" A HISTOLOGICAL STUDY OF THE GONADS AND THE PITUITARY OF THE AFRICAN LUNGFISH, PROTOPTERUS AETHIOPICUS "

BY

PETUNIA AUDREY FERNANDES

A thesis submitted in fulfilment for the degree of Master of Science in the University of Nairobi.

1982

This thesis is my original work and has not been presented for a degree in any other University.

Petunia Audrey Fernandes

.....

Petunia Audrey Fernandes

This thesis has been submitted for examination with my approval as University supervisor.

.....

Professor M. Hyder

ACKNOWLEDGEMENTS

I am deeply grateful to my supervisor, Professor Mohamed Hyder for his guidance and assistance throughout the course of the M.Sc. program and during the writing-up of the thesis.

To Mr. Kuria Kagori of G.I.T.C.E. and Mr. Daniel Kamau of the Department of Veterinary Anatomy, I offer my sincere appreciation for their help in histology and electron microscopy.

I am also very thankful to Messrs. E. Karithi-Njogu, James Gichiri and Mrs. Rozina Katema of the Department of Veterinary Anatomy and Mr. Joseph Mcilo and Mr. Isuvi of the Department of Zoology for their constant assistance with photographic and histological materials and with photography.

And lastly to my family I offer my love and gratitude for their sincere help and support, in completing this thesis.

TABLE OF CONTENTS

ABSTRACT

A histological study on the gonads and the pituitary of the African lungfish Protopterus aethiopicus was undertaken, aimed at determining the pattern of gonadal changes and changes in the state of the pars distalis that could be correlated with gonadal variation.

Testis: The germ cell generations in the testis of P. aethiopicus i.e. spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa, are described histomorphologically. Characteristics of the five maturational stages (I-V) are also described. In summary, the interstitial cells consisted of some amorphous cells and fibroblasts and exhibited no observable or evident variation in morphology or distribution (amount) with the different maturational stages. A clear cystic arrangement of the late secondary spermatocytes only, was evident in the post-spawning testis of Stage V. Ultrastructurally, the Sertoli cells appeared to be modified fibroblasts and revealed the presence of cisternal, rough endoplasmic reticulum, numerous large mitochondria with well-developed tubular cristae, Golgi complexes and a few lipid droplets within their cytoplasm. The interstitial cells however, appeared to be undifferentiated or undeveloped fibroblasts and their cytoplasm lacked developed or recognizable organelles such as those

present in the Sertoli cells. The possible steroidogenic and nutritive functions of the Sertoli cell and the steroidogenic capacity of the interstitial cells are discussed in the light of previous and recent research on the piscine and amphibian groups.

Ovary: Oocyte maturational stages from the preprotoplasmic stages, through the protoplasmic stages and to the various vitellogenic stages, are described. Cytoplasmic and nuclear characteristics or features including their dimensions are also noted. Furthermore, the pattern of yolk granule accumulation during the vitellogenic stages and the changes in the surrounding follicle cells with these maturational stages, are included in the definition of the different vitellogenic oocyte stages. The probable physiological significance of organelles such as nucleoli, yolk nucleus and "lampbrush" chromosomes, is discussed. In the ultrastructural study, the formation of the zona pellucida, its characteristics and those of the follicular layer with different stages of oocyte maturation, are further described. The follicle cells of vitellogenic oocytes revealed cisternal, rough endoplasmic reticulum, several mitochondria with developed tubular cristae, areas of Golgi vesicles, and other large vesicles, some of which contained only coarse, electron-dense granules while others contained a non-granular material together with the coarse granules. The possible steroidogenic function

of the follicle cells is discussed. The thecal cells or fibroblasts are undifferentiated as compared with the follicle cells, and contain undeveloped mitochondria and small Golgi complexes within their cytoplasm. Endoplasmic reticulum, either of a granular or agranular variety, is lacking.

Pituitary: In the pars distalis of all the lungfish ranging from 18.5cm to 66.5cm in body length, the type 2 basophils (as described by Kerr and van Oordt, 1966) are absent. Only basophils type 1 and 3 occur in the pars distalis, of which the former cell type exhibited obvious variation in distribution, granulation, extent and staining intensity of their chromophilic substance, with the different stages of gonadal maturation. From previous research by other workers, the type 3 basophils in the South American lungfish Lepidosiren paradoxa and in the amphibians, which are phylogenetically related to the lungfishes, were demonstrated by immunohistochemistry to be the ACTH-producing cells, while the types 1 and 2 basophils in the anurans were demonstrated to be the TSH- and FSH-producing cells respectively. The possibility whether the type 1 basophils are gonadotropin or thyroptopin-producing cells in P. aethiopicus, is discussed.

LIST OF FIGURES

Page No.

1. INTRODUCTION

The Devonian era i.e. 340-400 million years ago, was a period of evolutionary advancement, during which time all the fishes that lived in continental fresh waters, rivers, streams and lakes, established themselves as the dominant aquatic animals throughout the world (Colbert, 1969). The "Age of Fishes " as the Devonian is regarded, was a time of climatic extremities, where rainy seasons alternated with times of severe drought (Romer, 1954) and marked the era when fishes began evolving towards a terrestrial life, in response to the problems of survival imposed by constantly fluctuating or receding water levels of their fresh water habitats.

The first terrestrial vertebrates i.e. the ancestors of modern-day amphibians which so appeared at the end of the Devonian period, are derived from the subclass Sarcopterygia, represented today by the lungfishes (Order Dipnoi) and the coelacanths (Order Crossopterygii), the only surviving member of the latter Order being the coelacanth Latimeria (Romer, 1954; Colbert, 1969; Thenius and Terofal, 1974).

During the Devonian period, lungfishes and coelacanths occurred throughout the world. Today, lungfishes are found only in South America, Africa

and Australia, in parts of the primeval land-mass known as the "Gondwanaland". The only coelacanth species is found in deep waters around the Comoros Islands near Malagasy (Colbert, 1969; Thenius and Terofal, 1974). From fossil evidence, it appears that the present-day lungfishes and coelacanths have evolved little beyond those prevalent during the Devonian era. They were also both very similar and regarded as having a common origin (Barrington, 1968) since they showed convergence in many anatomical or morphological characters as they were traced back through this period (Romer, 1954; Barrington, 1968).

The periods of drought during the Devonian often created large stretches of dry land and left small, stagnant and foul dried-up pools of water. This led to the major problems of air breathing and locomotion on dry land to places of adequate water. The Sarcopterygians were said to already have had an advantage or a head-start over the other groups of fishes regarding the problem of air-breathing in that they already possessed highly vascular lungs (Thenius and Terofal, 1974). Of this group, the lungfishes solved the problem of locomotion and survival on completely dry land (as it is still today practised by the South American and African lungfishes) by burrowing into the ground and estivating within a "cocoon" formed with debris and mud and lined with mucus to prevent them

from dehydrating. A tiny hole at the top of the cocoon served as the breathing passage between the dry environment and the animal (Colbert, 1969; Thenius and Terofal, 1974). With the onset of sufficient rainfall and subsequent flooding of the burrow, the cocoon breaks down, thereby releasing the lungfish (Thenius and Terofal, 1974). On the other hand, the Australian lungfish is able to "walk" along the bottom of shallow rivers or pools by using its stout paired pectoral and pelvic fins like legs (Colbert, 1969). Yet in spite of such trends directed towards a terrestrial existence, the lungfishes are not on the direct line of evolution leading from fishes to the first tetrapods or land vertebrates since they possess too many specializations in order for them to occupy an intermediate evolutionary position between the fishes and the amphibians. These specialized features, unlike those of other bony fishes, include a reduction of their bony skeleton; numerous bony plates of the skull that have no homologies with the skull bones in bony fish; and a specialized dentition of tooth plates (Colbert, 1969).

It was the coelacanths of the suborder Rhipidistia, that were on the direct line of evolution to the first terrestrial vertebrates and which exhibited trends that would be expected in the first land-dwellers. In summary, these had no reduction of their endoskeletons as there was in the lungfishes;

the bony elements of the vertebral column were well developed and suggested a strong supportive fuction (for locomotion, etc); the skull and jaws were completely bony with well defined bony patterns, comparable to those in other bony fishes as well as in the early ancestral amphibians; their teeth were sharp and pointed, with the enamel being highly infolded to form a complex labyrinthine pattern - such a structure also having occurred in the teeth of the early amphibians. Furthermore, Rhipidistia possessed well-developed internal nostrils (nares) and as in the higher land vertebrates, the nasal passages led directly from the external nares into the mouth or pharynx. Most important in the skeletal system was the structural beginning of a tetrapod-like limb in each of the paired pelvic and pectoral fins (Colbert, 1969; Kuhn and Thenius, 1974).

Whereas other members of the Rhipidistia successfully colonized the terrestrial environment from their fresh water habitats, Latimeria eventually migrated into deeper marine waters, thereby eluding both the terrestrial environment and the imposed problems of locomotion and air breathing.

Thus the opinion that the first tetrapods have a monophyletic origin, is strongly supported and generally accepted. In summary, this concludes that

the Dipnoi and the Rhipidistia originated from a common rhipidistian stock in the Lower Devonian, which soon diverged independently into two streams, one giving rise to the lungfishes and the other giving rise to the various amphibian groups (Fox quoted by Barrington, 1968). A second opinion concerning the history of the Rhipidistia, considers that this Order differentiated into two main lines even before the Devonian era, one line, the Osteolepiformes giving rise to the anuran amphibians (frogs and toads), while the other, the Porolepiformes, gave rise to the urodeles (newts and salamanders). This view of a diphyletic origin of the first tetrapods, however, has not been generally accepted (Jarvik quoted by Barrington, 1968).

Despite the fact that the lungfishes are not the direct progenitors of the first amphibians, they are nevertheless very close to their ancestry. Their survival up to modern times provides us with a glimpse of the important vertebrates that formed the link between fishes and the first land vertebrates - that is to say that the lungfishes are the collateral uncles of the amphibians (Romer, 1954; Colbert, 1969). Their morphological and physiological adaptations to reproduction might indeed provide us with important clues in the evolution of the tetrapodan reproductive pattern and function and how these link or compare with those of the cyclostomes,elasmobranchs and

actinopterygians.

In this study, we aim to establish the pattern of gonadal changes in Protopterus aethiopicus that have probably not been significantly changed since the end of the Devonian period and which can be related to adaptations evolved at overcoming the problems imposed by an unstable environment. It is also of interest to see how the reproductive pattern of P. aethiopicus compares with that of the cyclostomes, elasmobranchs, actinopterygians and lastly with the amphibians with which the lungfishes share a close phylogenetic relationship. The cyclostomes represent the earliest aquatic vertebrates (as fossil records indicate); the elasmobranchs are generally considered to be the primitive fishes which appeared during the Devonian period and continued their evolutionary expansion until the Permian period of 160-200 million years ago; the actinopterygians or bony fishes also appeared during the Devonian and successfully dominated the waters of the earth up until recent times and best represented today by the teleosts (Colbert, 1969). Like the lungfishes and the amphibians, the cyclostomes, elasmobranchs and bony fishes have all had a long period of independent evolution (Barrington, 1968).

2. LITERATURE REVIEW

There have been several excellent reviews on the piscine and amphibian gonads as to the nature of the gonadal tissue, gametogenetic and steroidogenic tissue (Mathews and Marshall, 1956; Dodd, 1960; Barr, 1968; Lofts, 1968; Hoar, 1969; Franchi, 1962; Rowlands and Weir, 1977; Guraya, 1978; Jones, 1978; Tokarz, 1978; Callard, Callard, Lance, Bolaffi and Rosset, 1978; Grier, 1981) and on the piscine and amphibian pituitary in terms of anatomy and physiology (Dodd, 1960; van Oordt, 1968; Ball and Baker, 1969; Fontaine and Olivereau, 1975; Licht, 1979; Peter and Crim, 1979; Ball, 1981). This review will concentrate on bringing into focus certain work in so far as it sheds relevant light on our investigation of the lungfish gonads and pituitary.

2.1. Testis

For clarity and understanding, this review describes the structure of the testis of the piscine and amphibian groups, characteristics of the spermatogenetic cycle and the experiments or techniques aimed at identifying the steroidogenic components of the testicular tissue and those with a nutritive or germ cell-supporting function.

2.1.1. Cyclostomes

Both the lampreys (Order Petromyzoniformes) and the hagfishes (Order Myxiniformes) possess a single, unpaired and ductless testis (Dodd, 1960; Walvig, 1963; Callard et al, 1978). The mature testis of these cyclostomes consist of several large cysts or follicles containing germ cells and the cysts are grouped into lobules by vascular connective tissue. Germinal epithelium lines the lobules (Dodd, 1960; Walvig, 1963; Tsuneki and Gorbman, 1977a). When germ cells within the cysts are transformed into spermatozoa, the cysts break down and discharge spermatozoa directly into the body cavity (Dodd, 1960; Walvig, 1963; Callard et al, 1978) which then reaches the external environment through the duct of the urino-genital papilla via two pores in the urinary sinus (Dodd, 1960; Walvig, 1963).

Seasonal cyclical changes have been described in the testis of the hagfish Eptatretus burgeri which has an annual reproductive cycle with spawning occurring in October. Spermatogenesis in adult males is continuous throughout the year with the cyclical changes being similar to those in teleosts and amphibians. Spermatogenesis is asynchronous and testes contain all developmental stages from spermatogonia to spermatids prior to spawning (Patzner, 1977).

Hagfishes spawn repeatedly throughout their reproductive life (Walvig, 1963; Tsuneki and Gorbman, 1977a).

The interstitial cells of both Eptatretus stouti and the lamprey Lampetra fluviatilis possess ultrastructural characteristics indicative of steroidogenesis i.e. well developed agranular or smooth vesicular endoplasmic reticulum, mitochondria with tubular cristae, and lipid inclusions (Tsuneki and Gorbman, 1977a; Barnes and Hardisty, 1972). With different stages of testicular maturation, the interstitial Leydig cells of L. fluviatilis revealed cytological and histochemical variation. Thus in an immature testis, the interstitial tissue consists of isolated connective tissue cells, the cytoplasm of which contains small osmiophilic and sudanophilic granules. During the later stages of maturation, these interstitial cells were observed to have accumulated large areas of lipid and cholesterol-positive droplets, with their cytoplasm showing extensive vacuolization and an increase in the number of cells and formation of compact groups. Changes in the interstitium were also paralleled by histochemical and cytological changes in the modified fibroblasts of the lobule wall (lobule boundary cells) i.e. during the final stages of testicular maturation, there was an increase in cytoplasmic volume and a

change in nuclear shape from a spindle-shape fibroblast type to a swollen vesicular form with a large nucleolus. The cytoplasm of the lobule boundary cells also histochemically demonstrated the development of extensive lipid and cholesterol-positive inclusions (Hardisty, Rothwell and Steele, 1967). These findings suggested that the lobule boundary cells may also be involved in the steroid secretory activity of the testis. Later ultrastructural studies did indeed show the characteristics of steroidogenic activity in these cells immediately preceding spermiation (Barnes and Hardisty, 1972).

3β-Hydroxysteroid dehydrogenase (3β-HSD) activity, an enzyme involved in the synthesis of steroids, was also demonstrated in the interstitial cells of L. fluviatilis, the intensity of which reached a maximum just prior to the development of the secondary sexual characteristics and correlated with the abundance of lipids in the interstitium. The level of testosterone in gonadal extracts were reported to be the highest (24µg/kg of testicular tissue) with the appearance of the secondary sexual characters and just prior to the liquefaction of the testis and its release of androgen from the interstitial Leydig cells (Barnes and Hardisty, 1972). However the interstitial cells of E. stouti are suggested to have a low androgen secretory activity

as reflected by reported low plasma levels of testosterone, due to the fact that E. stouti possesses a low number of interstitial cells and comparatively lesser developed steroidogenic organelles within them as compared with the Leydig cells of higher vertebrates (Tsuneki and Gorbman,1977a).

Unlike the river lamprey L. fluviatilis, in which testicular extracts revealed the presence of testosterone (Barnes and Hardisty, 1972), this androgen could not be detected in testicular tissue of the sea lamprey Petromyzon marinus after incubation with either cholesterol or progesterone. In vitro incubation of the testis of the hagfish Myxine glutinosa with progesterone, led to the biosynthesis of testosterone and the conversion of this androgen to androstenedione and a number of metabolites similar in nature to 11β-hydroxytestosterone (Kime, 1980).

Sertoli cells of M. glutinosa (Walvig, 1963) and E. stouti (Tsuneki and Gorbman, 1977a) were reported as forming a "mantle" surrounding germ cells. Although these Sertoli cells contained organelles such as mitochondria - some with tubular cristae, rough and smooth endoplasmic reticulum, glycogen particles, lipid droplets and lysosomes, they were not well developed and the cells were

therefore not implicated with a steroidogenic function (Tsuneki and Gorbman, 1977a).

Spermatogenesis in L. fluviatilis is synchronous throughout the testicular cysts i.e. at any one maturation stage, all the germ cells in the testis are at the same stage of development (Larsen, 1973 quoted by Patzner, 1977). Since all lampreys die after spawning, a second phase of spermatogenesis never occurs and the testis produces only a single generation of spermatozoa (Lofts, 1968).

2.1.2. Elasmobranchs

Testes of elasmobranchs are paired and each is enclosed by a thick capsule of connective tissue - the tunica albuginea, the inner extensions of which divide the testis into lobes. Histologically, the elasmobranch testis possesses a zonate structure which appears to be unique among the vertebrates (Dodd, 1960; Hoar, 1969). Within each lobe are concentric zones of ampullae (cysts or spermatogenetic units), each ampulla within a zone having a central lumen, Sertoli cells and germ cells, the latter all being at the same stage of development of maturation (Dodd, 1960; Hoar, 1969; Callard et al, 1978). Ampullae are proliferated from nests of cells of the "germ-line" or "tubulogenic zone" which runs along

the medio-ventral border of each testis. These gradually move dorsally as they mature so that by the time they reach the dorsal surface of the testis, the ripe spermatozoa within the ampullae are ready for discharge into the vasa efferentia (efferent ducts) which also emerge from the dorsal surface (Dodd, 1960; Hoar, 1969).

Interstitial cells are derived from connective tissue (Dodd, 1960) and occur in all groups of elasmobranchs i.e. sharks, rays and skates (Hoar, 1969). 3β-HSD activity has been demonstrated in the interstitial cells of Torpedo marmorata and Scyliorhinus stellaria (Callard et al, 1978) and in the Sertoli cells of Scyliorhinus canicula and Squalus acanthias (Hoar, 1969), thereby suggesting a steroid-secreting capacity of these cell types.

The androgens testosterone, androstenedione and other steroid hormones, have been identified in the plasma of male S. canicula (Simpson, Wright and Hunt, 1969) and in the testicular extracts of S. stellaria. Testosterone and other metabolites have also been detected in testicular tissue of the skates Raja radiata and R. ocellata (Callard et al, 1978).

2.1.3. Teleosts

The Actinopterygians are the largest class of vertebrates and mostly represented today by the teleosts of which there are some 20,000 species. They exhibit a great diversity of their reproductive apparatus and several specializations which are unique to themselves and do not occur elsewhere among other vertebrates (Dodd, 1960). In spite of this diversity, a generalized description of the gross morphology of the testis can be made: testes of teleosts are paired and in many species, the vas deferens are fused posteriorly to form a single sperm duct. Within the testis are convoluted tubules which are limited by strands of connective tissue. The lumina of the tubules eventually drain into the lumen of the vas deferens which runs down the dorso-median length of the testis (Sundararaj, 1960; Henderson, 1962; Rai, 1965; Ruby and McMillan, 1970; 1972). The surface of the testis may be lobulated, as in the pike Esox lucius (Lofts and Marshall, 1957), Heteropneustes fossilis (Sundararaj, 1960) and Barbus tor (Rai, 1965).

Very recently, Grier (1981) has re-defined the structure of the teleost testis and distinguished two types according to the distribution of the sperma-togonia. In the "unrestricted spermatogonial" testis

type, which most teleosts possess e.g. E. lucius, E. niger, Cichlasoma nigrofasciatum, Mugil cephalus and Onchorynchus kisutch, spermatogonia occur all along the length of the tubules. However, in the second testis type i.e. the "restricted spermatogonial" type, spermatogonia are limited to the distal terminus of the tubule immediately beneath the tunica albuginea, where they are associated with the Sertoli cells. The Fundulus species possess this type of testis.

Teleosts possess cystic spermatogenesis i.e. germ cells occur in cysts within the tubules and in any one cyst, they are all at the same developmental stage and mature synchronously. Since many teleosts are seasonal spawners, the testis undergoes several different and distinct stages of maturation. This reproductive cycle was divided generally into the following stages by Grier (1981): (I) proliferation of spermatogonia, (II) early recrudescence - spermatogonia and spermatocytes present, (III) mid-recrudescence - all stages of sperm development present, (IV) late recrudescence - tubules filled with spermatozoa and number of developing spermatozoa cysts is declining, (V) maturity - tubules filled with spermatozoa and almost no spermatogenesis is occurring, (VI) post-spawning stage. Stage III is considered to be the developmental peak of the spermatogenetic cycle and characterized by the

presence of numerous cysts at all maturational stages i.e. from spermatogonia to ripe spermatozoa (Lofts and Marshall, 1956; Henderson, 1962; Lofts, Pickford and Atz, 1966; Chan and Phillips, 1967, Ruby and McMillan, 1970; de Vlaming, 1972; Gresik, Quirk and Hamilton, 1973).

