surgical hypophysectomy as far as the development of gonad and appearance of secondary sex characters are concerned.

The effect of 'methallibure' on the gonads and gonadal functions has been studied in only three species of teleost fish (Hoar et al 1967; Wiebe 1968). The gonosomatic index of 'methallibure' treated adult guppy is markedly reduced. This finding is in accord with Hoar et al (1967) and Wiebe (1968).

In the adult guppy, 'methallibure' treatment brings about significant changes in the percent composition of different stages of spermatogenesis. After eight weeks of treatment, there are few cysts containing spermatogonia, spermatocytes, spermatids and sperm but spermatophores are present in abundance (Table XVII). Since hypophysectomy completely blocks the transformation of spermatogonia into spermatocytes, the presence of spermatocyte-cysts in 'methallibure' treated testis suggests that a complete pituitary blockage of gonadotropin was not attained in adults at this dose level ($1:10^6$ parts) in eight weeks (Table XIX).

Hoar et al (1967) and Wiebe (1968) concluded that 'methallibure' effectively blocks the pituitary gonadotropic action. Their conclusion is based on the fact that the stages of reduction division and subsequent spermiogenesis are blocked. Wiebe (1968) found that in 'methallibure' treated seaperch, Cymatogaster aggregata, the spermatogonia and spermatocytes comprise 96% of the lobule area (10% of the

area in control) and the spermatophores disappear. Wiebe (1968) further noted that interstitial cells of Leydig and columnar epithelial (Sertoli) border cells atrophy following 'methallibure' treatment. The results of the present investigation are in contradiction to these findings. In the 'methallibure' treated adult guppy, spermatogonia and spermatocytes comprise 3.4% of the total testis area (16% of the area in control) and spermatophores are intact and cover 88% of the testis area (68% of the area in control Table XVII). The interstitial cells and Sertoli cells do not seem to be regressed. Perhaps, even a small release of gonodotropins prevents their regression (complete regression ensues after hypophysectomy). It seems likely that in the guppy the 'methallibure' treatment suppresses the transformation of spermatogonia into spermatocytes (Table XIX). Wiebe (1968) demonstrated that the block following 'methallibure' treatment is between primary and secondary spermatocytes. The differences in results might be attributed to two species of fish - Poecilia reticulata (ovoviviparous, monthly breeder) and Cymatogaster aggregata (viviparous, seasonal breeder).

In 'methallibure' treated male guppy, there is a marked decrease in lipophore pigmentation both on the sides of the body and on the tail. Hoar et al (1967) demonstrated that the height of kidney tubule of stickleback (a secondary sex character) is reduced following 'methallibure' treatment.

Wiebe (1968) found that 'methallibure' causes the atrophy of secondary sex modifications on Cymatogaster male anal fin. These results indicate that 'methallibure' treatment causes reduction in gonadal steroidogenesis.

Since 'methallibure' treatment of the adult guppy does not lead to complete regression of gametogenetic and steroidogenetic tissues of the testis, as is evident in the absence of the pituitary, it is concluded that 'methallibure' does not completely block the release of the pituitary gonadotropins.

The site of action of 'methallibure'

Although it is recognised that 'methallibure' blocks the action of pituitary gonadotropins on both the gametogenetic and endocrine tissues of testis (Hoar et al 1967; Wiebe 1968), the site of action of 'methallibure' is not known. 'Methallibure' treatment of both adult and juvenile guppies brings about a depletion of the aldehyde fuchsin positive granules from the cytoplasm of the pituitary gonadotropic cells. As in all similar studies, there is a problem of interpretation; is a cell which is depleted of granules inactive, or is the rate of synthesis of the granules simply equal to, or less than the rate of release? Examination of the mean cell diameter of the gonadotrophs and of the total number of cells indicates that there is a very significant ($p < 0.001$) reduction in the size of the gonadotrophs of the treated fish compared with those of the controls in both the

adult and juvenile stages which would tend to indicate hypo- rather than hyperactivity.

On this assumption, the gonadotropic blocking action of 'methallibure' may be effective in one of two ways: (a) it may block the hypothalamic control over gonadotropic hormone synthesis, or, (b) it may directly block the synthesis of the hormone within the intrinsic pituitary endocrine cells.