Grier (1981) has also sought to clarify the controversy regarding the cellular organization of the testis, in particular the interstitial Leydig cells and the so called lobule boundary cells. The latter were first described by Marshall and Lofts (1956) in the testis of E. lucius which was at that time thought to lack the typical interstitial cells. The lobule boundary cells which are modified fibroblasts surrounding the tubules (lobules), were regarded as being homologous with the interstitial Leydig cells. Such lobule boundary cells were also later reported in the testis of the brook trout Salvelinus fontinalis (Henderson, 1962) and in the Atlantic salmon Salmo salar (O'Halloran and Idler, 1970), these teleosts also seeming to lack interstitial cells. Other teleost species were said to possess both the lobule boundary and the interstitial cells i.e. B. tor (Rai, 1965), Belone belone (Upadhyay and Guraya, 1971) and C. nigrofasciatum (Nicholls and Graham, 1972), while some were observed as possessing only the typical interstitial

cells i.e. Fundulus heteroclitus (Lofts et al, 1966), Eucalia inconstans (Ruby and McMillan, 1970), Gillichthys mirabilis (de Vlaming, 1972) and Oryzias latipes (Gresik et al, 1973). However, ultrastructural studies of the testis of C. nigrofasciatum (Nicholls and Graham, 1972) and later of E. lucius, revealed that the lobule boundary cells were in fact intratubular and homologous not with Leydig cells but with Sertoli cells, these cells in E. lucius exhibiting identifying criteria such as phagocytosis of residual spermatid bodies and the occasional enveloping of sperm by their cytoplasm (Grier and Linton, 1977). The lobule boundary cells (Sertoli cells) of C. nigrofasciatum also revealed similar ultrastructural characteristics (Nicholls and Graham, 1972). According to Grier (1981), the interstitial Leydig cells typically occur in all teleosts and previous reports or observations that such cells are absent in certain species (as reported by Henderson, 1962 and O'Halloran and Idler, 1970), are incorrect. Furthermore, the lobule boundary cells where described (e.g. Henderson, 1962; Rai, 1965; O'Halloran and Idler, 1970; Upadhyay and Guraya, 1971) are in fact intratubular Sertoli cells.

Techniques or methods employed to determine possible steroidogenic function in certain testicular cell types have varied. Thus on the basis of

morphological changes alone, the interstitial cells of B. tor, G. mirabilis and E. inconstans were suggested to be homologous with the steroid-producing interstitial Leydig cells of higher vertebrates, those of the former teleost undergoing cytoplasmic vacuolization during spermiation (Rai, 1965), while cyclical morphological changes in cell and nuclear dimensions of the interstitial cells occurred in the latter two fish (de Vlaming, 1972; Ruby and McMillan, 1970).

Histochemical evidence suggesting steroidogenesis (i.e. a positive sudanophil reaction indicating the presence of lipids or cholesterol - these being the precursor materials for the synthesis of steroid hormones), was demonstrated in the interstitial cells of Monopterus albus (Chan and Phillips, 1967) and in the Sertoli cells of E. lucius (Lofts and Marshall, 1956; Grier, 1981), S. salar (O'Halloran and Idler, 1970) and B. belone (Upadhyay and Guraya, 1971). The activity of the enzyme 3β-HSD was also demonstrated in the Sertoli cells of S. salar (O'Halloran and Idler, 1970) and in the interstitial cells of E. lucius (Grier, 1981). Although the testis of B. belone also possesses interstitial cells, in the opinion of the authors (Upadhyay and Guraya, 1971), these cells revealed no morphological or histochemical evidence of a steroidogenic

function. However, Gresik et al (1973) stressed caution in interpreting negative results when employing histochemical methods for identifying steroidogenic tissue, after such tests proved negative in the interstitial cells of O. latipes but ultrastructural observations revealed that these cells did indeed possess organelles that suggested a steroidogenic function. 3β-HSD activity was later demonstrated in the interstitial cells of O.latipes (Grier, 1981).

The 11-oxygenated derivatives or metabolites of testosterone i.e. 11-ketotestosterone and 11β-hydroxytestosterone, are established as being the characteristic androgens of the testes of most teleosts (Callard et al, 1978; Kime, 1980). However, testosterone too was identified in the plasma of Serranus cabrilla and Pleuronectes platessa, the level of which increased as spawning neared. 11-ketotestosterone has been demonstrated as being the predominant androgen in the peripheral plasma of the sockeye salmon Onchorynchus nerka, S. salar and Pseudopleuronectes americanus, with 11β-hydroxytestosterone occurring in low concentrations. In S. cabrilla and Pagellus acarne however, 11β-hydroxytestosterone appeared to be the major circulating androgen. 11-ketotestosterone has been further demonstrated to be ten times more potent than

testosterone in promoting the male secondary sexual characters in the female O. latipes. The concentration of this androgen was found to increase in the plasma of S. salar with approaching sexual maturity and in the plasma of P. americanus just prior to spawning. In Salmo gairdneri, the level of 11-keto-testosterone also rose dramatically with the onset of testicular maturation (Callard et al, 1978).

Besides the formation of 11β-hydroxytestosterone and 11-ketotestosterone synthesized by the teleost testis, in some species a conjugate of testosterone - testosterone glucoronide, is also formed. This glucoronidation of testosterone is attributed to the liver and is considered to facilitate deactivation and excretion of this androgen. Thus with the testis of S. gairdneri incubated with testosterone, it was found that the formation of the 11-oxygenated androgens by the testis occurs between the incubation temperature range of 6° - 21° C. However, above 21° C incubation, there was a diminished output of the 11-oxygenated derivatives of testosterone. 11-ketotestosterone was converted to testosterone glucoronide by higher competition for testosterone by the glucoronyl transferase enzyme of the liver. 6° - 21° C reflects the breeding temperature of S. gairdneri and the formation of testosterone glucoronide beyond 21° C is said to reflect the

removal of testosterone at higher temperatures which are unfavourable for reproductive development and limit the secretion of free 11-oxygenated androgens to the environmentally preferred temperature range for reproduction (Kime, 1980).

2.1.4. Amphibians

There are some 2000 species which belong mainly to the subclasses of urodeles (newts and salamanders) and anurans (frogs and toads).

i). Urodeles

The testes of urodeles are paired and in some mature species e.g. Triturus cristatus (Lofts, 1968), Trituroides hongkongenesis (Tso and Lofts, 1977a) and Triturus viridescens, consist of several lobes joined together by thread-like segments. In other species e.g. Diemyctylus, there is a single lobe (Callard et al, 1978). The lobe(s) eventually communicate(s) with the vasa efferentia and thereafter with the Wolfian ducts (Dodd, 1960). Each lobe consists of several tubules (lobules) within which are cysts of germ cells which mature in a gradient or zonation. This gradient of cyst maturation occurs in a cephalo-caudal wave and the histological appearance of the testis varies seasonally or with the stage of

maturation. The least advanced cysts of germ cells occur anteriorly in each lobe or in the single lobe, while the most advanced or mature cysts occur caudally, such that in a fully mature testis, the large caudal section is occupied by cysts of mature spermatozoa held together by Sertoli cells and lobule boundary cells, the latter in urodeles regarded as being homologous with interstitial Leydig cells. The smaller cephalic section or immature zone consists of cysts of spermatogonia (Callard et al, 1978). Prior to spermiation, the lobule boundary cells hypertrophy and together with the residual Sertoli cells, condense to form a mass of tissue called the yellow zone, glandular tissue or interstitial gland. This glandular yellow zone has been demonstrated to possess 3β-HSD activity in a variety of urodele species and further revealed ultrastructural characteristics of steroid-secreting cells (Callard et al, 1978). Thus 3β-HSD activity occurs in both the Leydig and the Sertoli cells of Ambystoma mexicanum. During regeneration of the tubules after spawning the Sertoli cells exhibit a strong 3β-HSD activity, this activity diminishing with the onset of spermatogonial meiosis and the greater part of spermatogenesis. In the Leydig cells, 3β-HSD only occurs during the final steps of spermiogenesis i.e. during spermiation and the active secretion of androgens by the glandular tissue which

is related to stimulation of the secondary sexual characters. This delayed correlation between the Leydig and Sertoli cells had been said to suggest that the stimulation of enzyme activity in the Sertoli cells may depend to a certain extent on a steroid factor synthesized by the Leydig cells (Lazard, 1979).

In the newt T. hongkongenesis, the lobule boundary cells are fibroblast-like which show no evident morphological changes during the process of spermatogenesis. However, with maturation of the spermatozoa , the lobule boundary cells were noted as having increased in size. On discharge of the spermatozoa, the nuclei of the lobule boundary cells became ovoid and the cytoplasm increased in quantity. 3β-HSD activity occurred in the lobule boundary cells of mature tubules containing spermatozoa and in evacuated ones in which the lumina were filled with Sertoli cells and residual bodies. Furthermore, cholesterol-positive lipid droplets occurred in Sertoli cells supporting spermatids and spermatozoa prior to spermiation, in the lobule boundary cells of mature tubules and in the lumina of evacuated ones. The lobule boundary cells of evacuated tubules are however, completely devoid of lipid droplets (Tso and Lofts, 1977a).

Ultrastructural studies of the lobule boundary cells of T. hongkongenesis revealed characteristics typical of steroid-producing cells. Morphologically developed lobule boundary cells (during spermatozoa maturation and spermiation) had an abundance of well-developed agranular endoplasmic reticulum, several lipid droplets, Golgi complexes and mitochondria with elaborate cristae. These features were said to probably indicate that the secretory phase of the lobule boundary cells begins when spermatozoa are ready for spermiation, with steroidogenic activity of these cells being the highest when spermiation has just occurred. Both these ultrastructural findings and the previous histochemical findings (Tso and Lofts, 1977a) suggest that the lobule boundary cells are the major site of steroid production, though not necessarily the only component involved in this activity (Tso and Lofts, 1977b).

Testosterone has been detected in the plasma of Necturus maculosus, Pleurodeles waltlii (Callard et al 1978) and Taricha granulosa (Specker and Moore, 1980). In the testis of T. cristatus, it was shown that testosterone could be converted to 11-ketotestosterone and 11-dihydrotestosterone, the latter androgen also having been detected in the plasma of T. granulosa (Specker and Moore, 1980) and

N. maculosa (Callard et al, 1978). Plasma levels of testosterone and dihydrotestosterone were measured in T. granulosa during seasonal changes in the testis. It was found that during the initial stages of spermatogenetic activity i.e. after spawning and during the proliferation of spermatogonia, the concentration of the plasma androgens was low (less than 5ng/ml) and remained so until spermatogenesis was almost complete. Spermiation coincided with a dramatic increase in the plasma androgens (54.7±7.9 ng/ml) which could reflect an increased capacitation of the lobule boundary cells to synthesize androgens or an increased output of pituitary gonadotropin, presumably luteinizing hormone (LH) (Specker and Moore, 1980).

ii). Anurans

The testes of anurans are paired, ovoid and consist of a mass of seminiferous tubules lined with germinal epithelium and containing germ and Sertoli cells (Callard et al, 1978). The tubules eventually drain into the vas efferens, this then leading to the Wolfian duct (ureter) (Mathews and Marshall, 1956; Dodd, 1960).

Anurans possess cystic spermatogenesis - germ cells occur within membrane-bound cysts and in any

one cyst, the germ cells are all at the same stage of development or maturation. Unlike the urodele testis where different regions contain cysts at a different maturation stage, the entire anuran testis is uniform in the compostion of the germ cells, at any stage of testicular maturation. The typical higher vertebrate pattern of interstitial cells occurs, which displays a well-marked variation histologically and histochemically (Callard et al, 1978). Histological variation of the interstitial cells of Rana temporaria and R. esculenta with different maturational stages of the testis, has been reported. Prior to breeding or spawning, the nuclei of the interstitial cells were large, rounded or oval with fairly coarse clumped chromatin i.e. possessing a secretory appearance. After spawning, nuclear size of the interstitial cells decreased and the nuclei became somewhat shrunken and strongly chromophilic (Lofts, Wellen and Benraad, 1972; Mathews and Marshall, 1956).

The absence of lipids in the interstitial cells of R. temporaria and R. esculenta during the winter or non-breeding season,paralleledor coincided with a lack of cholesterol and absence of 3β-HSD activity in these cells. Active spermatogenesis was accompanied by the appearance of lipids, cholesterol, 3β-HSD activity and the ultrastructural features

characteristic of active steroid-producing cells. Post-spawning changes included a dramatic increase in the cholesterol and lipid content of the interstitial cells and a decrease in 3β-HSD activity. During later post-nuptial changes, these lipids gradually disappeared from the interstitial cells and the latter regressed and could not be distinguished from connective tissue cells. Depletion of the lipids correlated with a resurgence of spermatogenesis (Callard et al, 1978). In R. cyanophlyctis, 3β-HSD activity also occurred mainly in the interstitial Leydig cells (Saidapur and Nadkarni, 1973).

Sertoli cells of the anurans also possess the ultrastructural features of steroidogenic cells (Callard et al, 1978) and like the interstitial cells, there is a well-defined histochemical cycle with varying levels of cholesterol-positive lipids. Towards the spawning season, Sertoli cells become glandular in appearance and lipid droplets accumulate in their cytoplasm (Lofts and Boswell, 1960; Lofts, 1964). Furthermore, their cytoplasm reveals both 3β-HSD and glucose 6-phosphate dehydrogenase (G6-PDH) activity (van Ooodt and Brands, 1970; Saidapur and Nadkarni, 1973). The presence of G6-PDH in both the Leydig and Sertoli cells of R. cyanophlyctis and in the Sertoli cells of R. temporaria (van Oordt and Brands, 1970), is said to provide additional but

indirect evidence of steroid synthesis, since this enzyme is supposed to be the principal one that provides NADPH employed in hydroxylations during spermatogenesis (Saidapur and Nadkarni, 1973).

The appearance of 3β-HSD activity in the interstitial cells of the anurans and the lobule boundary cells of the urodeles, correlates with the appearance of the secondary sex characters, suggesting that these cells secrete the androgens. 3β-HSD activity in the Sertoli cells can be correlated with spermatogonial division, which may take place when the peripheral plasma androgen titres are low or absent. Sertoli cells possibly secrete steroids within the cysts and stimulate spermatogenesis without affecting the secondary sex characters (Callard et al, 1978).

Despite demonstrated histochemical evidence of steroidogenesis in the interstitial Leydig cells of the anurans (i.e. 3β-HSD activity), in not all species do vast quantities of agranular endoplasmic reticulum occur in these cells. Thus in the active interstitial cells of R. temporaria, rough endoplasmic reticulum predominated. In Bufo bufo, the well-developed endoplasmic reticulum that occurs in the interstitial cells prior to the breeding season, is mainly of the rough, cisternal variety, with only

some agranular endoplasmic reticulum and dilated vesicles also occurring. Unsicker (1975) has suggested that the predominance of rough endoplasmic reticulum in these cells could be due to the fact that the high rate of synthesis of the protein enzyme that is required for steroid synthesis, requires rough endoplasmic reticulum and not the smooth variety.

Pregnenolone, progesterone and testosterone were all converted into dihydrotestosterone in the testis of R. esculenta, R.catesbeiana and Bufo marinus (Kime, 1980). Dihydrotestosterone also seems to be the major metabolite formed from testosterone in R. temporaria, Discoglossus pictus and Nectophyrnoides occidentalis (Callard et al, 1978). In R. pipiens, the major metabolite of testosterone is also dihydrotestosterone (Kime, 1980). During active spermatogenesis in R.catesbeiana, the level of testosterone was lower than dihydrotestosterone, but at other maturational stages, testosterone was higher than dihydrotestosterone (Callard et al, 1978).

2.2. Ovary

In this section of the literature review, the structure of the ovary in the piscine and amphibian groups is reviewed, including characteristics of the oocytes with maturation. Furthermore, the histochemical and other techniques aimed at determining possible steroidogenic activity or steroidogenic characteristics in either the follicle or the thecal cells of the oocytes, are also reviewed.

2.2.1. Cyclostomes

The adult lamprey has an unpaired, elongated ovary which is lobulated and consists of cortical tissue only (Franchi, 1962). Stromal connective tissue derived from the peritoneal epithelium, covers the ovary. A mature ovary of L. planeri and L. fluviatilis has a large number of oocytes which are all at the same stage of maturation i.e. oocyte development is synchronous. No atretic oocytes are formed (Rowlands and Weir, 1977; Jones, 1978). During its lifetime, each female lamprey has only one ovarian cycle and dies after spawning. Mature oocytes are discharged from the surface of the ovary directly into the body cavity (Dodd, 1960; Jones 1978) and exuded through the gonopore since cyclostomes lack genital ducts (Rowlands and Weir, 1977; Dodd, 1960).

Adult hagfish also possess a single right ovary - the left one fails to develop (Franchi, 1962; Tsuneki and Gorbman, 1977b). Unlike the lampreys, the hagfishes are continuous breeders and possess asynchronous oocyte development and do not die after spawning. The oocytes of M. glutinosa are arranged serially on stalks (Jones, 1978) and as they mature, they acquire a layer of follicle cells, two layers of connective tissue and an outer peritoneal epithelium. Mature oocytes are surrounded by a tough, horny shell secreted by the follicle cells (Walvig, 1963; Jones, 1978).

In the lampreys, the follicle cells are restricted to the basal or vegetative pole of the oocytes (Tokarz, 1978). Early stages of oocyte development (oogenesis) have been described in the brook lamprey L. planeri and in the landlocked sea lamprey P. marinus where early oocyte growth is associated with increased cytoplasmic basophilia (Hardisty, 1965a; 1965b). Oocytes in the diplotene stage were characterized by a large, deeply basophilic nucleolus and deep staining chromosomes - suggestive of the "lampbrush" chromosomal stage (Hardisty, 1965a).

Hardisty and Barnes (1968) reported the presence of 3β-HSD activity in the follicle cells

of L. fluviatilis. This activity, suggesting steroidogenesis, was noted to be more intense when the follicle cells were at their maximum size and development (Guraya, 1978). Thecal cells of L. fluviatilis revealed no histochemical evidence of steroidogenesis (Hardisty and Barnes, 1968). On the other hand, the hypertrophied thecal cells but not the follicle cells of the oocytes of L. planeri showed ultrastructural characteristics of steroidogenesis i.e. elements of agranular endoplasmic reticulum and lipid droplets (Guraya, 1978). The follicle and thecal cells of the hagfish E. stouti were not regarded as being actively steroidogenic. Both granular and agranular endoplasmic reticulum occurred in the follicle cells while the thecal cells possessed dilated granular endoplasmic reticulum with a homogeneous material within the cisterns (Tsuneki and Gorbman, 1977b).

The ultrastructural morphology of the zona pellucida (chorion or egg envelope) has been described in L. fluviatilis (Afzelius, Nicander and Sjoden, 1968) and in L. planeri (Guraya, 1978).

2.2.2. Elasmobranchs

The elasmobranch ovary is differentiated into both cortex and medulla. Many species possess only a

large, developed right ovary, but in some, the ovaries are paired (Franchi, 1962). Oocytes occur in the cortical region and as they grow and accumulate yolk, they become surrounded by a layer of follicle cells and a two-layered (Jones, 1978) or a multiple-layered theca (Dodd and Dodd, 1980). The single ovary of the basking shark Cetorhinus maximus has a somewhat unique structure. It has little stroma and millions of oocytes which are attached to lamellae covered with germinal epithelium. The latter proliferates these oocytes which are arranged in a size hierarchy. Tubules formed from the lamellae radiate from an anterior pocket or opening on the right side of the ovary (Jones, 1978). The ovary is invested in a fibrous tunica albuginea and the oocytes communicate with the abdominal cavity or oviduct through the anterior opening (Dodd, 1960).

Morphological and histochemical changes in the follicle cells with oocyte maturation, have been described in the dogfish Scoliodon sorrakowah. Such changes involved an increase in follicle cell size and aggregation of sudanophilic lipid droplets with increase in oocyte growth and yolk accumulation (Guraya, 1978). An increase in the frequency of lipid droplets and 3β-HSD activity was also reported in the follicle cells of S. acanthias (Lance and Callard, 1969), suggesting that these cells may be the

possible sites of steroid biosynthesis. Ultrastructural studies of the ovarian follicle of S. canicula revealed that the cells of the theca externa were rich in agranular or smooth endoplasmic reticulum, had lipid droplets and numerous mitochondria, some with tubular cristae (Dodd and Dodd, 1980), such features being typical of steroidogenic cells.

Histochemical and morphological changes in the zona pellucida and theca of S. sorrakowah with oocyte maturation, have also been described (Guraya, 1978). The estrogens estradiol-17β and estrone were shown to occur in the ovary of S. canicula , each in a concentration of 19µg/ml. Most of these steroids i.e. approximately 80% are located within the oocytes, the remainder occurring in the ovarian stroma (Simpson, Wright and Hunt, 1963).

2.2.3. Teleosts

Many teleosts possess paired ovaries which are mostly of the cystovarian type i.e. the ovarian tissue is enclosed within a muscular tunica albuginea (Jones, 1978). The ovary may be either compact or hollow (Dodd, 1960) and in the latter case, the cavity is lined by germinal epithelium. Numerous ovigerous folds made up of connective tissue lamellae, traverse the ovarian tissue and project into this

cavity. Oocytes develop along the lamellae which are also lined by germinal epithelium and proliferate oogonia (Jones, 1978). Mature oocytes are ovulated into the ovarian cavity (if present), the lumen of which is continuous with the lumen of the oviduct, the latter being a posterior continuation of the ovarian tunica albuginea (Dodd, 1960).

The developmental range of oocyte stages, including the nuclear and cytoplasmic features of each stage, have been described at the light microscopy level in the teleosts Scomber scomber (Bara, 1965), Gobius giuris (Rajalakshmi, 1966), the pipefish Syngnathus fuscus (Anderson, 1968), the goby G. mirabilis (de Vlaming, 1972), Mystus tengara (Guraya, Kaur and Saxena, 1975) and at the ultrastructural level in Blennius pholis (Shackley and King, 1977). Oocyte features of suggested physiological importance or significance, included the presence of several peripheral nucleoli, either of a uniform or duplex nature in the early oocytes of G. giuris (Rajalakshmi, 1966), S. fuscus (Anderson, 1968), G. mirabilis (de Vlaming, 1972) and M. tengara (Guraya et al, 1975), and a cytoplasmic body referred to as the yolk nucleus or Balbiani body and consisting variously of mitochondria or protein and ribonucleic acid (RNA) in the early oocytes of S. fuscus (Anderson, 1968) and the trout S. gairdneri (Beams

and Kessel, 1973). Guraya et al (1975) also observed a yolk nucleus with a homogeneous structure in the immature oocytes of M. tengara.

On the basis of histochemical evidence i.e. the presence of 3β-HSD activity, or ultrastructural evidence, the thecal cells of the oocytes of S. scomber (Bara, 1965), Sarotherodon niloticus (Yaron, 1971), C. nigrofasciatum, Haplochromis multicolor (Nicholls and Maple, 1972) , the coho O. kisutch, pink salmon O. gorbuscha (Nagahama, Clarke and Hoar, 1978) and the follicle cells of the guppy Poecilia reticulata (Lambert, 1970), Acanthobrama terrae-sanctae and S. niloticus (Yaron, 1971), were suggested to have a function in the synthesis of ovarian steroids. Other functions attributed to the follicle cells are those of nutrition, specifically yolk formation and its subsequent transport to the developing oocytes (Yaron, 1971, Hirose, 1972).

Ultrastructural changes in the follicle cells and formation of the zona radiata, have been described in the medaka O. latipes (Hirose, 1972) and in B. pholis (Shackley and King, 1977). The follicle cells were implicated in having a function in the formation of the zona radiata (Shackley and King, 1977). Furthermore, phagocytosis of the contents of

atretic oocytes by the follicle cells is well documented (Rajalakshmi, 1966; Yaron, 1971; de Vlaming, 1972; Guraya et al, 1975).

Mature oocytes of the cod Gadus callarias were found to contain estradiol-17β in a concentration of approximately 4.8µg/kg and estrone in a concentration of 1µg/kg. Only traces of these hormones occurred in the immature oocytes (Gottfried, Hunt and Simpson, 1962). Estrone and estradiol-17β were also reported to occur in the ovaries of Sarotherodon aureus, the concentration of the latter steroid being 91 ± 7µg/kg. As such, the three major mammalian estrogens are said to occur in the ovaries of the teleosts - estrone, estradiol-17β and estriol, or their oxygenated derivatives (Katz, Eckstein, Ikan and Gottlieb, 1971).

2.2.4. Amphibians

The ovaries of amphibians are paired and covered by the mesovarium which forms the squamous germinal epithelium. Each ovary is divided into lobes or sac-like structures which are hollow (Jones, 1978). Stromal tissue is absent (Dodd, 1960). Oocytes lie in the cortical region of the lobes and in a breeding female, the oocytes can be divided into several maturational stages i.e. very small

primordial germ cells, pre-vitellogenic oocytes, vitellogenic oocytes and post-vitellogenic oocytes (Jones, 1978).

Oogenesis has been described in X. laevis, incorporating changes in the nucleus, nucleoli, the single layer of follicle cells, zona radiata (vitelline envelope) and the pattern of yolk platelet accumulation (Dumont, 1972). In the immature oocytes of both the anurans and the urodeles, the follicle cells are fibroblast-like (flattened and spindle-shaped) and with oocyte maturation, undergo morphological changes, eventually becoming cuboidal and later columnar. In large, mature oocytes, the follicular epithelium becomes thin and the follicle cells are reduced in size (Guraya, 1978).

A cytoplasmic juxtanuclear mass or aggregation of mainly mitochondria, has been reported in the primordial germ cells and oogonia of X. laevis (Al-Mukhtar and Webb, 1971) and in the immature oocytes of X. laevis (Dumont, 1972). This is considered to be the precursor of the Balbiani body (yolk nucleus) which later condenses to give the characteristic well-developed yolk nucleus of pre-vitellogenic oocytes (Al-Mukhtar and Webb, 1971).

Histochemical evidence of steroidogenesis

(i.e. 3β-HSD and G6-PDH activity) has been demonstrated in the follicle cells of R. cyanophlyctis, R. tigrina, R. verrucosa, Cacopus systoma (Saidapur and Nadkarni, 1974) and X. laevis (Redshaw and Nicholls, 1971). Furthermore, ultrastructural observations of lipid loss in the follicle cells of B. bufo prior to ovulation, has suggested the preovulatory secretion of a steroid hormone by these cells (Thornton and Evennett, 1973).

Hope, Humphries and Bourne (1963) have described the ultrastructural changes in the follicle cells and formation of the zona radiata or pellucida in the developing oocytes of the salamander T. viridescens. The activity of both the developing oocyte and its follicular epithelium were regarded as being involved in the formation of the zona pellucida (Guraya, 1978). The absence of extensive areas of agranular endoplasmic reticulum and other organelles specific to steroidogenic tissue in the follicle cells of the amphibians, has suggested the possibility that different pathways of steroid hormone synthesis may occur in the follicle cells, which need further investigation (Guraya, 1978).

Ovarian tissue of X. laevis has been shown to synthesize (in vitro) both estrone and estradiol-17β in almost equal quantities (Redshaw and Nicholls,

1971), while the ovarian steroid content of the urodele Triturus cristatus carnifex has been shown to consist of progesterone, 17-hydroxyprogesterone, androstenedione and testosterone (di Prisco, Delrio, Chieffi, Cardellini and Polzonetti, 1971).

2.3. Pituitary

The pituitary generally reflects variation in gonadal maturation by structural variation of certain basophils in the pars distalis, such basophils hence suggested to be the gonadotropin-producing cells. For clarity and understanding, this section of the review describes the anatomy of the pituitary in the piscine and the amphibians groups, the hypothalamo-hypophysial anatomical relationships and the various research techniques aimed at identifying the gonadotropin-release factors and the gonadotropic cells. The structure of the lungfish pituitary and the cell types in the pars distalis are also reviewed.

2.3.1. Cyclostomes

The pituitary of lampreys (Fig. 1) and ammocoete larvae possesses an unusual morphology in that the various zones are serially arranged instead of forming a compact structure as in the pituitary of higher vertebrates (Dodd, 1960). The adenohypo-

physis consists of two parts situated one behind the other - the pars distalis (anterior region) and the pars intermedia (posterior region or meta-adenohypophysis) (van Oordt, 1968; Ball, 1981), these being separated by a thick layer of connective tissue (van Oordt, 1968; Ball and Baker, 1969). The pars intermedia underlies the neurohypophysis and is separated from it only by basal membranes and a thin network of capillaries - the vascular plexus (van Oordt, 1968; Ball and Baker, 1969; Ball, 1981). A thick sheet of avascular connective tissue also separates the pars distalis and the overlying infundibular element (Ball, 1981). The hypophysial cavity is extensive and lies not between the pars distalis and pars intermedia, but below the pituitary i.e. between it and the mouth cavity (Dodd, 1960). Septa of connective tissue and capillaries divide the pars distalis dorso-ventrally into several lobules, and on the basis of differences in cell type, the pars distalis can be divided into a rostral zone or pro-adenohypophysis and a caudal or proximal zone (meso-adenohypophysis) (van Oordt, 1968; Ball and Baker, 1969).

Hagfishes possess a more primitive and an extremely reduced pituitary (Fig. 2) with no contact existing between the neuro- and the adenohypophysis (Dodd, 1960; Ball and Baker, 1969; Fontaine and

Olivereau, 1975). There is no subdivision of the adenohypophysis into separate zones, the cells of which occur in nests or clusters and separated from each other by connective tissue (Dodd, 1960; Ball and Baker, 1969; Fontaine and Olivereau, 1975; Ball, 1981). The entire adenohypophysis is separated from the overlying infundibulum or neurohypophysis by a thick sheet of connective tissue (Fontaine and Olivereau, 1975; Ball, 1981).

In the cyclostomes, there appears to be no or a very poorly developed hypothalamo-hypophysial anatomical relationship, either of a vascular or neural type that would provide transfer of hypothalamic influences to the adenohypophysis (Crim,Urano and Gorbman, 1979a). Thus the lamprey pars distalis and hagfish adenohypophysis are not innervated although a posterior region of the hagfish adenohypophysis is linked by axons to modified glial cells in the neurohypophysis. The ventral floor of the hagfish neurohypophysis and its anterdorsal wall has been suggested to be a possible rudimentary median eminence since it contains monoamines and both peptidergic and aminergic nerve endings on ependymal processes lying against the connective tissue that lies above the adenohypophysis. In lampreys too, the infundibular floor above the pars distalis has a typical median eminence structure, containing mono-

amines, specialized transporting cells (tanycytes) and peptidergic and aminergic nerve endings ventrally on the avascular connective tissue between the infundibulum and the pars distalis, or on ependymal processes close to the pars distalis. Therefore, it has been suggested that material from these nerve endings could reach the adenohypophysis via the connective tissue. The hagfish E. burgeri, unlike other hagfishes e.g. E. stouti and Myxine, possesses some vascular system between its neuro- and adenohypophysis, which may offer another route of material transport although the direction of blood flow is not known (Ball, 1981).

Immunoreactive luteinizing hormone-releasing hormone (irLH-RH) has been demonstrated in brain extracts of Entosphenus tridentata and L. richardsoni, located over perikarya in the preoptic nucleus with reactive axons passing ventro-posteriorly through the infundibulum and terminating in the neurohypophysis (Crim et al, 1979a; 1979b). Ball (1981) has suggested that in lampreys the gonadotropin-releasing factor may travel from the neurohypophysis to the pars distalis via the systemic circulation.

Specific staining of irLH-RH however, did not occur in any part of the hagfish E. stouti central nervous tissues (Crim et al, 1979a). The authors

have suggested that the hagfish may possess an LH-RH-like substance which remained undetected by the immunocytochemical technique (due to "masking" of immunoreactivity, presence of quantities below the level of detection, etc) or that E. stouti simply lacks LH-RH-like material in its central nervous tissues. Since most hagfishes reside in an unchanging, dark, deep-sea environment which is at a relatively constant temperature and appear to exhibit no seasonal breeding, their hypothalamus may have little function in intergrating environmental cues (Crim et al, 1979a). Furthermore, since it was not possible to correlate morphological features of the Myxine adenohypophysis with maturational stages of the gonads or with gonadectomy, it was suggested that the hagfish adenohypophysis exerts no considerable control over gonadal functions and the feed-back systems involving the neuro-endocrine reflexes, are not required (Fernholm and Olsson, 1969). However, hypophysectomy implicates a gonadotropic function in both male and female E. burgeri but not in E. stouti. This may be due to the fact that E. burgeri unlike other myxinoids, has seasonal gonadal cycles and lives mostly in shallow waters and therefore exposed to environmental cues that would lead to hypophysiotropic signals and call for endocrine adjustments (Ball, 1981).

In L. planeri, only one type of basophil in the rostral pars distalis revealed changes with ovarian growth, vitellogenesis and spawning and was hence considered to be the source of the gonadotropic hormone. Prior to spawning, clear vacuoles were present in the cytoplasm of the basophils and regarded as features of extrusion. These basophils start to develop in growing larvae and increase in number and staining affinity during metamorphosis. However, the basophils in the proximal pars distalis were also found to be active at the beginning of maturation or in lampreys with immature gonads. In the adult lamprey, a reduction of secretory activity in the proximal basophils occurred, resulting in a storage of the secretory products. It was therefore concluded that the basophils of the proximal pars distalis primarily produced the gonadotropin and to a lesser extent, the basophils of the rostral pars distalis (van Oordt, 1968).

2.3.2. Elasmobranchs

The pituitary gland of elasmobranchs consists of distinct and well-defined lobes (Fig. 3). The neuro-intermediate lobe consists of interdigitated tissues of the neurohypophysis and the large pars intermedia and lies in a dorso-caudal position (Dodd, 1960; van Oordt, 1968; Ball and Baker, 1969). The

pars distalis is an elongated, tongue-like structure and due to the segregation of its cell types, can be divided into proximal, medial and rostral zones (van Oordt, 1968). In the sharks and dogfishes, the rostral pars distalis consists of several vesicles and tubules communicating with the spacious hypophysial cavity. The median pars distalis consists of folded walls around this cavity. In rays and skates, the pituitary possesses a reduced hypophysial cavity, compact tubules and vesicles, giving a solid appearance to the pars distalis (van Oordt, 1968; Ball and Baker, 1969)

Above the median and rostral zones of the pars distalis, lies the elaborate and elongated median eminence, this having a rostral region with a palisade zone projecting into the rostral pars distalis, and capillaries continuous with those of the rostral pars distalis, without intervening portal vessels. The caudal region of the median eminence has capillary loops which form distinct portal vessels supplying the median pars distalis, and in some species, the neuro-intermedia (Ball, 1981). A unique feature is the ventral lobe which is unpaired, lies ventral to the pars distalis and the neuro-intermediate lobe and connected by an interhypophysial stalk to the median zone of the pars distalis (Dodd, 1960; van Oordt, 1968; Ball, 1981).

The ventral lobe receives little or no portal blood and since none of the pars distalis is innervated, it remains unclear as to how the hypothalamus might control the ventral lobe, this region being the source of both the gonadotropin and the thyrotropin. It has been suggested that the systemic circulation may provide the route between the hypothalamus and the ventral lobe (Ball, 1981). Extirpation of the ventral lobe in dogfishes led to an atrophy of the gonads and cessation of ovulation, and again in female dogfishes, this lobe increases in size considerably at sexual maturity (Dodd, 1960; van Oordt, 1968). However, seasonal changes seen in regions of the pituitary other than the ventral lobe, suggests that the ventral lobe may not be the only part of the pituitary concerned with the control of reproduction (Della Corte quoted by Firth and Vollrath, 1973)

Ultrastructural observations have also shown that the median pars distalis contains certain cells which are morphologically similar to those in the ventral lobe, suggesting the presence of the same hormone in both regions (Knowles, Meurling and Vollrath quoted by Firth and Vollrath, 1973). This was confirmed by the demonstration of lutenizing hormone-like gonadotropic activity in the ventral and median lobes of the dogfish S. canicula pituitary.

Since the median lobe (with its gonadotropic activity) unlike the ventral lobe, receives blood from the hypothalamus via a portal system, it was suggested that the pituitary-gonadal system in elasmobranchs may be partially under direct hypothalamic control (Firth and Vollrath, 1973).

Evidence of hypothalamic control comes from the demonstration that extensive hypothalamic lesions (which probably damaged the median eminence) caused a dramatic increase in the frequency of ovulation,thus suggesting the removal of some hypothalamic gonadotropin-inhibiting factor, this probably reaching the ventral lobe via the systemic circulation. Evidence also of a gonadotropin-releasing hormone in elasmobranchs, has come from the demonstration of irLH-RH in the hypothalamic extracts of Poroderma africanum and in the forebrain of S. acanthias (Ball, 1981).

The gonadotropin from the ventral lobe of S. canicula has been purified and found to have biological activities similar to mammalian follicle stimulating hormone (FSH). Since only one gonadotropin was purified specifically from the ventral lobe, it does not follow that only a single gonadotropin is present in the elasmobranch pituitary (Sumpter, Follett, Jenkins and Dodd, 1978).

2.3.3. Teleosts

The neurohypophysis and the adenohypophysis of the teleost pituitary are easily recognizable, with the adenohypophysis being the largest part of the pituitary and divided into three regions situated one behind the other - the pro-adenohypophysis or rostral pars distalis, the meso-adenohypophysis or proximal pars distalis and a meta-adenohypophysis or pars intermedia (Fig. 4). The neurohypophysis is not a discrete lobe and is in the form of anastomosing strands or protrusions which penetrate the pars intermedia and to a lesser extent, the two regions of the pars distalis. A typical hypophysial cavity is absent in the teleost pituitary (Dodd, 1960; van Oordt, 1968; Fontaine and Olivereau, 1975).

Teleosts are regarded as unique in having incorporated the median eminence and portal vessels as the rostral neurohypophysis, this being adjacent to the pars distalis and connected to it with short capillaries. The pars distalis is more or less directly innervated and it has been suggested that in the primitive teleosts such as the salmonids, nerve endings on the capillary plexus in the rostral neurohypophysis, exert control over the pars distalis - the hypophysiotropic factors being carried into the pars distalis by the short capillaries. However, in most of

the teleosts that have been studied, hypophysiotropic nerve fibres pass to the pars distalis and either terminate on the neurohypophysis-pars distalis interface basement membrane, or penetrate into the pars distalis and form nerve endings, often synaptic, impinging on the endocrine cells. In any one species, the hypothalamo-hypophysial system may involve various combinations of all routes (Ball, 1981).

In several teleost species, the characteristics displayed by certain basophils in the pars distalis in response to various stages of gonadal maturation, have been closely consistent. Thus at peak testicular maturity, degranulation, regression and chromophobia occurred in the basophils of the proximal pars distalis of H. fossilis (Sundararaj, 1960) and also in the basophils of the proximal pars distalis of the black surfperch Embiotoca jacksoni (Lagios, 1965). In the pituitary of a gravid female H. fossilis, the basophilic β cells in the proximal pars distalis again showed a regression in cell and nuclear sizes and contained little granulation (Baker, Keshavanath and Sundararaj, 1974). Moreover, in these species with either maturing testes or actively vitellogenic oocytes, the basophils of the proximal pars distalis exhibited hypertrophy or an increase in cell and nuclear dimensions, increase in number, and intensity of granulation, and were hence suggested to be the

gonadotropin-producing cells.

Barr and Hobson (1964) reported that the number of basophils in the proximal pars distalis of the plaice Pleuronectes platessa closely paralleled gonadal development and gonadotropic potency of the pituitary i.e. with maturing gonads which have the greatest rate of development, the proximal pars distalis revealed the largest number of basophils and pituitary extracts had the greatest gonadotropic potency. Pituitaries of immature and spawned fish lacked these basophils and their extracts contained little or no gonadotropic activity.

Some controversy exists as to the number of gonadotropic cell types in the teleost pituitary. The technique of immunofluorescence identified or stained only one gonadotropic cell type in the pars distalis of the Atlantic salmon S. salar (Ekengren, Peute and Fridberg, 1978a; Lindahl, 1980) and in the meso-adenohypophysis of the Poecilinae, especially the black molly Mollienisia latipinna (Goos, Seldenrijik and Peute, 1976). In the proximal pars distalis of the goldfish Carassius auratus, the administration of synthetic mammalian LH-RH helped identify the gonadotropic cells and evidence indicated only one type (Kaul and Vollrath, 1974). The administration of LH-RH in S. salar, led to degranulation and

vacuolization of the gonadotropic cells (Ekengren et al, 1978a) as it did to the gonadotropic cells of C. auratus (Kaul and Vollrath, 1974), these features suggesting high activity and release of gonadotropin. Ovariectomy in the rainbow trout S. gairdneri also led to degranulation in most of the gonadotropic cells and an increase in the plasma gonadotropin level, probably due to the decrease in the level of sex steroids in the blood. Again, degranulation implied a secretory activity (Peute, Terlow, Breton, Goos and van Oordt, 1980). Ekengren et al (1978b) again employing the technique of immunohistochemistry, identified two types of gonadotropic cells in the proximal pars distalis of Rutilus rutilus, which were cytologically different from each other.

irLH-RH has been demonstrated in the forebrain, neurohypophysis and its nerve fibres leading to the proximal pars distalis of S. gairdneri and in the anterior neurohypophysis close to the gonadotropic cells of Anguilla japonica and the puffer Fugu niphobles (Ball, 1981). Furthermore, relatively purified gonadotropin has been obtained from the pituitaries of several teleosts - the carp Cyprinus carpio, chinook salmon O. tshawytscha, chum salmon O. keta, S. gairdneri and Sarotherodon mossambicus, which was found to be a glycoprotein hormone with structural properties similar to mammalian LH and

FSH. At present, it is uncertain as to the number of gonadotropins present in the teleost pituitary. Thus from extensive gonadotropin fractionation preparations, it was found that O. tshawytscha, O. keta and S. mossambicus, all possess two forms of gonadotropin, an LH-like and and FSH-like fraction occurring in the latter teleost, while the gonadotropins of the former two fish possess some male and female specificity. On the other hand, a single glycoprotein gonadotropin of the American plaice Hippoglossoides platessoides possessed both FSH and LH-like properties in that it induced oocyte maturation as well as ovulation. Furthermore, a non-glycoprotein fraction that stimulates vitellogenesis was obtained from the pituitary glands of the winter flounder Pseudopleuronectes americanus and the salmon (Peter and Crim, 1979).

2.3.4. Amphibians

The amphibian adenohypophysis is situated ventro-caudally of the neurohypophysis and consists of the pars intermedia, pars distalis and pars tuberalis. The pars intermedia lies dorsally or dorso-caudally of the pars distalis with its rostral surface attached to the caudo-ventral surface of the pars nervosa (neurohypophysis). In anurans, the pars intermedia is connected at its caudo-ventral rim

with the dorso-caudal surface of the pars distalis (Fig. 5), while in urodeles, the pars intermedia is continuous with the pars distalis (Fig. 6) (van Oordt, 1968). The pars distalis is well-developed and rich in vascular sinusoids which subdivide the cells into cord-like aggregations (Dodd, 1960). In anurans, its width usually exceeds its length while in urodeles, this lobe is more elongated (van Oordt, 1968). The pars tuberalis in urodeles consists of a pair of processes formed from a small group of cells and attached to the ventral wall of the tuber cinereum that lies rostrally of the pars distalis. Such a connection between the pars distalis and the pars tuberalis is absent in anurans, the two lobes being separated by a layer of flattened cells which may be reduced to a membrane (Dodd, 1960; van Oordt, 1968).

The typical median eminence portal system route of communication between the hypothalamus and the pars distalis, occurs in the amphibians, this being best developed in the anurans. The pars distalis of the adult is not directly innervated, but R. temporaria tadpoles have a pars distalis which is sparsely innervated by hypothalamic monoaminergic neurones, which disappear at metamorphosis (Ball, 1981).

The results of direct biochemical investigations (chemical fractionation) on the hormones of the amphibian pituitary, have established that two separate and chemically distinct glycoprotein gonadotropins occur in both the anurans (Licht and Papkoff, 1974; Farmer, Licht, Papkoff and Daniels, 1977) and in the urodeles, which appear to be homologous with the LH and FSH of mammals (Licht, 1979). Recent studies have demonstrated irLH-RH in the brain and hypothalamus of Rana (Ball, 1981) and in the hypothalamus of X. laevis, and studies have confirmed that amphibian gonadotropin-releasing hormone is identical to mammalian LH-RH. In X. laevis, the hypothalamic LH-RH content varies seasonally with the reproductive cycle, being low in sexually quiescent frogs during the non-breeding winter season and high in reproductively active frogs during the spring or breeding season (King and Millar, 1979).

Five histomorphologically different cell types occur in the pars distalis of the amphibians i.e. two acidophils and three basophils. Basophils type 1 occur as small groups in the centro-ventral region while type 2 occur in greater quantities throughout the pars distalis and differ in staining properties of their granules as from the type 1 basophils. Type 3 basophils characteristically occur

around the rostral pars distalis and possess different staining properties altogether as from the types 1 and 2.