The hypothalamic nucleus lateralis tuberis (NLT) (Florentin 1934; Hild 1950; Stahl 1957; Brehm 1958; Billenstein 1962; Oztan 1963) and pre-optic nucleus (PON) (see Dodd, Perks and Dodd 1966 for review) have been implicated in the regulation of the sexual cycle. Olivereau and Ball (1966) demonstrated by means of autotransplants of the pituitary that an intimate contact with the hypothalamus is necessary for the maintenance of gonadotrophs. It is possible that 'methallibure' blocks the synthesis or release of factors produced by these nuclei. However, most of the above authors describe changes in the appearance of the neurons of the nuclei or changes in the amount of stainable material in the neuro-hypophysis associated with the sexual cycle. In the present investigation no such changes are apparent in the nuclei following the block of gonadotrophic activity, offering no support for the first hypothesis. On the other hand, a decrease in the number and size of gonadotroph cells as well as a depletion of aldehyde fuchsin positive granules in their cytoplasm, lend support to the second hypothesis.

Large blood sinuses are found in the ventral region of meso-adenohypophysis of 'methallibure' treated fish (group II) and in those control groups in which the gonadotrophs are developed and presumably functional (groups I and IV). The role of sinuses or capillaries is not clear although their close spatial association with the gonadotrophs suggests a related function. An increased blood supply to the gonadotrophs may facilitate a rise in the release of gonadotropic hormones, and the control of hypothalamus over the intrinsic endocrine cells of the pituitary may be controlled in this way (Leatherland 1967). Thus a negative feed-back mechanism following a decline in testosterone or estrogen production after treatment with 'methallibure' would result in an enlargement of the blood sinuses controlled by the hypothalamus. An enlargement of these vessels would also be anticipated in those fish actively secreting gonadotropic hormones (groups I and IV).

There is little difference in the number or appearance of the somatotrophs in the adult groups (I and II), which may be expected in more or less fully grown fish. There were, however, fewer of these cells in the juvenile fish killed at the commencement of the experiment (group III) compared with controls killed eight weeks later (group IV). Somatotrophs were not identified in any of the 'methallibure' treated juvenile fish, which may indicate a direct or indirect influence of the compound on the production of growth

hormone in the juvenile fish. The very limited growth of the 'methallibure' treated juveniles compared with the controls would similarly indicate an effect on growth hormone synthesis and/or release.

The thyrotrophs of the adult 'methallibure' treated fish are both significantly larger and more numerous than those of the controls. The thyrotrophs of the 'methallibure' treated juvenile fish are similarly larger and more numerous compared with the controls killed at the beginning of experiment, but not when compared with the control fish killed eight weeks later. It is probably more consistent to compare the 'methallibure' treated juveniles with the group III controls which are of a similar weight rather than group IV controls of a similar age. 'Methallibure' has been shown to have an 'anti-thyroid' effect in mammals with two distinct actions, one at the level of the thyroid blocking the production of the thyroid hormone, and simultaneously at the level of the pituitary and/or hypothalamus tending to cause thyroid involution (Walpole 1965; Tulloch et al 1963). However, the clear increase in number and size of the thyrotrophs in 'methallibure' treated adult and juvenile fish is indicative of an increased TSH production rather than a reduced activity.

There is a direct correlation between the thyroid cell epithelial height (TEH) and the number and size of the pituitary thyrotrophs within the five groups, the TEH of the 'methallibure' treated adult group is greater than that

of the controls. In the juvenile groups, the TEH is largest in the fish of group IV which are rapidly developing and which, during the course of the experiment, achieved sexual maturity, and small in the control group III killed at the beginning of the experiment. The thyroid follicles of the 'methallibure' treated groups have a much reduced colloid content compared with the controls, consistent with a decreased thyroid hormone production. The apparent hyperactivity of the pituitary thyrotrophs in the 'methallibure' treated fish thus appears to result from a fall in thyroid hormone production, and that of the control group (IV) from an increased thyroid activity at this stage of the sexual cycle (Stolk 1959).

It is concluded that 'methallibure' treatment of adult and juvenile guppies causes a decrease in both the number and mean cell diameter of gonadotrophs, an increase in the number and mean cell diameter of the thyrotrophs and a decrease in the number of somatotrophs of treated juveniles.