Previous studies aimed at determining the function of the basophils, involved correlating changes in the target organs with parallel changes of these cells in the pars distalis. Thus in the anurans X. laevis, B. bufo, P. waltlii, Salamandra salamandra taeniata and T. cristatus, the type 1 basophils were regarded as the source of thyrotropin (TSH) after variously revealing changes in activity with the state of the thyroid in larval amphibians during metamorphosis and in those larvae and adults that were thyroidectomized and goitrogen treated. Upon blockage of thyroid function in adults amphibians, a degranulation, vacuolization and enlargement of the type 1 basophils occurred (van Oordt, 1968).

Several workers have accepted the type 2 basophils as being the gonadotropin-producing cells. These were noted to be absent in larval amphibians but develop at the onset of gonadal maturation. Gametogenetic variations also coincided with changes in the type 2 basophils (van Oordt, 1968). Other experimental techniques aimed at identifying the gonadotropic cells in the amphibian pars distalis, involved gonadectomy and subsequent gonadotropin-

replacement therapy in X. laevis (Kerr, 1965) and in R. esculenta (Rastogi and Chieffi, 1970). This again led to a variation in number, granulation, dimension and staining reaction of the type 2 basophils in both X. laevis (Kerr, 1965) and R. esculenta (Rastogi and Chieffi, 1970).

Fairly recently, the technique of immunohistochemistry confirmed that the amphibian type 2 basophils were indeed the gonadotropic cells (stained with LH antiserum) (Evennett and Thornton, 1971; Doerr-Schott, 1976) and that the type 1 basophils in the anurans were the TSH producing cells. In the urodeles, the type 1 basophils were not identified or stained by the immunohistochemical technique. The type 3 basophils in amphibians are the ACTH (corticotropin) producing cells (Doerr-Schott, 1976). Previously, LH production was ascribed to the type 3 basophils after atrophy of the interstitial tissue in the testes of anurans led to a regression of these basophils. Moreover, cyclical changes in the interstitial tissue also seemed to correlate with changes in the type 3 basophils (van Oordt, 1968).

2.3.5. Dipnoi

The close phylogenetic relationship that is said to exist between the lungfishes and the amphi-

bians, is reflected in the structural similarities of their pituitaries, especially the pars distalis (Fig. 7). An interesting difference however, is the presence of a distinct hypophysial cavity which incompletely separates the pars distalis and pars intermedia, in both the South American lungfish Lepidosiren paradoxa (Hansen, Hansen and Hummer, 1980) and in adult P. aethiopicus (Kerr and van Oordt, 1966). The organization of the hypothalamo-hypophysial complex in lungfishes also resembles that of the amphibians in that the lungfishes too have a well-developed median eminence portal system, with the portal vessels connecting the capillary bed of the median eminence to that of the pars distalis (Hansen et al, 1980; Ball, 1981). Like the tadpoles of R. temporaria, the pars distalis of lungfishes is also sparsely but directly innervated by aminergic fibres which probably originate from scattered aminergic nerve cells in the tuberal hypothalamus (Ball, 1981).

Kerr and van Oordt (1966) identified three types of basophils and two types of acidophils in the pars distalis of P. aethiopicus, these cell types closely resembling the five cell types in the amphibian pars distalis in staining reaction, distribution and development (Fig. 7). In the pars distalis of L. paradoxa, three basophilic cell types were also

described, whereas the identification of two acidophilic cell types was uncertain. It was noted however, that the histological features, staining reaction and distribution of the basophils were similar to those in Protopterus (Hansen et al, 1980). In their study, Kerr and van Oordt (1966) correlated the state of the pars distalis with the size or body length of the lungfish, but in the absence of experimental evidence or correlation of the pituitary with changes in its target organs, they stressed that the functions attributed to the cell types remain tentative. Two size ranges of P. aethiopicus were studied i.e. young specimens ranging from 3cm to 35cm and an adult range of 65cm to 170cm, the intermediate range of 35cm to 65cm being absent in their study. In summary, the type 1 basophils were tentatively regarded as the TSH producing cells due to their early appearance in a lungfish of size 11cm and having comparable staining reactions with the TSH cells of amphibians. Type 2 basophils were suggested to be the FSH cells because of their absence in the juvenile range and predominance in the adult range. Due to the similarity in distribution, shape and staining reaction with the type 3 basophils in the amphibian pituitary, the type 3 basophils of P. aethiopicus were tentatively regarded to be the LH producing cells. However, in L. paradoxa, immunocytochemical staining of ACTH was located in the type 3 basophils in the rostral pars

distalis (Hansen et al, 1980), and therefore does not agree with the deduction of Kerr and van Oordt (1966). The aim of the present study is to identify the basophil(s) that reveal(s) a histomorphological variation with the different stages of gonadal maturation and which therefore can be regarded with some accuracy as the gonadotropin-producing cells.

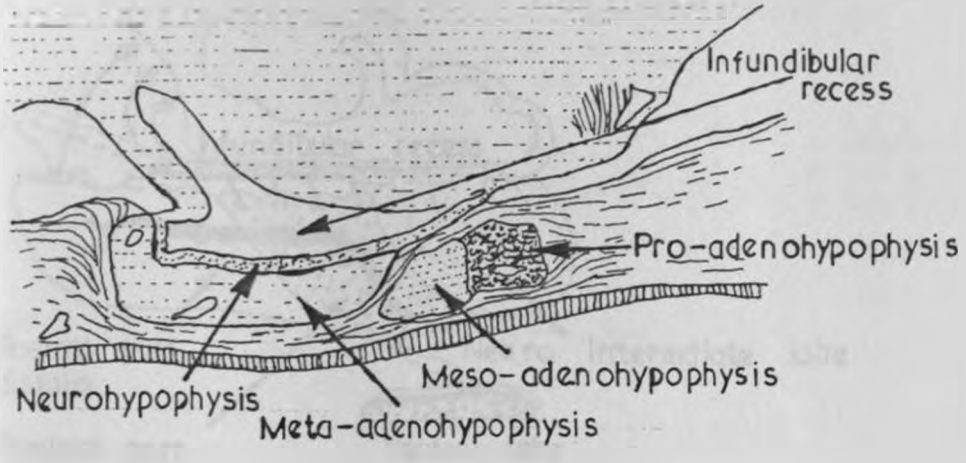


FIG. 1. Sagittal section of Lamprey pituitary (from Lanzing, 1959)

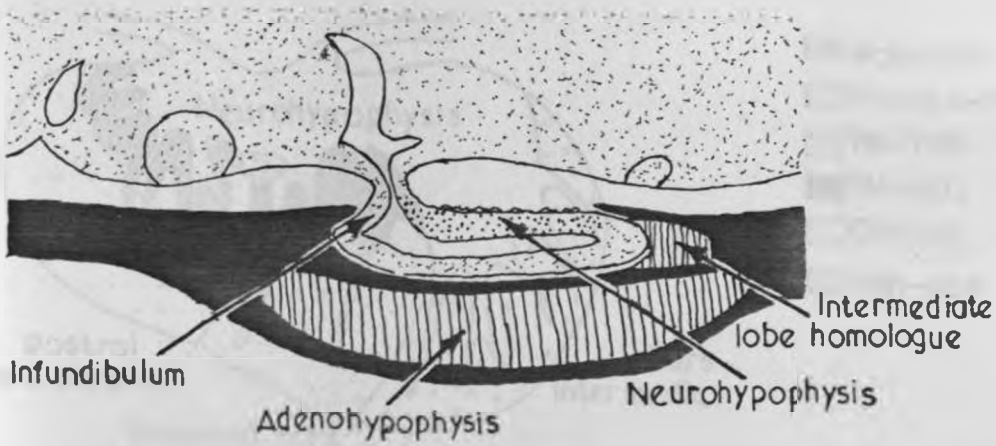


FIG. 2. Median sagittal section of Myxine pituitary (from Adam, 1959). Connective tissue is in black.

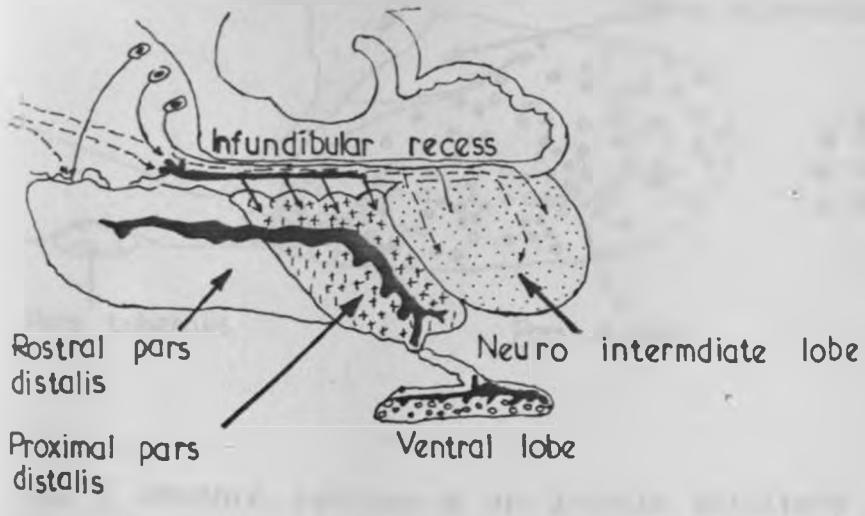


FIG. 3. Diagrammatic representation of a median sagittal section of Scyliorhinus pituitary (as modified from Mellinger, 1966 by Fontaine and Olivereau, 1975)

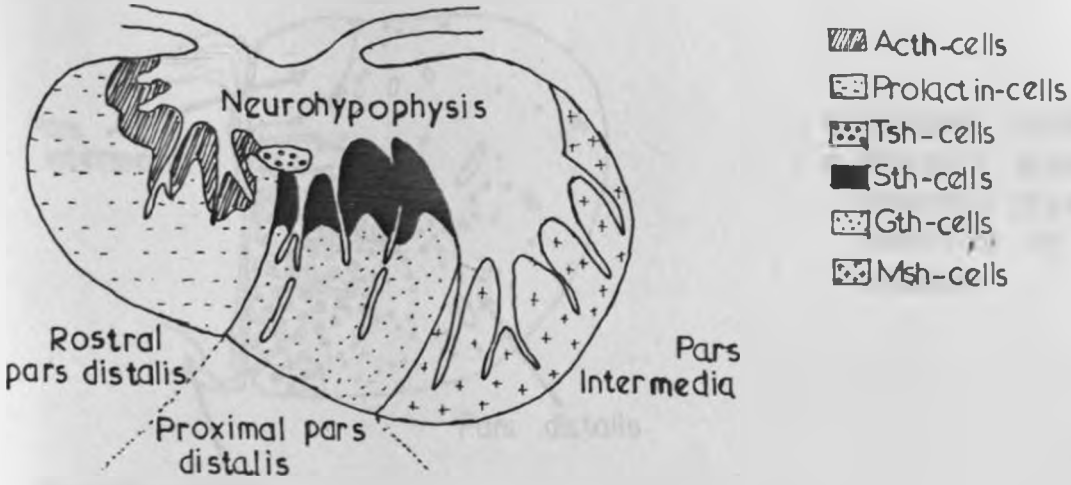


FIG. 4. Diagrammatic representation of a median sagittal section of Tilapia pituitary (from Bern, Nishioka and Nagahama, 1974)

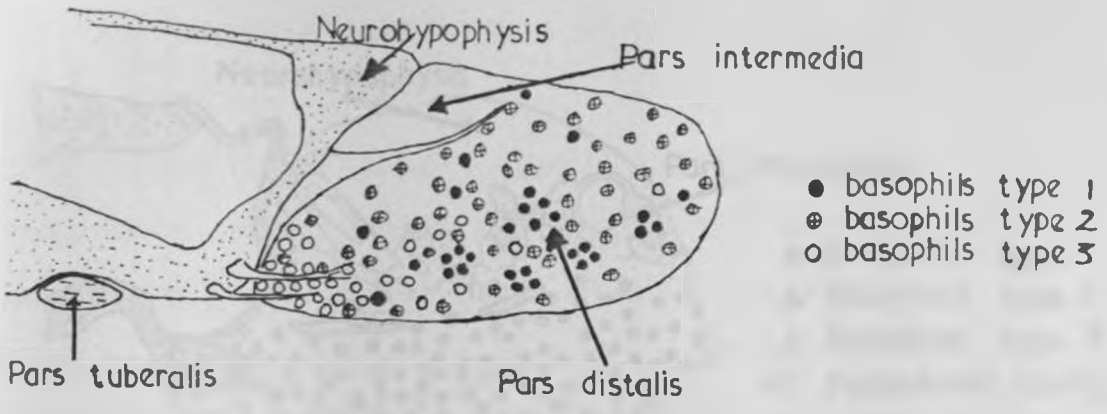


FIG. 5. Median section of an anuran pituitary showing the distribution of the basophil types in the pars distalis (from Kerr, 1965)

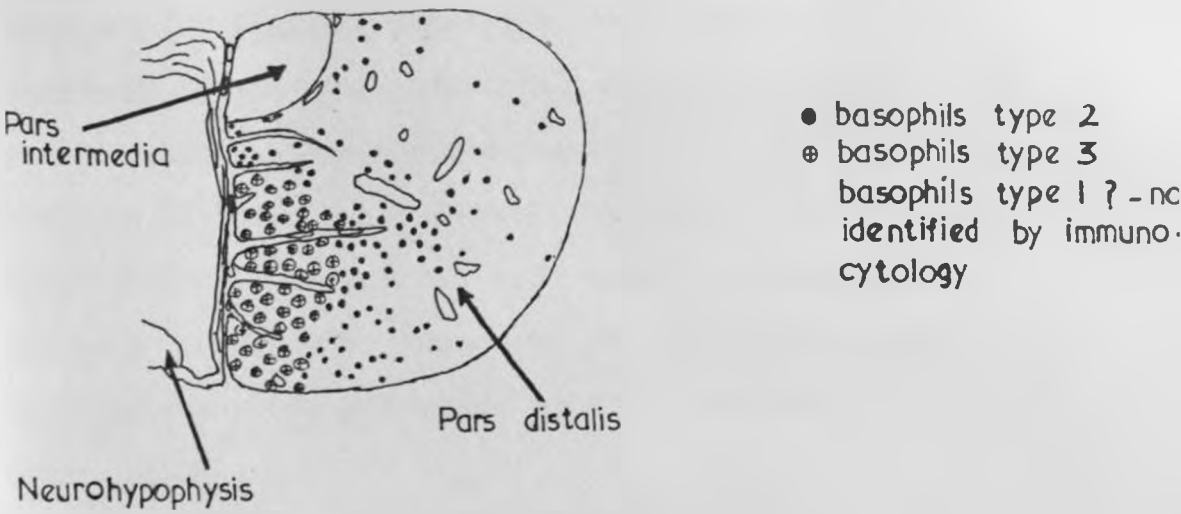


FIG. 6. Schematic representation of a median section of a urodele pituitary showing the distribution of the basophil types in the pars distalis (from Doerr-Schott, 1976)

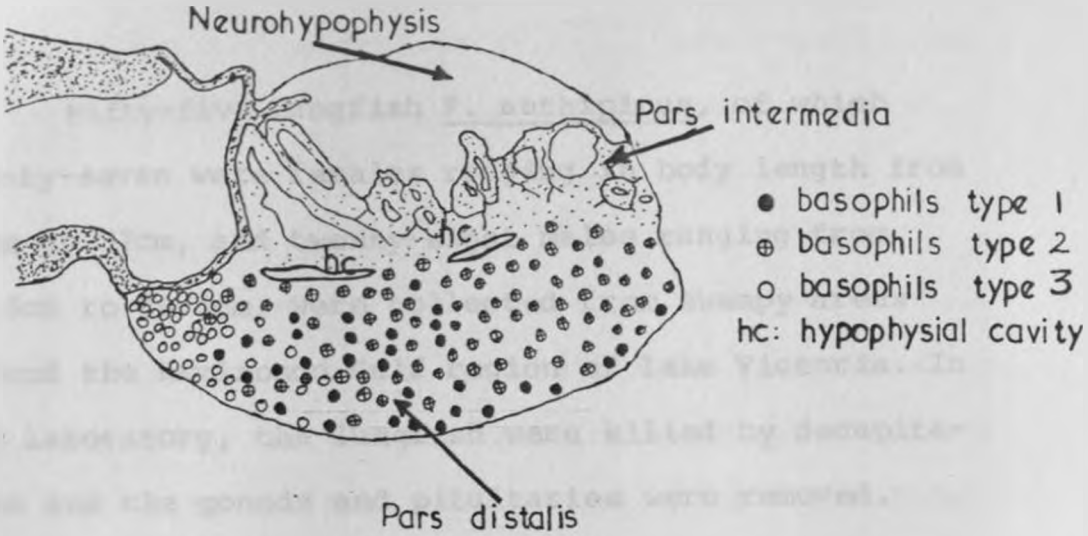


FIG. 7. Diagram of a sagittal section of an adult Protopterus pituitary (135cm fish) showing the distribution of the basophil types in the pars distalis (from Kerr and van Oordt, 1966)

3. MATERIALS AND METHOD

Fifty-five lungfish P. aethipicus, of which twenty-seven were females ranging in body length from 26cm to 57cm, and twenty-eight males ranging from 18.5cm to 66.5cm, were collected from swampy areas around the Kavirondo Gulf region of Lake Victoria. In the laboratory, the lungfish were killed by decapitation and the gonads and pituitaries were removed.

3.1. Light Microscopy

Since development was noted to be consistent throughout the length of the gonads, random portions of the tissue were fixed in Bouin's fixative. Pituitaries were fixed in Bouin's Hollande fixative (Humason, 1972). Routine dehydration in a graded series of alcohols, clearing and paraffin wax embedding, followed. Gonad specimens were sectioned transversely at 5-7μ thickness, stained with Ehrlich's haematoxylin and counterstained with Eosin. Sagittal sections of the pituitaries at 5μ thick, were stained with Alcian blue-Periodic acid-Schiff's reagent-Orange G (AB-PAS-OG) (Humason, 1972) in accordance with the staining procedure employed by Kerr and van Oordt (1966).

Mean diameter of the gametogenetic cells,

nuclei, nucleoli, yolk granules, inclusions, etc. was determined by the formula $\sqrt{M_1 \times M_2}\,\mu$ where $M_1\,\mu$ is the longest diameter and $M_2\,\mu$ is the shortest diameter.

3.2. Electron Microscopy

Tiny portions of gonadal tissue were fixed in Karnovsky's paraformaldehyde-glutaraldehyde fixative in phosphate buffer at 4°C (Hayat, 1970), postfixed in 1% osmic acid in phosphate buffer also at 4°C and rinsed in 0.85% of sodium chloride solution. Tissues were then dehydrated in a graded series of alcohols, cleared in propylene oxide and embedded in Epoxy Resin - Epon 812 (Griffin, 1972). Sections were cut in a Porter-Blum Ultramicrotome MT-1 at a thickness of 500Å to 750Å units and stained in a 3% solution of uranyl acetate, followed by Reynold's lead citrate solution (Griffin, 1972). Mature oocytes of P. aethiopicus were not included in the present study since their large size prevented rapid penetration and hence intact and satisfactory fixation by the E.M. fixative.

4. RESULTS

4.1.1. Histology of the Testis of P. aethiopicus

The testes are paired and each is surrounded by stromal tissue on its lateral and ventral sides (Figs. 8 and 11), while dorsally, fibres of connective tissue connect the testis with the renal tissue (Fig.11). Except for its ventral surface, the renal tissue is also surrounded by tissue which is histologically indistinguishable from the testicular stroma. A continuous fibrous tunica albuginea envelops the stroma of both the testis and the renal tissue. The histological appearance of the testis varies with its stage of maturation, and on this basis has been classified to five stages i.e. from the very immature Stage I to the post-spawning testis of Stage V. The various germ cell generations have been described in terms of cell and nuclear dimensions, morphology, nuclear inclusions and basophilic reaction or affinity of these cells.

Spermatogonia: These are large and spherical with a visible cell membrane. The cytoplasm is very pale and the nucleus is either lobed (Fig. 9) or spherical (Fig.13) with lightly basophilic nucleoplasm and deeply basophilic nucleoli. Mean diameter of spermatogonia measures as much as 24.6μ while that of the

spherical nucleus may measure upto 16.2μ.

Primary spermatocytes: These are produced from the mitotic divisions of spermatogonia and have a large nucleus ranging from 14.8μ to 16.0μ in mean diameter and containing a fine network of basophilic chromatin material. The surrounding cytoplasm is scant, very pale and can only be made out with reference to the faint cell membrane. Mean diameter of primary spermatocytes measures upto 19.6μ (Fig.15).

Secondary spermatocytes: After the first meiotic division of the primary spermatocytes, the secondary spermtocytes produced are smaller (upto 13.3μ in mean cell diameter) and also contain very little and pale cytoplasm. The nucleus initially has a mean diameter range of 9.3μ to 11.3μ and contains a condensed network of the chromatin material, thereby appearing more basophilic than the nucleus of primary spermatocytes. In later secondary spermatocytes, the nucleus becomes condensed homogeneously and extremely basophilic. Mean nuclear diameter of late secondary spermatocytes remains about 9.6μ (Fig.15).

Spermatids: These produced after the second meiotic division initially assume a blunt, spear-head shape, having maximum length and width of 13.4μ and 6.1μ respectively. During their differentiation into spermatozoa, a tiny head-piece adherent to the outer rounded edge, becomes visible and is faintly basophilic. It appears vaguely cuboidal (Figs.15 and 16).

Spermatozoa: Spermiogenesis i.e. the differentiation of spermatids into ripe or mature spermatozoa, takes place either in the lumina of the tubules or within the epithelium of the tubules. Mature spermatozoa, however, lie freely in the lumina with their head-piece still often discernible and the extended tail-piece having a narrow width of about 1.3μ and a length which may exceed 21.2μ (Fig.15).

Stage I: Very immature testis

Testicular tissue initially appears or forms along one side of the efferent duct and individual tubules are barely discernible (Fig. 8). Fibroblasts of the connective tissue occur along the periphery of the tubules. Their nuclei are basophilic and spindle-shape i.e. narrow and elongated, with coarse clumps of chromatin material within and having length and width ranges of 25.2μ to 27.8μ and 3.7μ to 4.9μ respectively. The cell membrane and cytoplasm of fibroblasts are non-staining. The only germ cell generation present in the very immature testis are the spermatogonia. Other cell types in the testis are mostly those with amorphous and spherical nuclei, which histologically resemble the fibroblasts in that they also contain clumps of basophilic chromatin material. Such cells appear to be differentiating fibroblasts and where discernible, especially with the

regular spherical forms, the mean diameter of the cell measures 21.5μ while that of the nucleus measures 15.9μ. Modified or differentiated fibroblasts also occur around the outer cell membrane of spermatogonia, closely following their contours and sometimes almost completely surrounding a spermatogonium. These cells probably act as supportive or "nurse cells" of the spermatogonia and therefore may be regarded as being homologous with Sertoli cells (Fig. 9).

The testicular stroma appears to be the source of the germ cells since it contains spermatogonia and mitotic or chromosomal figures (Fig.10).

Stage II: Immature testis

Testicular mass increases due to formation of tubules around the efferent duct (Fig.11). Although the tubules are still closely massed together, individual ones and their lumina are more apparent at this stage (Fig.12). Paralleling the increase in tubule formation, is their expansion and the increased occurrence of spermatogonia and the modified fibroblasts (Sertoli cells). The spindle-shaped fibroblasts characteristically occur along either side of the basement membrane of the tubules (Figs.12 and 13). The extent of the seminiferous epithelium or tubule epithelium (i.e. from the basement membrane to the

periphery of the lumen) may measure as much as 28.1μ (Fig.12).

Stage III: Maturing testis

At this stage, the testis becomes divided into lobes by the inner extensions of the fibrous connective tissue that occurs at the periphery of the testicular tissue. Within each lobe, most of the tubules are clearly separated from each other, with the spindle-shaped fibroblasts and some amorphous cells occupying some of the interstitial spaces between the tubules (Figs.14 and 15). At this stage too, the testis appears to be at the height of spermatogenetic activity since it is characterized by the presence of all the germ cell generations i.e. spermatogonia, primary and secondary spermatocytes, spermatids (Figs.15 and 16) and spermatozoa (Fig.15).

The tubule epithelium increases further to a maximum height of 95.0μ. During this stage, the lumina of the tubules remain narrow and contain sparse amounts of spermatozoa. Chromosomal figures are also characteristic of a maturing testis, indicating active spermatogenesis and representing transformation of spermatogonia into spermatocytes and spermatocytes into spermatids (Figs.15 and 17).

Due to the non-staining nature of cell membranes in the testicular tissue, spermatogenesis appears to be non-cystic i.e. each generation of germ cells does not appear to be contained within a cyst and limited by a cyst wall. However, the fact that each germ cell generation from primary spermatocytes to spermatids, does tend to occur in a cluster or group (Fig.15), may imply a synchronous development within such a group, similar to that which occurs in germ cells within a cyst in cystic spermatogenesis. Furthermore, with excessive histological staining, there does appear to be some suggestion of a cystic arrangement of the germ cells.

The Sertoli cells are usually located along the inner periphery of the tubules, of which the spherical forms are observed most frequently. Spermatogonia are not so frequently seen at this stage. Again, due to the non-staining nature of the cytoplasm and cell membranes, any cytoplasmic relationship that the Sertoli cells may have with the germ cells other than the spermatogonia, is not evident.

Stage IV: Mature testis - nearing spermiation

Most of the lumina of the tubules are filled with spermatozoa (Figs.18 and 19) such that their accumulation causes the tubules to expand, thereby

stretching the epithelium and reducing it to a maximum height of 59.3μ (Fig.20). While the late secondary spermatocytes occur in only a few tubules, spermatids differentiating into spermatozoa are more frequently seen in the lumina, suggesting active spermiogenesis. Often the epithelium contains mostly Sertoli cells and primary spermatocytes. As in the previous stage, only some of the interstitial areas are occupied by small groups of the amorphous cells and fibroblasts (Fig.19). These interstitial cells reveal no histomorphological variation or a variation in distribution or amount as from those of the previous stage.

Stage V: Post-spawning testis

Following spawning or spermiation, most of the tubules appear collapsed and contain sparse amounts of spermatozoa within their lumina (Figs.21 and 22). The presence of relatively more spermatozoa in some of the tubules may suggest that spermiation is partial and extended over a period of time. Prior to spermatogenetic recovery, the epithelium of spawned or evacuated tubules, has a maximum height of 29.5μ (Fig. 22).

The tubules contain mostly spermatogonia with associated Sertoli cells, primary spermatocytes,

spherical Sertoli cells and late secondary spermatocytes. Cystic arrangement of the latter is commonly seen (Fig.22).

HISTOLOGY OF THE TESTIS

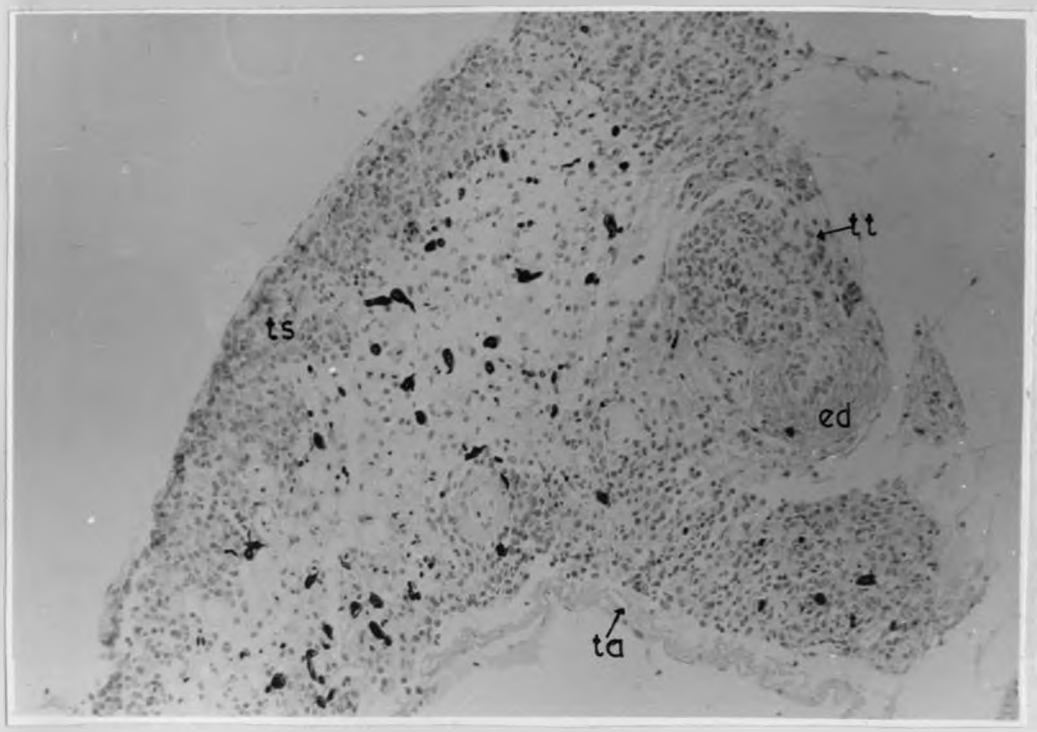


Fig. 8: Low power view of a very immature testis - Stage I. Testicular stroma (t.s.) surrounds the testicular tissue (t.t.) laterally and ventrally. e.d.: efferent duct; t.a.: tunica albuginea
Mag.x 239

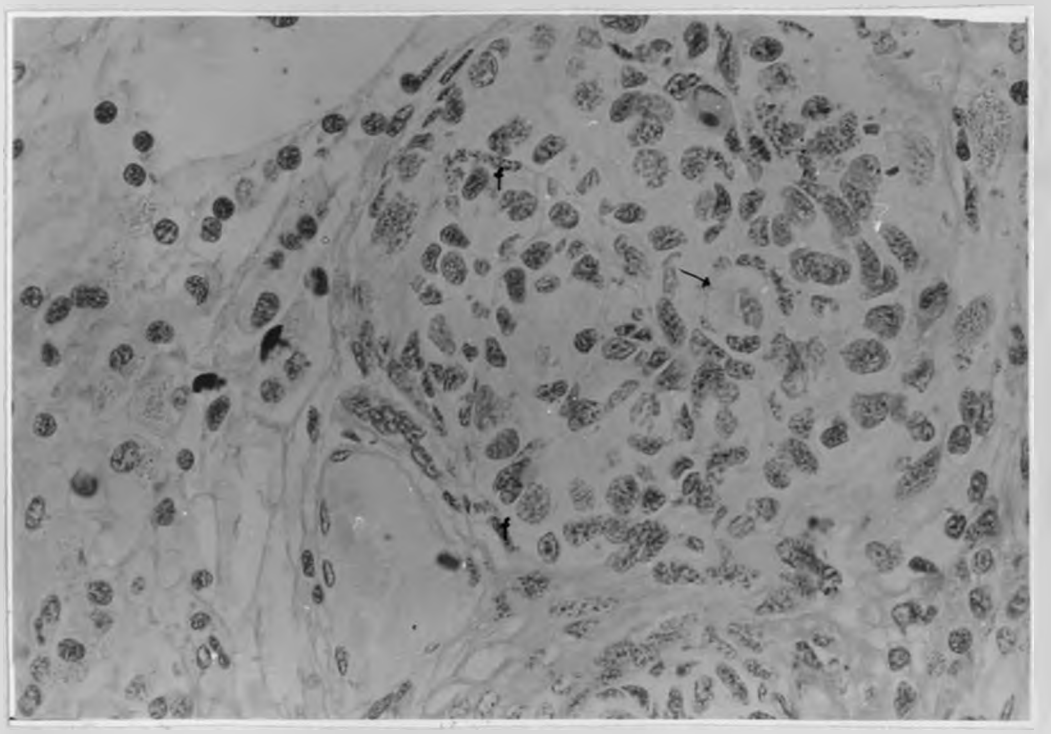


Fig. 9: Close up of the testicular tissue showing a spermatogonium with a lobed nucleus and surrounded by Sertoli cells (arrow). Fibroblasts (f) and other differentiating cells comprise the testicular tissue at this stage. Mag.X 950

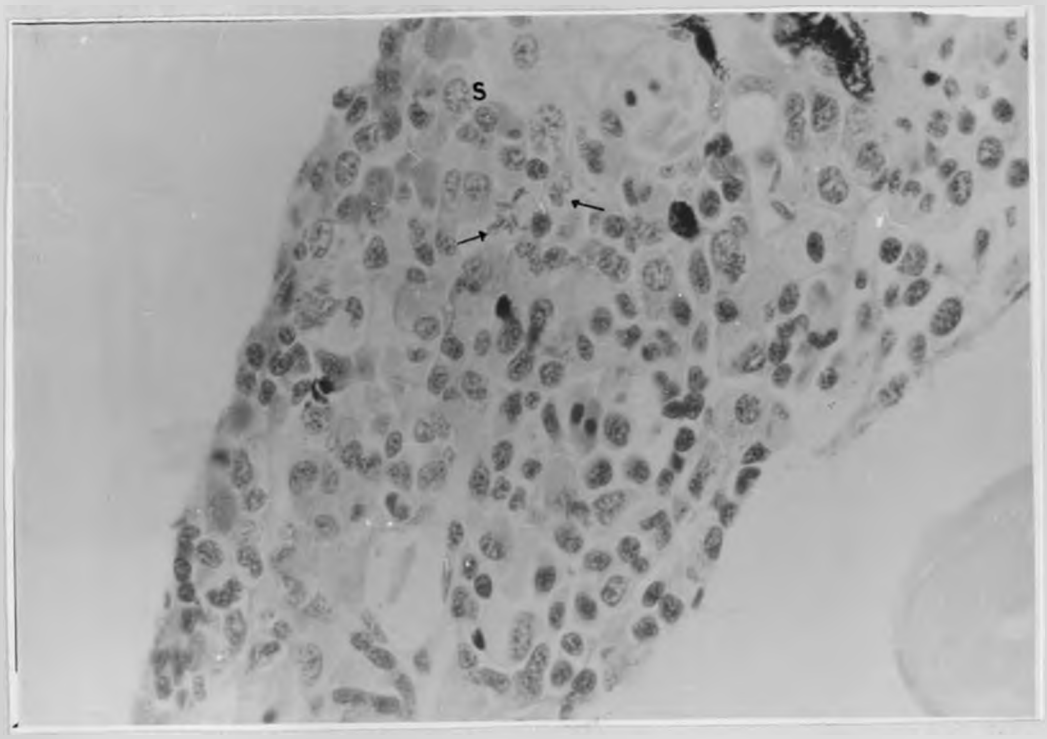


Fig.10: Testicular stroma of the very immature testis revealing spermatogonia (s) and mitotic or chromosomal figures (arrow) which probably represent dividing spermatogonia. Mag. X 950

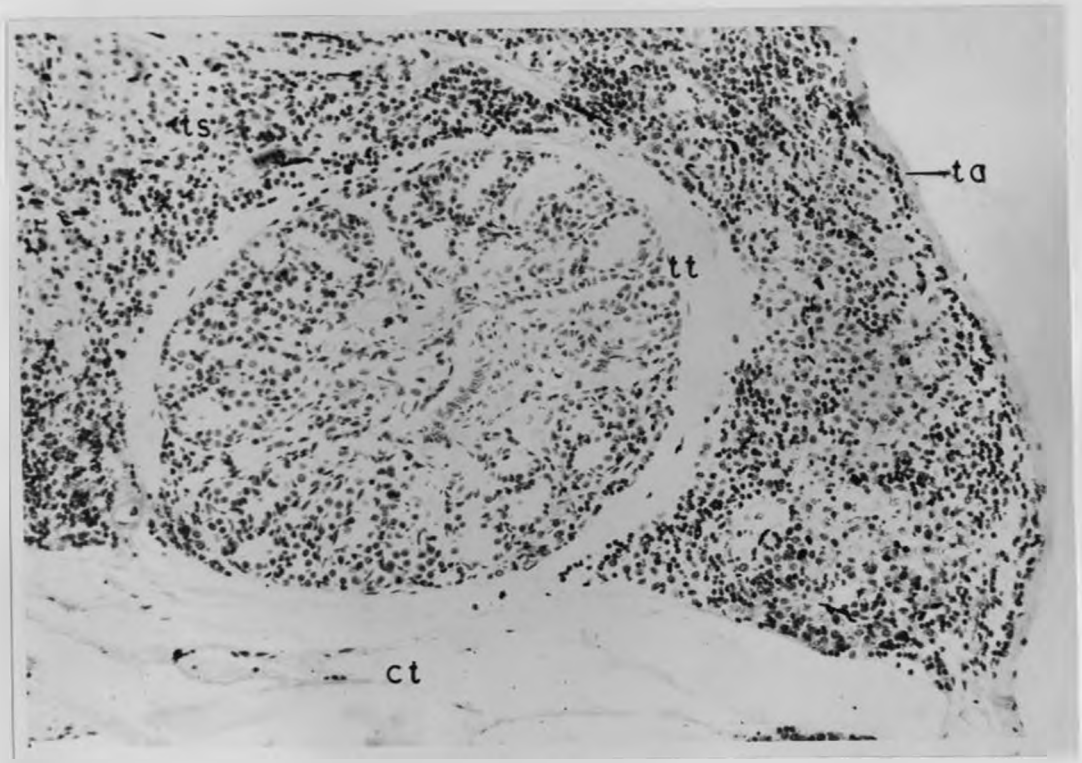


Fig.11: Low power view of the immature testis - Stage II, showing the increase in the amount of testicular tissue (tt). Testis is separated from the renal tissue by fibrous connective tissue (ct). ta: tunica albuginea; ts: testicular stroma. Mag.X 245.

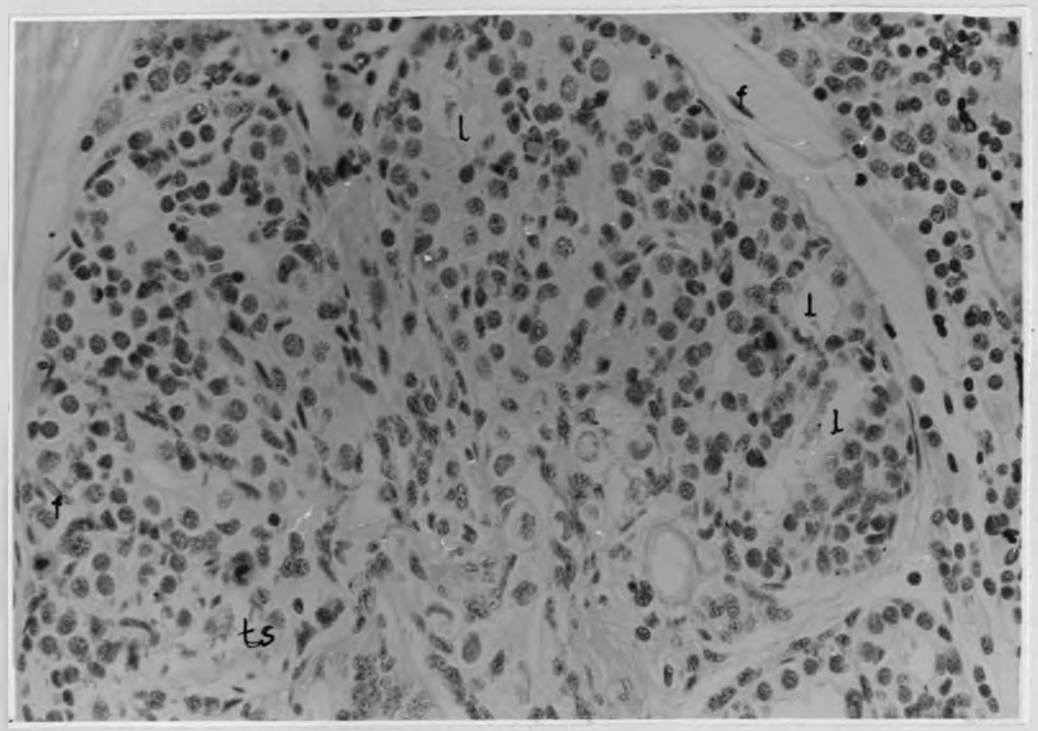


Fig.12: Testicular tissue of Stage II. Formation of some individual tubules and their lumina (l) is barely discernible. f: fibroblast; t.s.: testicular stroma. Mag. X 608

Fig.13: Spermatogonium (Sp) with a spherical nucleus and an associating Sertoli cell (S). d.f.: differentiating fibroblasts; f.:fibroblast. Mag. X 1520

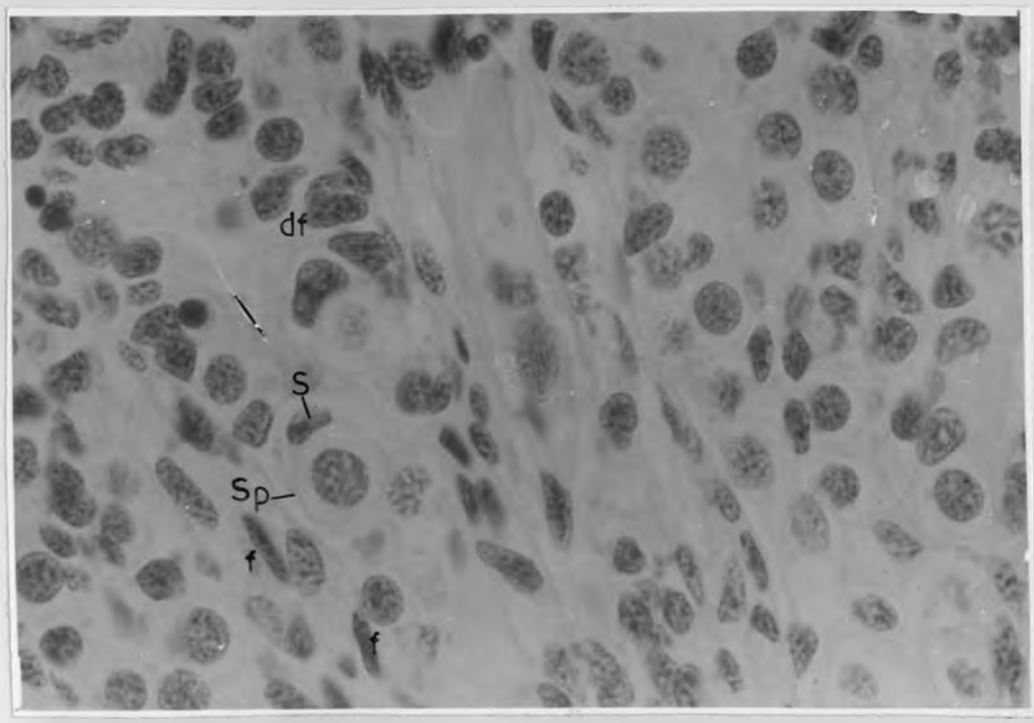


Fig.14: Low power view of a maturing testis - Stage III, showing the presence of several tubules within a lobe. Note scant interstitial tissue between the tubules. Mag. X168.