SUMMARY

1. Hypophysectomy of the adult guppy causes marked regression in the testis. The testis contains only spermatogonia and spermatophores because the spermatocytes, spermatids and sperm present at the time of operation transform into spermatophores. The mitotic divisions of spermatogonia and their transformation into spermatocytes cease. It is concluded that the division of spermatogonia and their transformation into spermatocytes are pituitary-dependent but the transformation of spermatocytes, spermatids and sperm into spermatophores are pituitary-independent.

2. In the absence of the pituitary, the spermatophores rupture after eight weeks and the resulting sperm instead of being discharged, are phagocytosed within the regressed efferent ducts and the main sperm duct. It seems that the release of spermatophores is under the control of the pituitary.

3. After hypophysectomy, Sertoli cells, interstitial cells and epithelial cells lining the efferent ducts and the main sperm duct regress. Methyl testosterone treatment of hypophysectomized animals causes the hypertrophy of the epithelial cells lining the efferent ducts and the main sperm duct; regressed Sertoli cells and interstitial cells are also restored to normal. It is concluded that Sertoli cells, interstitial cells and epithelial cells lining the duct are maintained by androgens and not by pituitary gonadotropins.

4. Hypophysectomy and methyl testosterone treatment of the adult guppy do not bring about any change in the structure of the gonopodium. It suggests that once morphogenesis of the gonopodium is complete, it becomes independent of the pituitary and the androgens. The lipophores, on the other hand, become obscure or entirely disappear in the absence of the pituitary but there is moderate recovery in the content of lipophores after testosterone treatment. It is concluded that the lipophores are regulated by androgens.

5. Exogenous methyl testosterone appears to have a direct spermatokinetic effect on the testis of hypophysectomized animal. Spermatogonial cysts divide rapidly and transform into spermatocytes but later stages of spermatogenesis do not appear.

6. Hypophysectomy of the juvenile guppy prevents the mitotic division of spermatogonia in the testis; no other stages of spermatogenesis appear and the interstitial cells and Sertoli cells are not differentiated. Testosterone treatment of the hypophysectomized juveniles do not initiate spermatogenesis (in contrast to the adult) and the interstitial cells and Sertoli cells are not evident. It is concluded that the spermatogenesis and the differentiation of the interstitial cells and Sertoli cells are under the control of pituitary gonadotropins only.

7. In the absence of the pituitary, the sperm ducts do not differentiate and the secondary sex characters do not appear in the juvenile guppy. Following testosterone treatment the sperm ducts are well differentiated and the secondary sex characters become evident. It is concluded that the androgens cause the differentiation and maintenance of the sperm ducts and the secondary sex characters (similar to the adult).

8. The testis of the adult guppy treated with 'methallibure' contains few cysts of earlier stages of spermatogenesis. The spermatophores remain intact. The epithelial cells lining the efferent ducts are reduced in size but there are no changes in Sertoli cells and interstitial cells. There is a marked decrease in the amount of lipophore pigmentation. Since 'methallibure' treatment of the adult guppy does not lead to complete regression of the gametogenetic and steroidogenetic tissues of testis, as is evident in the absence of the pituitary, it seems that 'methallibure' does not completely block the release of pituitary gonadotropins.

9. No developmental changes occur in the testis of the juvenile guppy following 'methallibure' treatment. The secondary sex characters are not differentiated. Since 'methallibure' produces identical effects to hypophysectomy, it is concluded that gonadotropin secretion of the juvenile guppies is entirely blocked with 'methallibure'.

10. 'Methallibure' treatment does not cause any change in the size or appearance of the cells of pro- and meta-adenohypophysis of the adult or juvenile guppies but the cell types of meso-adenohypophysis (gonadotrophs, thyrotrops and somatotrophs) show changes. A clear decrease in both the number and mean cell diameter of gonadotrophs, and an increase in the number and mean cell diameter of the thyrotrophs is evident in 'methallibure' treated adult and juvenile fish, whereas a decrease is noticed in the number of somatotrophs of the juveniles. The gonadotropic blocking activity of the compound is considered to occur at the level of hormone synthesis. The activity of the thyrotrophs is perhaps, the result of a block in thyroid hormone synthesis. The mode of action of the compound on the somatotrophs is not known.

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