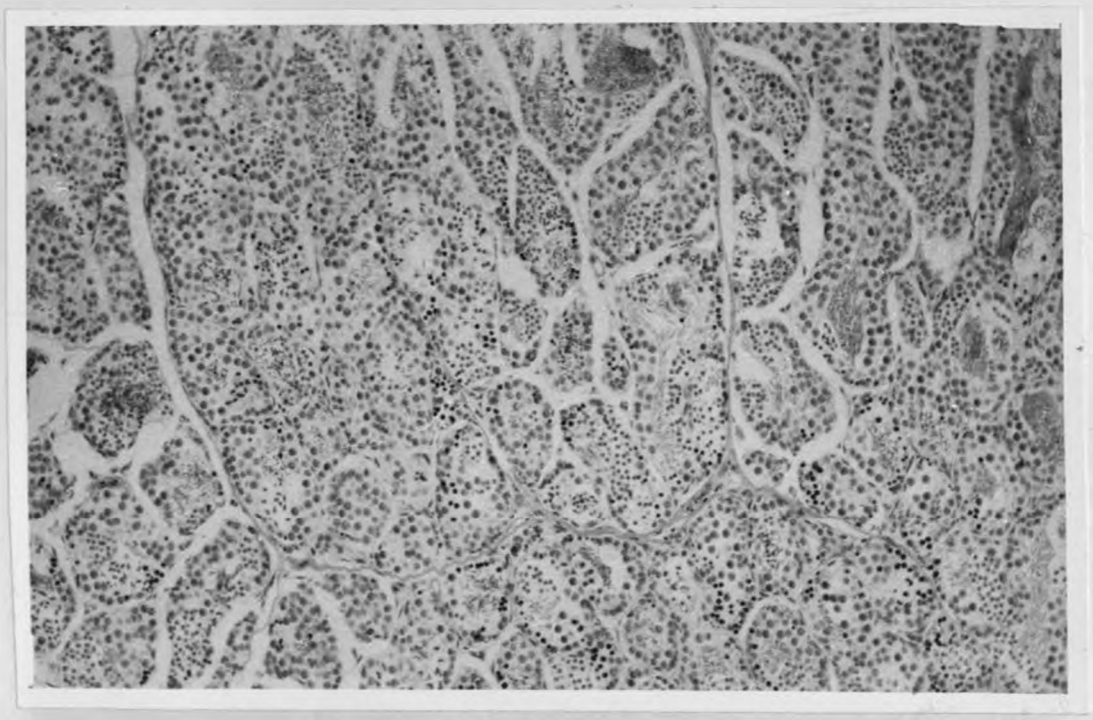


Fig.15: Presence of all spermatogenetic stages - primary spermatocytes (ps), early secondary spermatocytes (ess), late secondary spermatocytes (lss), spermatids (sp) and spermatozoa (sz). i.t.:interstitial tissue; m.f.: mitotic figures; f.: fibroblast; S.: Sertoli cell. Mag. X 950

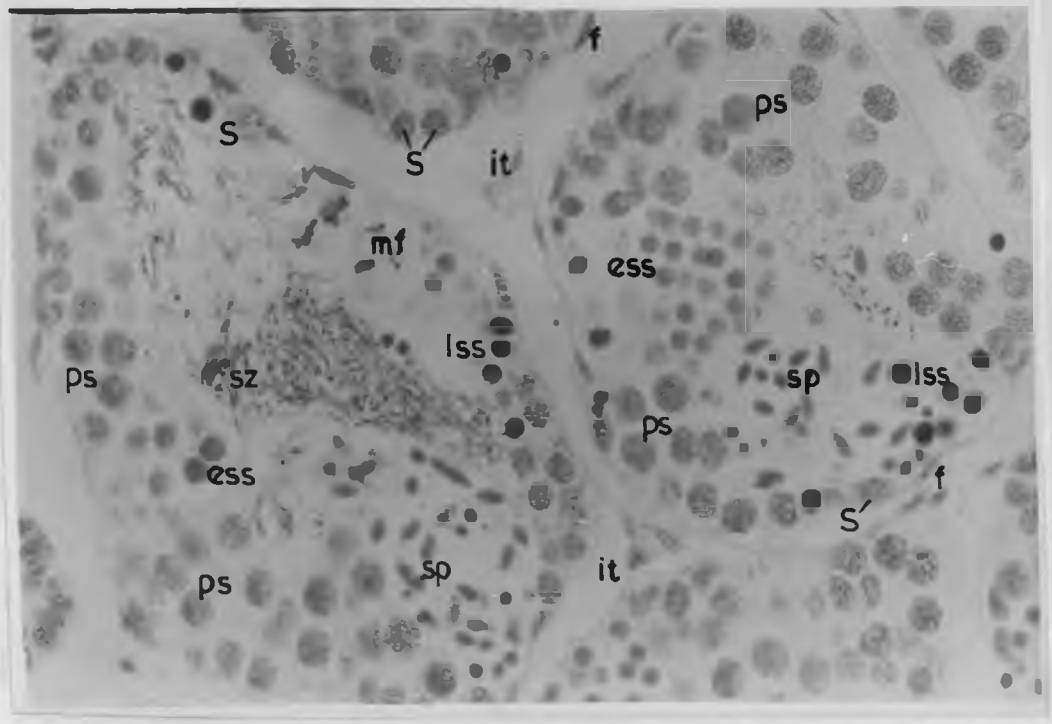


Fig.16: Spermatids (sp) differentiating into spermatozoa. Arrows indicate tiny head-piece. ps.: primary spermatocytes; ess: early secondary spermatocytes; lss:late secondary spermatocytes. Mag. X 1640

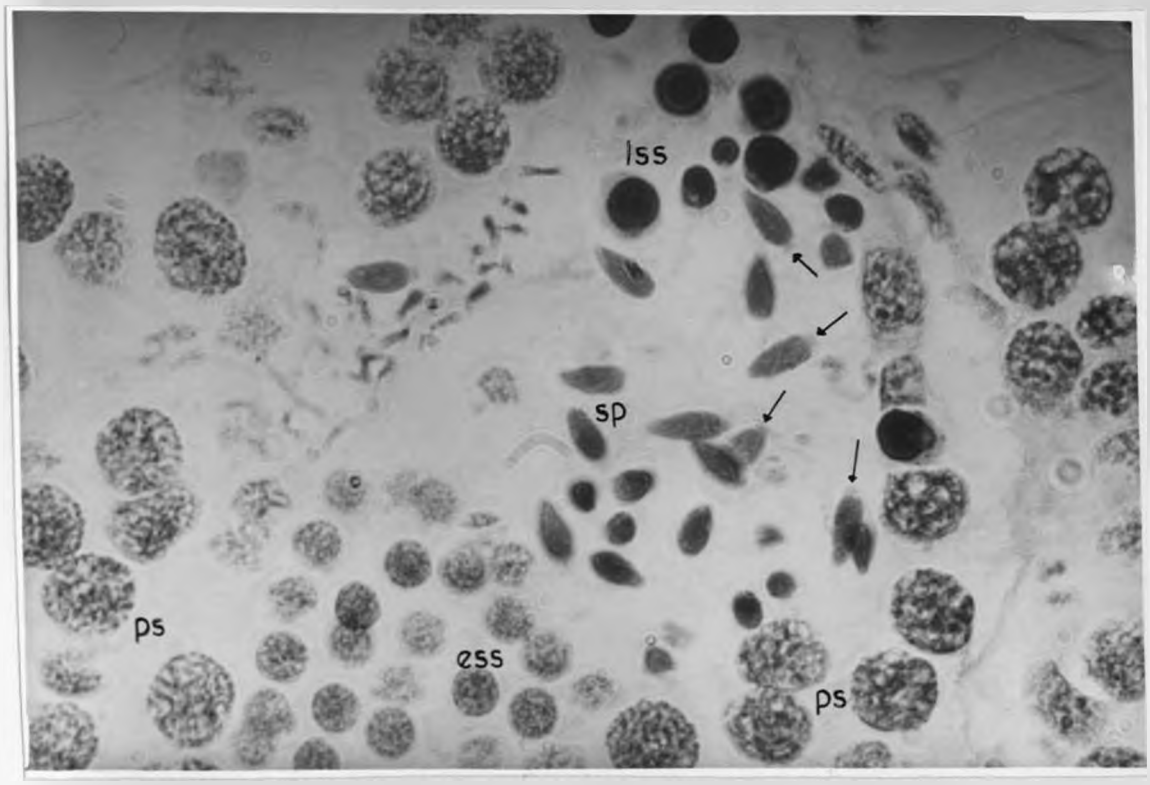
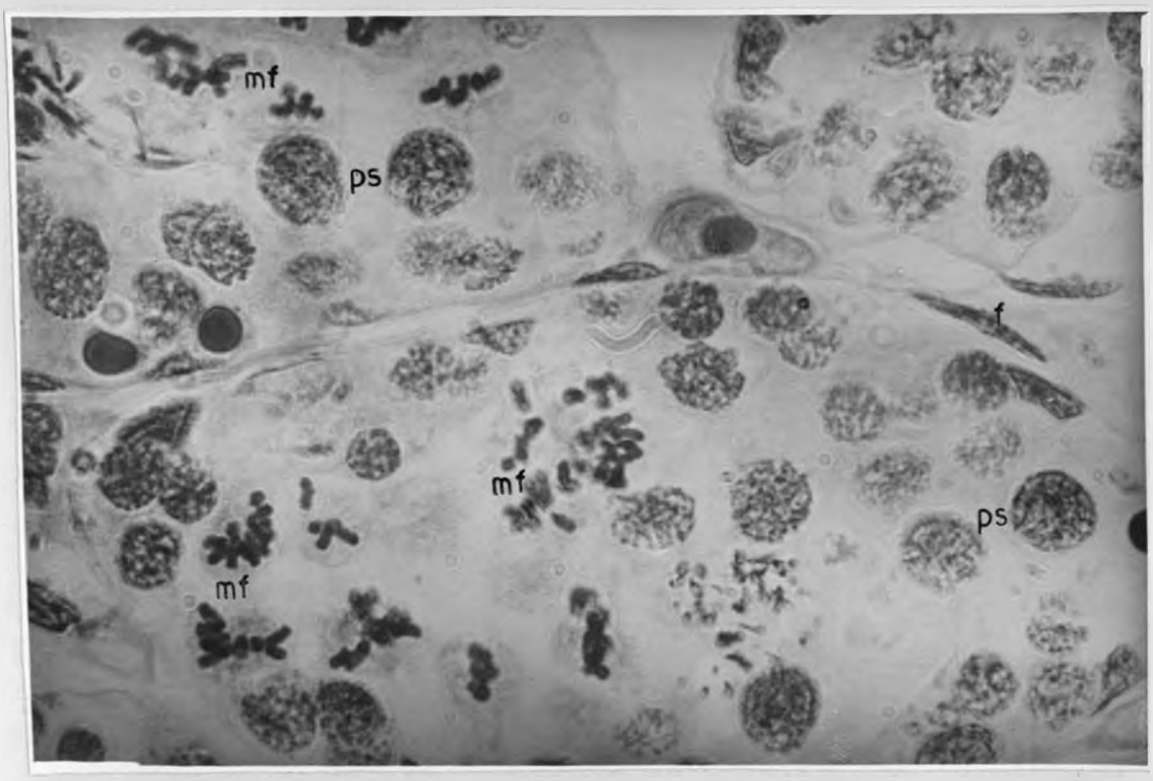


Fig.17: Mitotic figures (mf)- a common occurrence in the maturing testis of Stage III. f.:fibroblast; ps: primary spermatocyte. Mag. X 1640



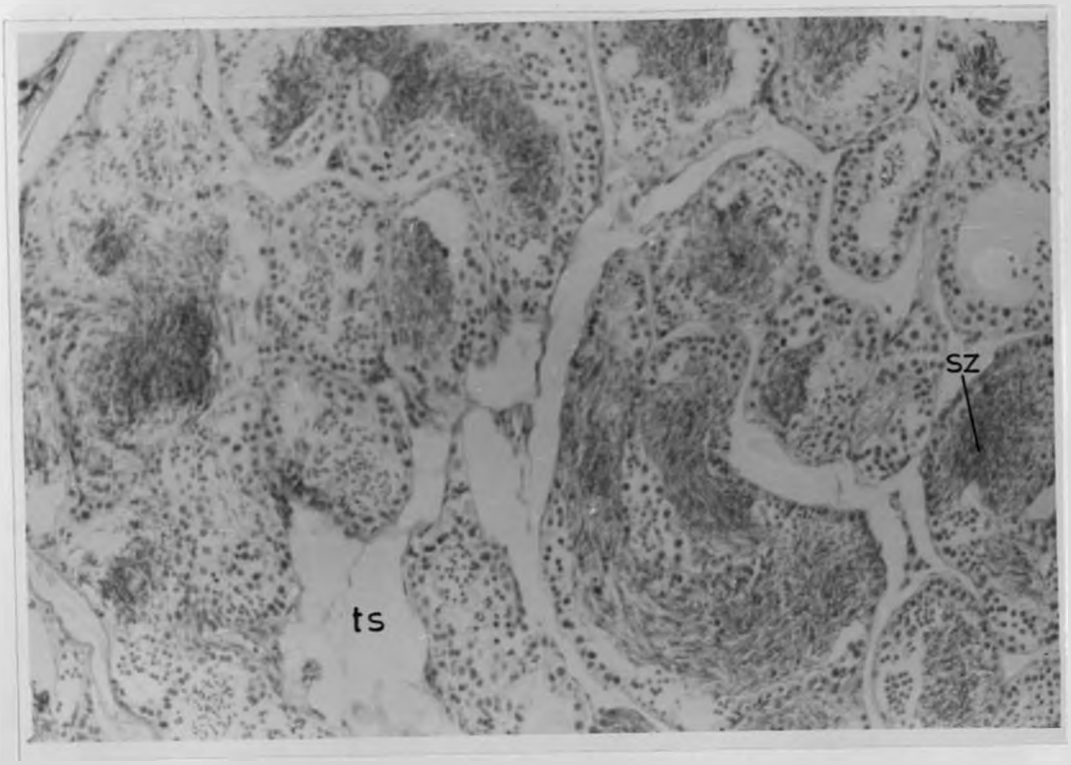
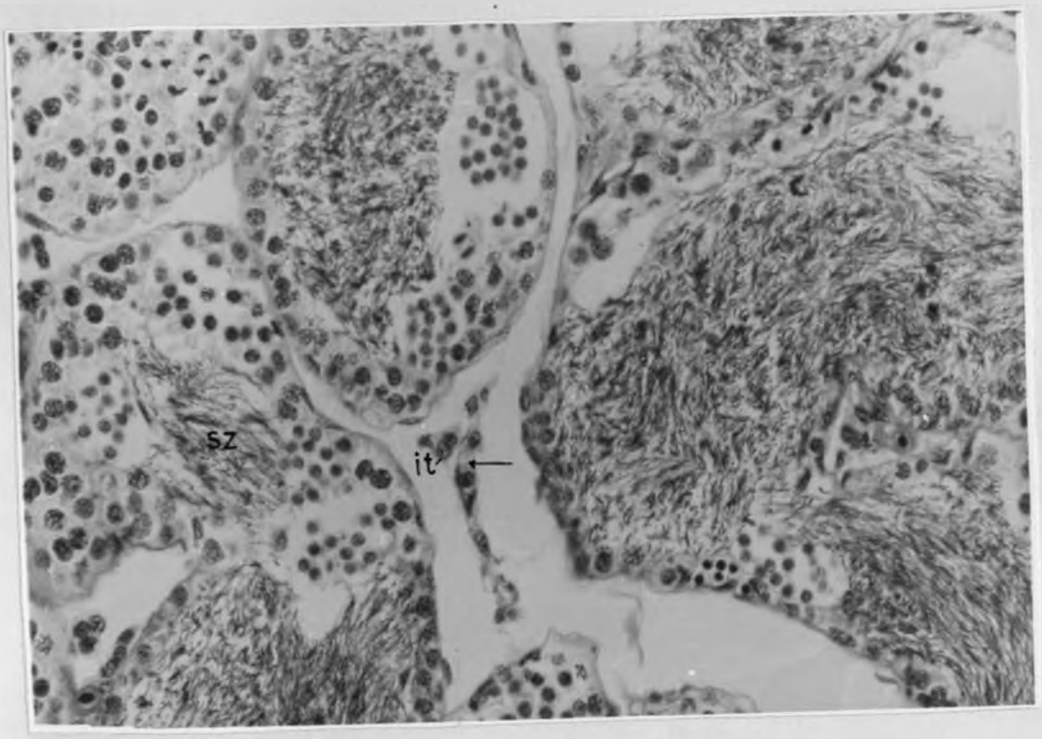


Fig.18: Low power view of a mature testis - Stage IV. Tubules are large and most are filled with spermatozoa (sz); ts: testicular stroma; Mag. X 241

Fig.19: Interstitial tissue (it) remains scant. What appears to be a large mass of interstitial tissue (arrow), is in fact a surface section of a tubule. sz: spermatozoa. Mag. X 608



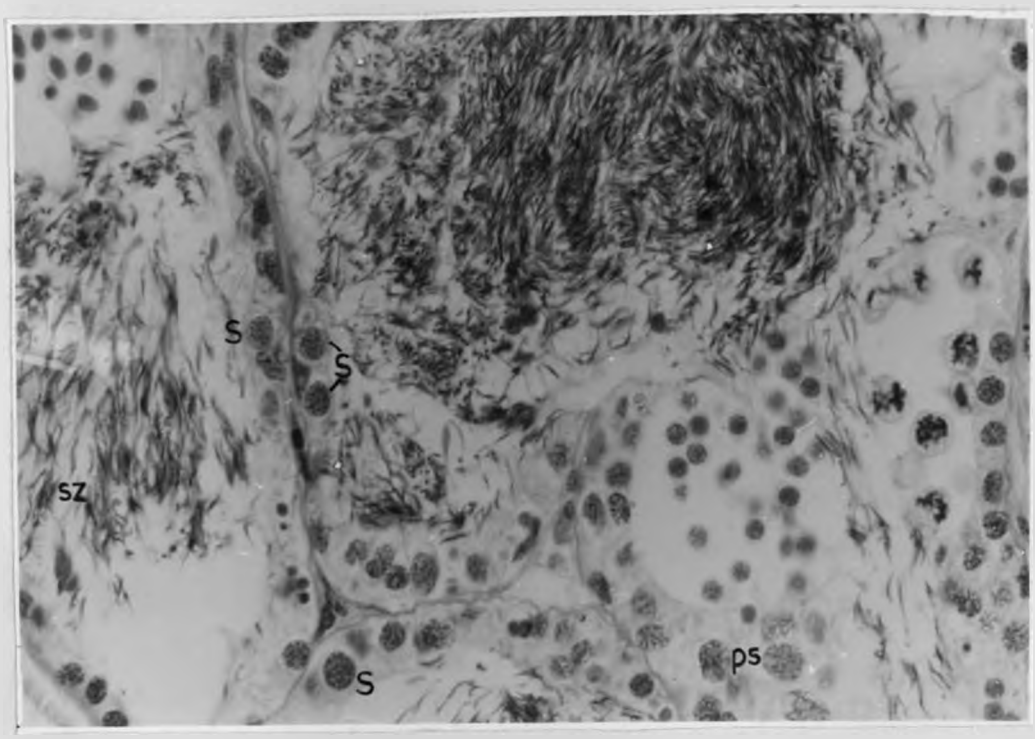
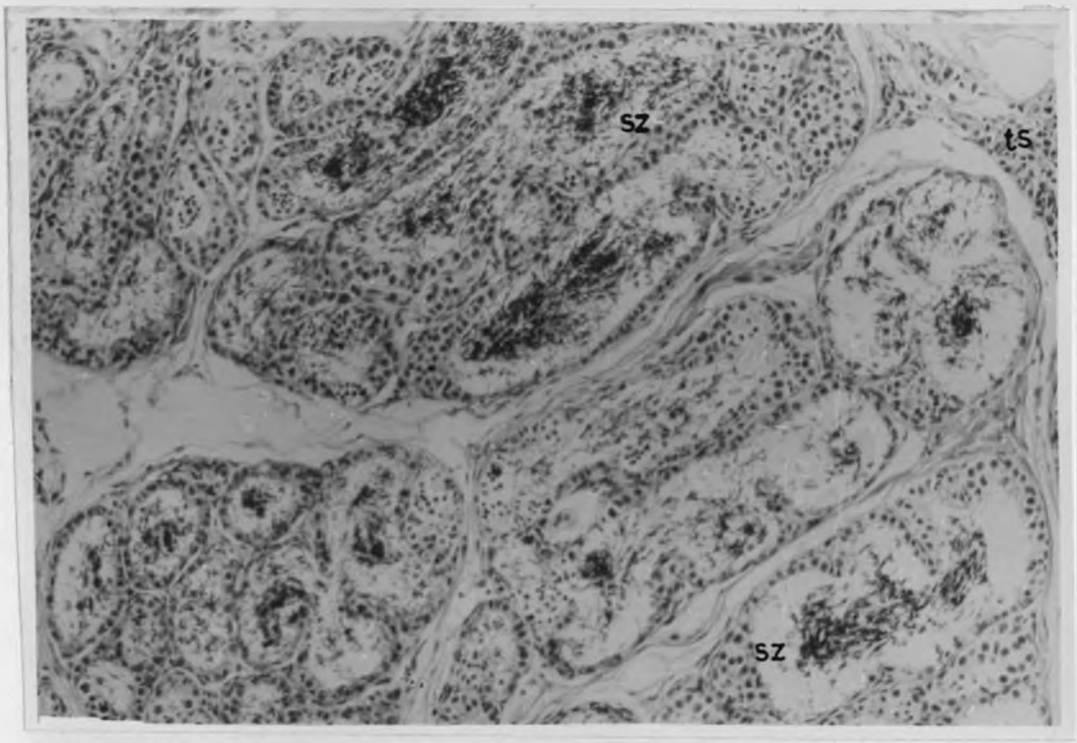


Fig.20: Epithelium of the tubules is reduced in height and contain mostly Sertoli cells (S) and primary spermatocytes (ps). sz: spermatozoa. Mag. X 950

Fig.21: Low power view of a post-spawning testis - Stage V. Most of the tubules appear collapsed and contain sparse amounts of spermatozoa (sz). ts; testicular stroma. Mag. X 241



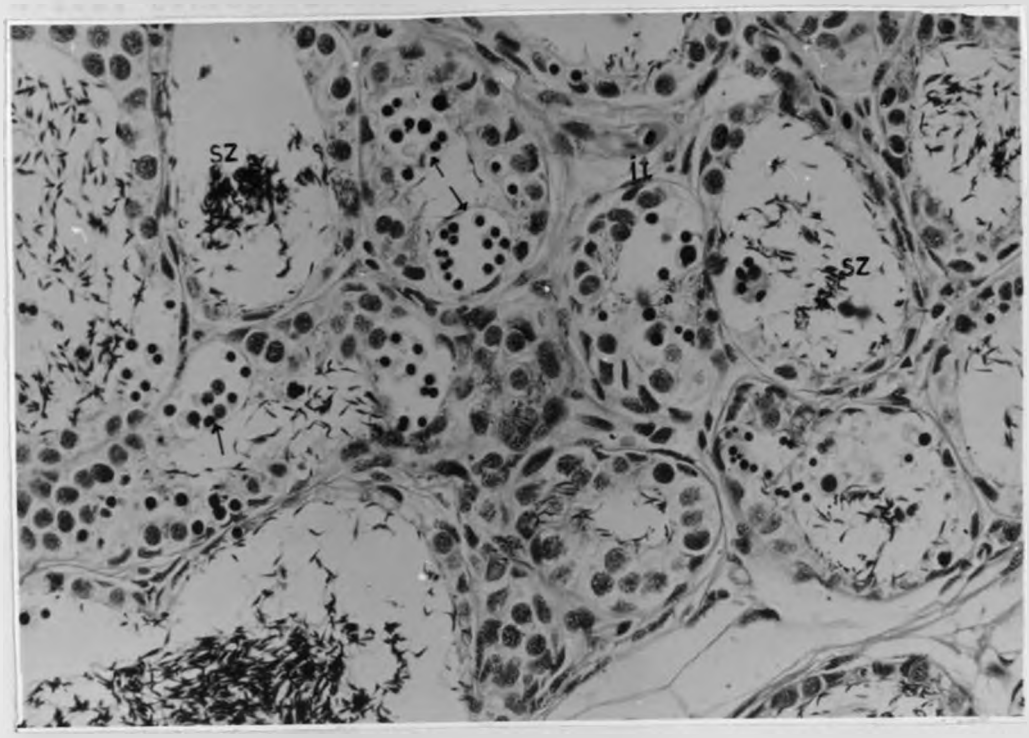


Fig.22: Collapsed tubules lying very close to each other and containing residual spermatozoa (sz). Cysts of late secondary spermatocytes (arrows) commonly occur. it: interstitial tissue. Mag. X 608

4.1.2. Ultrastructure of the Testis of P.aethiopicus

Since the cells in the interstitial areas of the testis of P. aethiopicus appeared to consist only of amorphous cells and spindle-shaped fibroblasts which showed no gross or observable variation in histomorphology with the different stages of testicular maturation, an ultrastructural study of these cells was undertaken in order to ascertain whether or not they possessed steroidogenic characteristics as did the interstitial Leydig cells of C. nigrofasciatum (Nicholls and Graham, 1972) and O. latipes (Gresik et al, 1973). Furthermore, since the Sertoli cells appeared to be modified fibroblasts, it was of interest to see the ultrastructural features of these cells, whether nutritive or steroidogenic.

The amorphous cells in the interstitial spaces and within the tubules of immature testes, have similar nuclear characteristics as the spindle-shaped fibroblasts (Fig.23). These include an uneven distribution of the coarse nuclear granules (ribosomes?) into areas of light and heavy density and such cells appear to be fibroblasts in the process of differentiation. Fibroblasts multiply or increase in number by fragmenting (Fig.23) and within the tubules form the Sertoli cells, of which the spherical forms seem to be

the most developed (Figs.24 and 25). In contrast to the spindle-shaped and amorphous fibroblasts, the spherical Sertoli cells have a considerable amount of cytoplasm which is granular (Figs.24 and 25), the granules resembling those of the nucleus (Fig.26). The nuclear pores are filled with a dense material that may represent the secretion of nuclear granules and other substances into the cytoplasm. Fine particles occur in the peri-nuclear spaces (Fig.26).

Granules are present in certain dilated inter-membranal spaces of the Sertoli cells (Figs.24 and 25) and appear to have been exuded from the cytoplasm into these spaces via a pore or rupture in the inner membrane layer. Such granules also appear to be secreted into the intercellular spaces via a rupture in the outer membrane layer (Fig.27). This feature may suggest a secretory activity of the Sertoli cell. Pinocytosis of granules also occurs at the Sertoli cell membrane (Fig.28) and accurate determination as to whether this process is exocytotic or endocytotic, must await further research techniques, employing the use of marker dyes.

The Sertoli cytoplasm contains several, large mitochondria which are oval or elongated in shape and have well-developed tubular cristae (Figs.25 and 26). Golgi complexes usually occur in the vicinity of

mitochondria (Figs.26 and 27) and the ends of the Golgi lamellae are visibly dilated and appear to have pinched off to form small vesicles. At higher magnifications, the lamellae reveal slight constrictions at intervals along their entire length (Fig.29), probably suggesting that the formation of vesicles is an almost simultaneous process along the Golgi lamellae. A fine, granular or particulate material occurs within the cisterns of the lamellae, the vesicles and in the surrounding cytoplasmic area (Figs. 26, 29 and 30) which may suggest a secretory activity of the Golgi complex. Some Golgi areas consist almost entirely of the pinched off vesicles (Fig.30).

The endoplasmic reticulum of the Sertoli cell is granular, cisternal and may be quite extensive (Figs.24 and 25). A fine particulate material occurs within the cisterns (Fig.31). Neither the Sertoli cells nor the amorphous fibroblasts show evidence of agranular, vesicular endoplasmic reticulum. Lipid droplets are more frequently seen in the cytoplasm of the amorphous, intratubular cells (Fig.23) whereas in the well-developed Sertoli cells, they are rare (Fig. 24). This appears to suggest that the Sertoli cells could be actively steroidogenic, resulting in no accumulation or storage of the precursor lipid material. On the other hand, the presence of several

lipid droplets in the amorphous fibroblasts perhaps suggests an inactivity of these cells, resulting in no steroid output and subsequently an accumulation of the lipid precursor material (Fig.23).

More commonly present in the cytoplasm of Sertoli cells are membranous organelles of varying morphology, some associated with a lipid droplet (Fig.25). The homogeneously electron-dense membrane-bound organelles, may be lysosomes (Fig.28).

Besides the presence of lipid droplets and lysosomes in the cytoplasm of the amorphous fibroblasts, portions of membranes usually occur which may suggest formation of cisternal endoplasmic reticulum. Also present in these undifferentiated cells are partially membrane-bound organelles with microtubule-like structures within them (Fig.32).

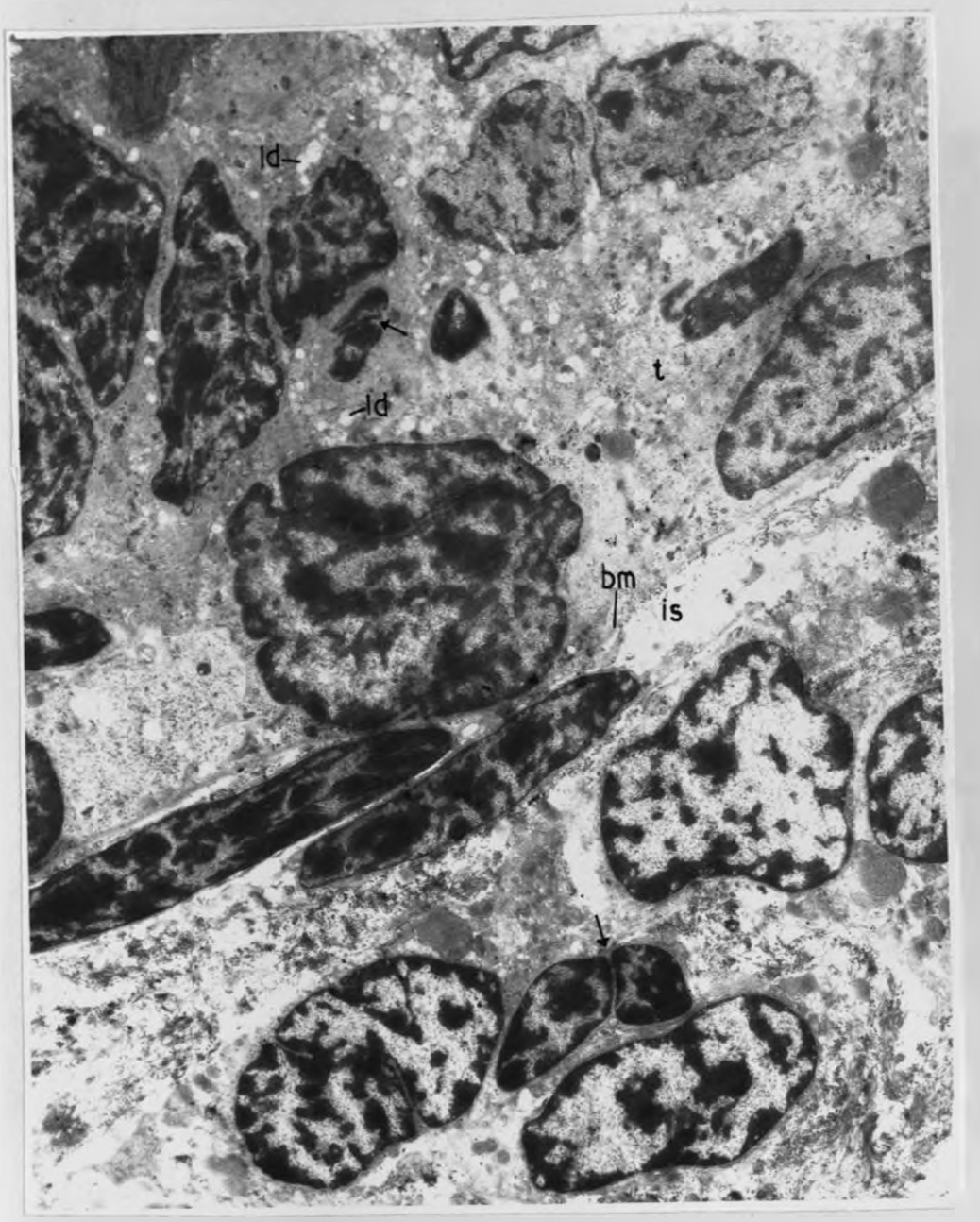


Fig.23: Spindle-shaped and amorphous (undifferentiated fibroblasts. Some of the latter appear to be fragmenting (arrows). bm: basement membrane; is;interstitial space; ld: lipid droplet; t:tubule. Mag. X 3400

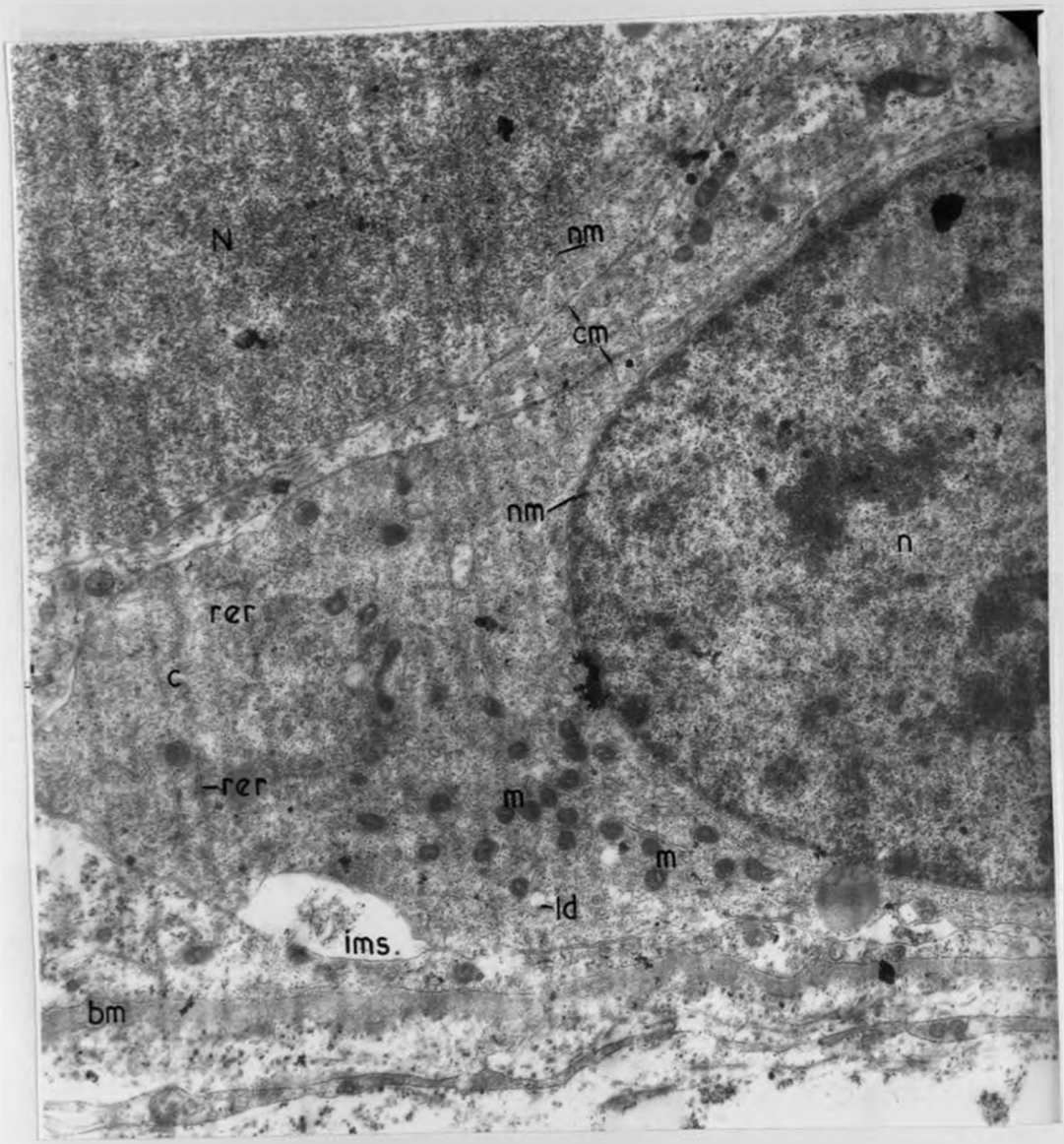


Fig.24: Developed Sertoli cell. Cytoplasm (c) is very granular, contains cisternal rough endoplasmic reticulum (rer), numerous mitochondria (m) and a few lipid droplets (ld). Nucleus (N) of primary spermtocyte is at upper left-hand corner. Note that nuclear membranes (nm) and cell membranes (cm) of both cell types are very pale. bm: basement membrane; ims:intermembranal space; n; nucleus of Sertoli cell. Mag. X 10,250.

Fig.25: Developed Sertoli cell. Lipid droplets are associated with membranous organelles (arrows). bm:basement membrane; ims; intermembranal space; n: nucleus; m: mitochondria; GC: Golgi complex; gc: granular cytoplasm. Mag. X 15,000.

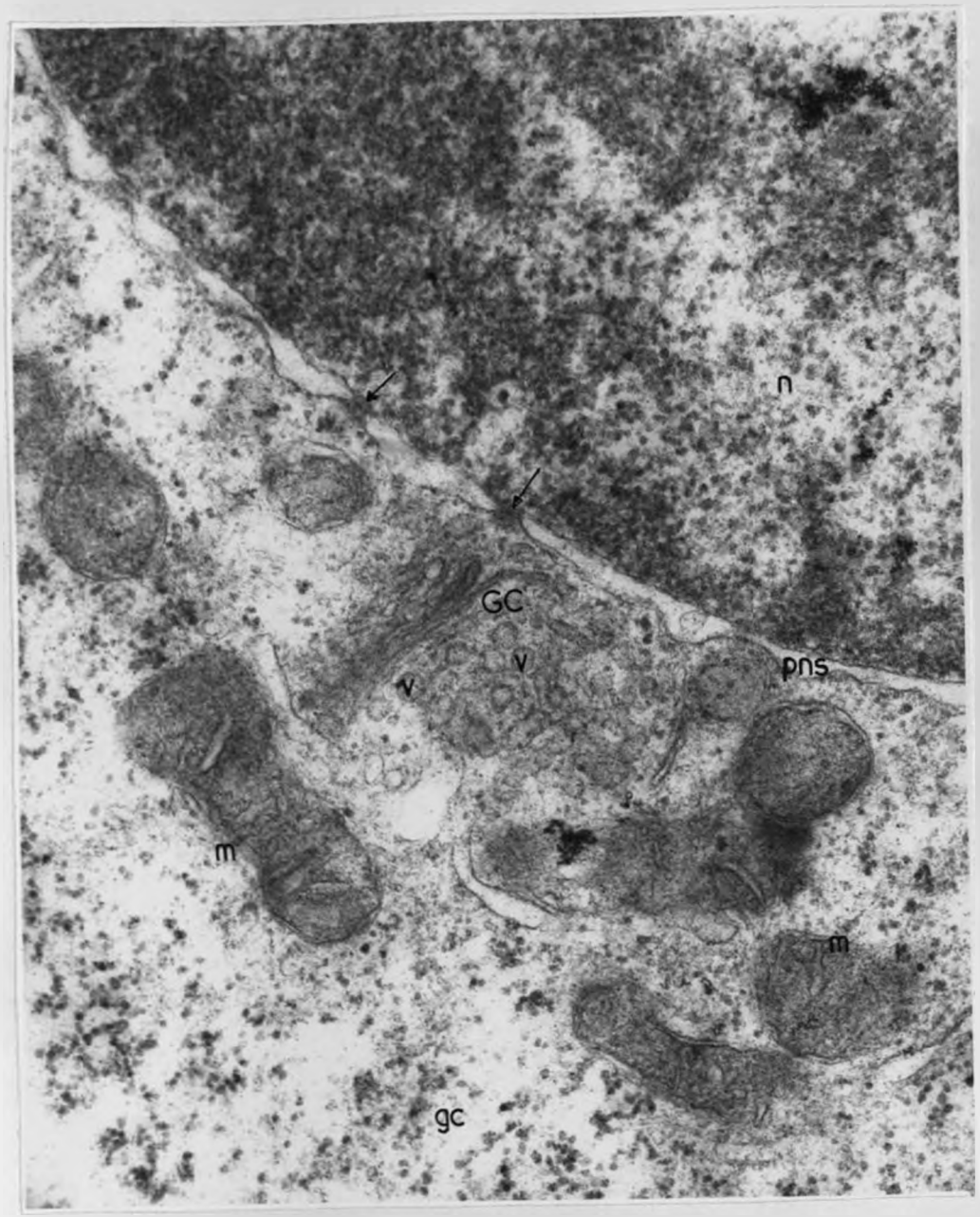


Fig.26: Nuclear pores of a Sertoli cell filled with a dense material (arrows). gc: granular cytoplasm; m: mitochondria; n: nucleus; GC: Golgi complex with vesicles (v); pns: peri-nuclear space.Mag. X 46,250

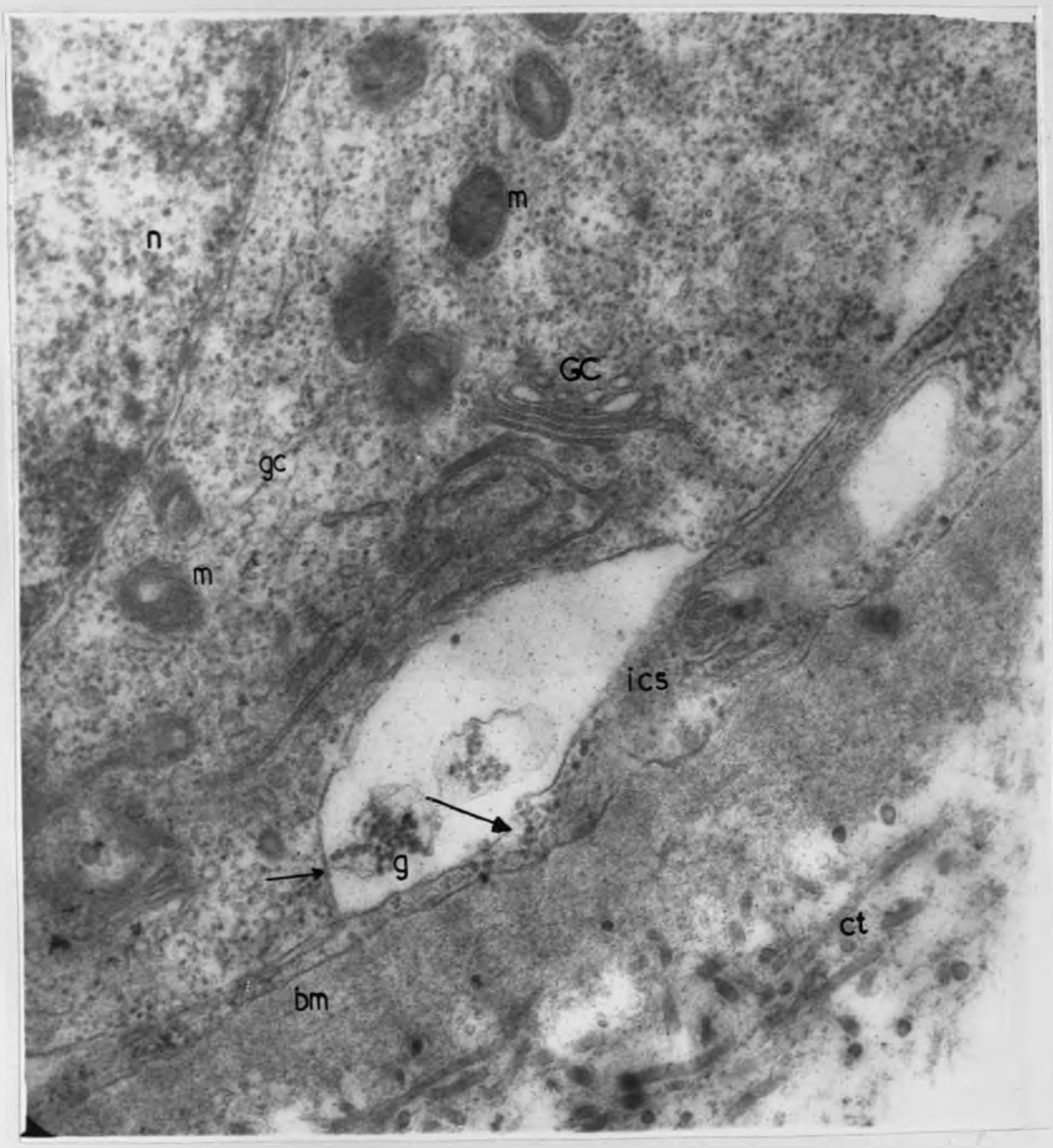


Fig.27:Granules (g) present in certain dilated intermembranal spaces, appear to have been exuded from the granular cytoplasm (gc) of the Sertoli cell via a pore in the inner membrane (arrow). These granules are also exuded into the intercellular space (ics) via a rupture in the outer membrane layer (long arrow).
bm: basement membrane; ct: fibres of connective tissue; GC: Golgi complex; m: mitochondria; n: nucleus.
Mag. X 38,600

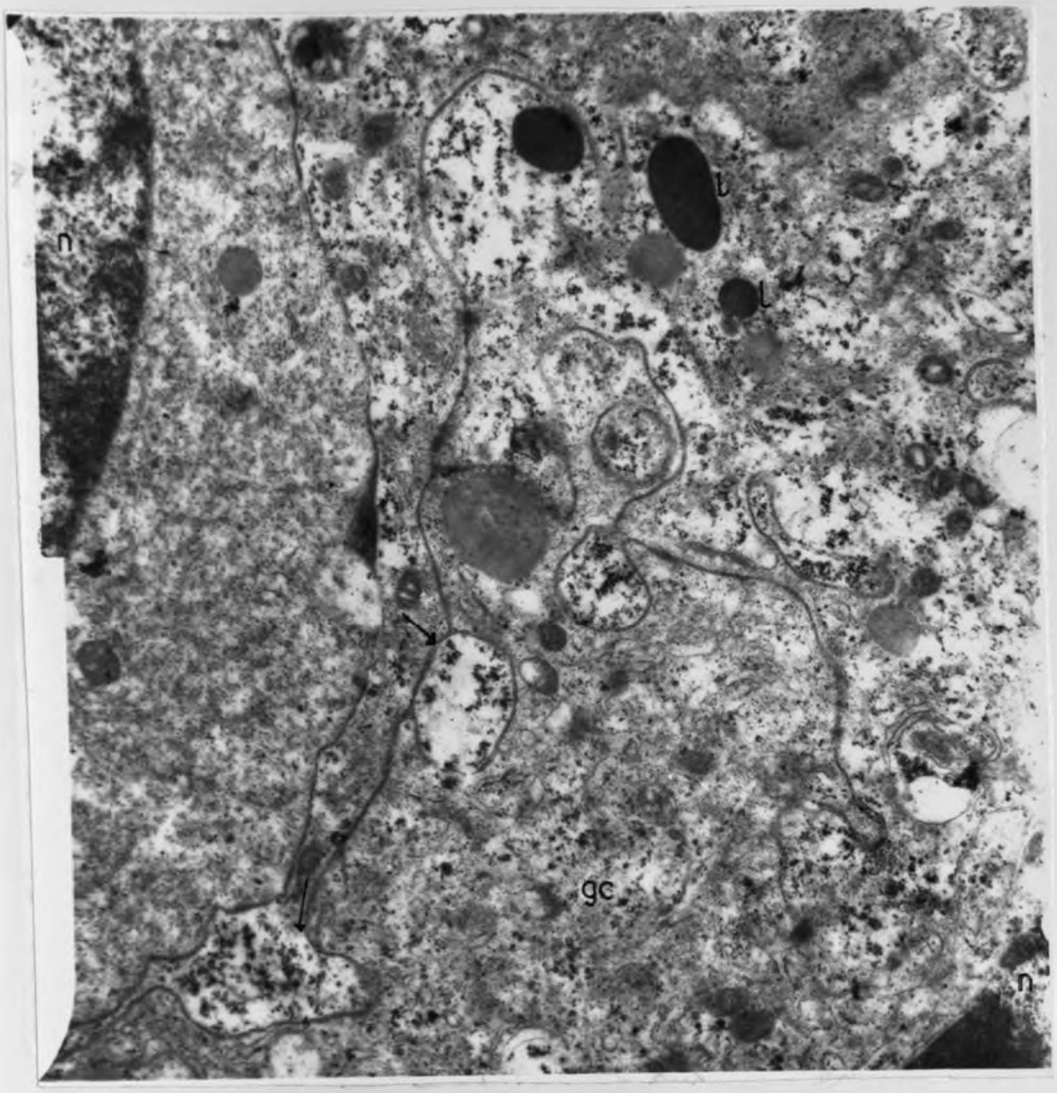


Fig.28: Pinocytosis of granules at the cell membrane of the Sertoli cell (arrows). gc: granular cytoplasm; l: lysosomes; n: nucleus. Mag. X 14,350.

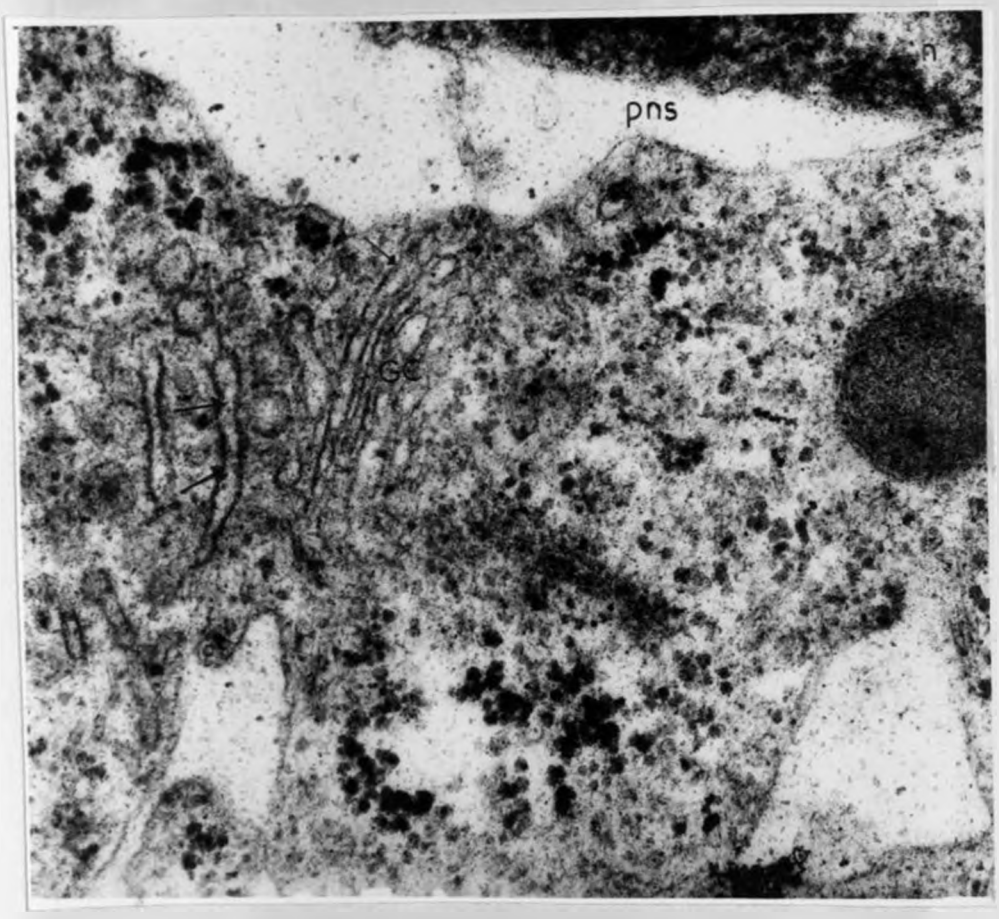


Fig.29: Golgi complex (GC) in the cytoplasm of a Sertoli cell showing slight constrictions along the length of the lamellae (arrows) and formation of vesicles. n: nucleus; pns: peri-nuclear space. Mag. X 62,500

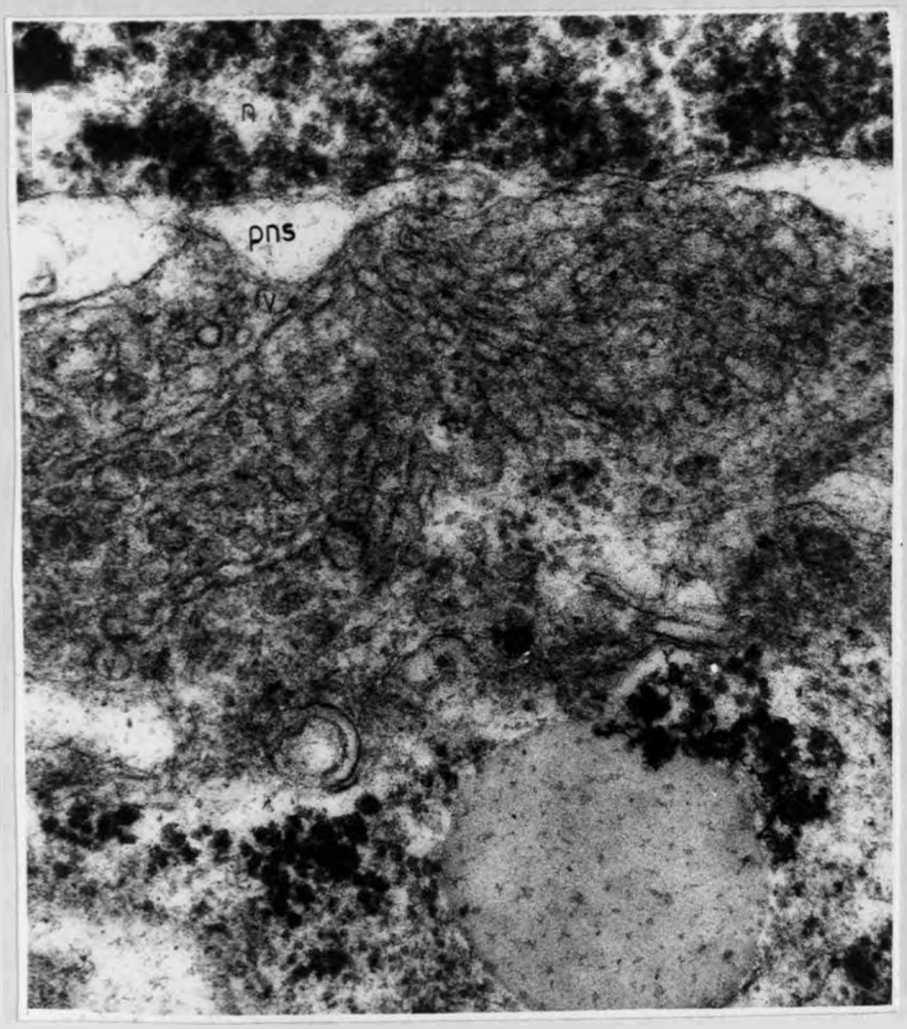


Fig.30: A Golgi area consisting almost entirely of the pinched off vesicles (v). n: nucleus; pns: perinuclear space. Mag. X 58,800.

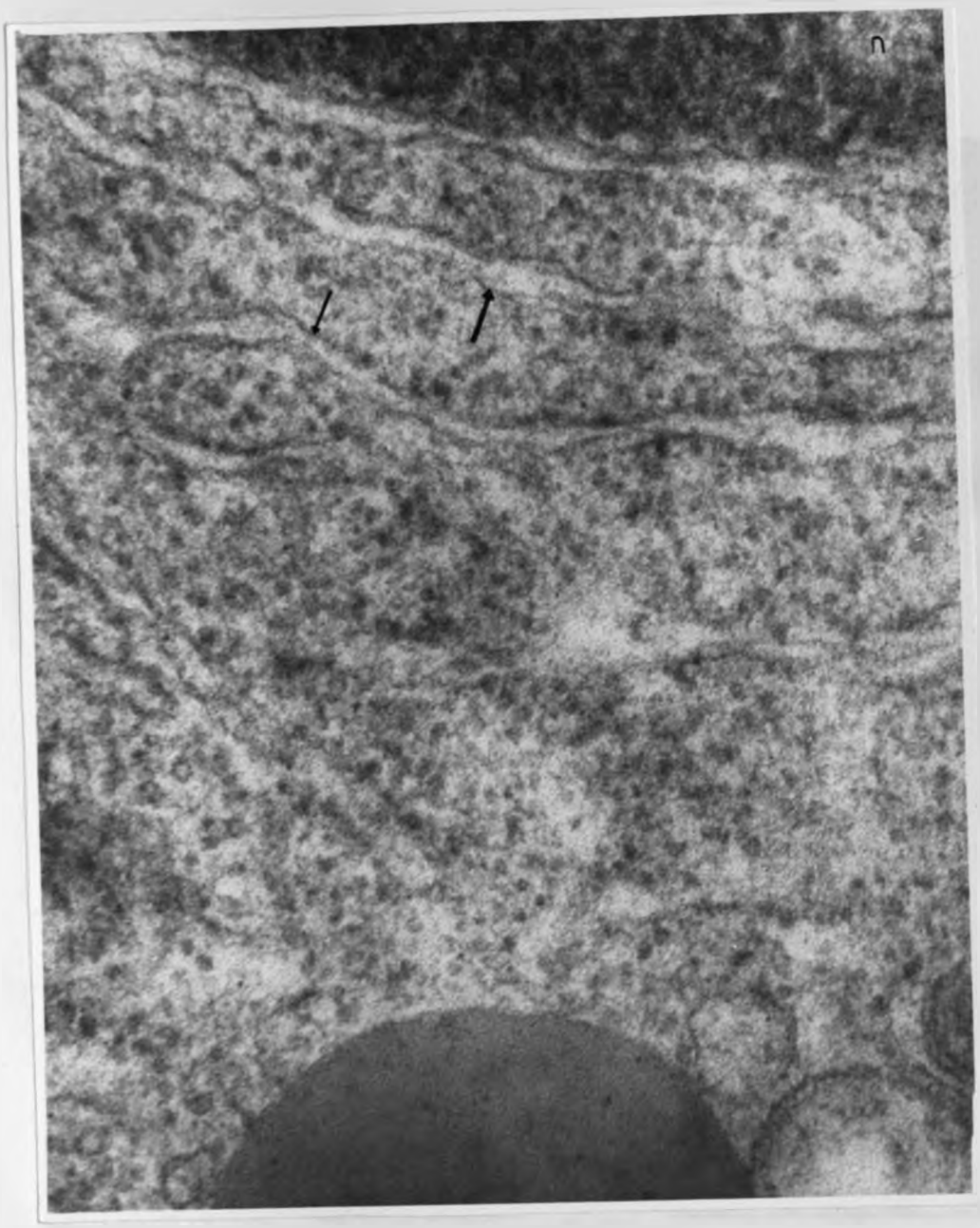


Fig.31: Cisterns of rough endoplasmic reticulum (arrows) containing fine, granular material within. n: nucleus of Sertoli cell. Mag. X 142,800.

Fig.32: Cytoplasm of an amorphous cell or undifferentiated fibroblast revealing portions of membranes or cisternal reticulum (arrows) and a partially membrane-bound organelle (o) within which are microtubule-like structures. n: nucleus. Mag. X 64,000.

4.2.1. Histology of the Ovary of P. aethiopicus

The ovary of the lungfish P. aethiopicus exists in the gymnovarian condition i.e. a "naked" or "open" ovary, with the oocytes aggregating into lobes and held together within a meshwork of fibrous connective tissue of stromal origin.

Oocyte development in individual lobes is asynchronous i.e. each lobe contains oocytes at more than one stage of development or maturation. Associated with the fibrous connective tissue are fibroblasts with deeply basophilic and granular nuclei, similar cells also occurring at the periphery of the oocytes, in which case they are referred to as follicle cells. The cytoplasm and cell membrane of follicle cells are very pale and therefore not discernible with light microscopy. The actual three-dimensional appearance of follicle cells or their nuclei appears to be coin or discus-shaped, with only the diameter being measurable in sagittal section. In transverse section however, the follicle cell appears narrow and elongated or cuboidal, depending on the stage of oocyte maturation, and both the thickness or height and the diameter can be determined.

Oocyte development has been classified broadly

into two phases - Protoplasmic and Vitellogenic. The first phase incorporates the early and late protoplasmic stages while the second phase includes the yolk stages and describes the pattern of yolk granule accumulation. For all stages, the characteristics of the oocyte are cited i.e. cell and nuclear morphology, dimensions, inclusions (including perinuclear nucleoli size, morphology and tincture), cytoplasmic tincture, thickness of the zona pellucida - where measurable, and dimensions of the surrounding follicle cells.

Oogonia: These precursors of oocytes occur in the ovarian stroma and have a basophilic, lobed nucleus. These cells are spherical, have very pale cytoplasm and cell membrane and a mean diameter of 21.5µ.

Pre-protoplasmic oocytes: These cells usually occur at the periphery of the lobes (Fig.33). They have a mean cell diameter of 45.4µ, are roughly spherical to oval cells with their cytoplasm and cell membrane being very faint. The large, centrally located nucleus of mean diameter 25.3µ, has deeply basophilic chromatin material and nuclear membrane. At this early oocyte stage, a few follicle cells are present at the outer cell membrane or oolemma (Fig.33). Pre-protoplasmic oocytes grow further, acquiring mean cell and nuclear diameters of 72.8µ and 37.1µ respectively before their cytoplasm adopts the basophilic

staining affinity (Fig.34). A maximum of two, deeply basophilic nucleoli which measure upto 4.3μ in mean diameter, have been observed in the nuclei of these oocytes and characteristically positioned at the inner edge of the nuclear membrane (Fig.34).

I. PROTOPLASMIC PHASE

1a. Early protoplasmic stage - dark staining oocytes

These somewhat roughly spherical to oval shaped oocytes have deeply basophilic ooplasm. The spherically-shaped nucleus is usually centrally located and strands of chromatin material and several amorphous nucleoli are visible within the lightly basophilic nucleoplasm. The amorphous nucleoli are usually acidophilic and appear to fragment and form tiny spherical nucleoli which position themselves at the inner edge of the nuclear membrane (Fig.35). These peri-nuclear nucleoli or peripheral nucleoli, when first discernible, measure only 1.0μ in mean diameter. Larger, lightly basophilic nucleoli of upto 12.0μ in mean diameter, also occur within the nucleus. Within each, there is usually a single, spherical sub-nucleolus which is extremely basophilic and measures 3.9μ in mean diameter (Fig.36). It is positioned either at the inner edge of the large nucleolus or half-way over its edge. It appears that the

sub-nucleolus is a product of the larger nucleolus and is eventually extruded into the nucleoplasm.

In the ooplasm, a single, spherical yolk nucleus is often observed. This has a duplex structure consisting of a basophilic or acidophilic cortical region and a very pale central region (Fig. 37). It is usually surrounded by a clear, non-staining area or vacuole. A yolk nucleus may measure upto 12.0μ in mean diameter and since they have only been observed in oocytes of this stage and not in later ones, it is suggested that towards the end of the early protoplasmic stage, they disintergrate and are reabsorbed into the ooplasm.

The few follicle cells at the outer oolemma are spindle-shape (long and narrow), having a mean length (diameter) and height of 22.2μ and 2.4μ respectively. Early protoplasmic oocytes grow further acquiring a mean cell diameter of 385.0μ and mean nuclear diameter of 119.0μ.

1b. Mottled vacuolar stage - cytoplasmic vacuoles

In the larger, darkly staining protoplasmic oocytes of mean cell diameter 207.0μ and mean nuclear diameter of 101.0μ, vacuoles appear in the ooplasm, giving it a reticulated appearance (Fig.38). These

vacuoles ease out by the time the oocytes attain their maximum dimensions and may represent lipid inclusions which have dissolved out of the ooplasm during the histological processes employed in this study.

The zona pellucida of early protoplasmic oocytes is extremely thin and is therefore not accurately measurable.

2a. Late protoplasmic stage - light staining oocytes

As the early protoplasmic oocytes grow beyond 385.0μ, their ooplasm gradually becomes weakly basophilic such that towards the end of this stage, when the oocytes have attained a maximum mean cell diameter of 1286.9μ and a mean nuclear diameter of 367.8μ, the ooplasm is very pale (Fig.39). Strands of chromatin material looking like "lampbrush" chromosomes, are easily discernible within the lightly basophilic nucleoplasm (Fig.40).

The peripheral nucleoli aggregate, thus becoming fewer but larger. Newly formed aggregates tend to be irregular in shape, but the larger, fully formed spherical peripheral nucleoli may be as large as 7.0μ in mean diameter (Fig.41).

During this stage, the ooplasm around the nucleus transforms into a finely reticulated consistency. This transformation occurs in a consistent pattern and has a predominant "outflow" region (Fig. 42).

There appears to be a dramatic increase in the number of follicle cells which are now thicker and shorter and closely adjacent to each other. Their length ranges from 15.0μ to 16.0μ while the height or thickness ranges from 3.6μ to 4.7μ. The zona pellucida remains barely visible (Fig.39).

II. VITELLOGENIC PHASE

Stage A: initial accumulation of yolk granules

Yolk granules make their first appearance in the ooplasmic transformational region, with greater granule accumulation occurring in the outflow region (Fig.43). At this initial stage, they tend to aggregate in small, loose clumps although individual granules are evident and measure about 1.4μ in mean diameter (Fig,44).

Both the ooplasm and nucleoplasm are weakly basophilic and the few peripheral nucleoli are all irregular in shape and consist of a duplex nature

i.e. deeply basophilic areas and very pale vacuole-like regions (Fig.44).

Follicle cell dimensions alter with the initial accumulation of yolk granules, becoming slightly longer (16.4μ in mean length), but visibly thicker (5.5μ in mean height). The zona pellucida appears homogeneous, weakly acidophilic and measures about 2.4μ thick. Oocytes of this first vitellogenic stage reach a mean cell diameter of 1003.4μ while the mean diameter of the nucleus measures 339.9μ.

Stage B:

The tiny yolk granules migrate from the ooplasmic trasformational region to almost all parts of the ooplasm except for the region immediately below the oolemma. The ooplasmic transformational region however, still possesses rather more granules, especially in its outflow region (Fig.45). It therefore appears to be the site of yolk granule formation and initial accumulation. At this stage, there appears to be no growth of the yolk granules which still have a mean diameter of about 1.4μ.

Faint lampbrush chromosomes are still evident in the pale nucleoplasm. The duplex-structured peripheral nucleoli appear nearly spherical, with

the largest measuring 10.4μ in mean diameter (Fig. 46).

Although the mean length of the follicle cells remains about the same as in the previous stage, the mean height is reduced to 4.3μ. This may be due to compression or slight stretching of the follicular epithelium caused by the rapidly growing oocytes which attains a large mean cell diameter of 1283.0μ and mean nuclear diameter of 374.1μ. The zona pellucida is also reduced to a height of 2.0μ, again probably caused by stretching due to increased oocyte growth.

Stage C:

The accumulation of yolk granules in the ooplasmic transformational region has eased out, resulting in a greater distribution of these granules in the ooplasm (Fig. 47). A portion of the granular ooplasmic transformational region, however, still persists at this stage. At most places, the yolk granules do not migrate all the way to the oolemma (Fig. 47 and 48), and their accumulation is slightly denser at the periphery of their distribution than in the rest of the ooplasm. Furthermore, yolk granules in the peripheral region are also larger (Fig. 48), having a mean diameter of 3.25μ, while the rest of the granules remain at 1.4μ in mean diameter. This appears to suggest growth of the yolk granules on reaching the peripheral regions of

the oocyte or simultaneous migration and growth, the latter only being detectable and measurable once the granules have reached the near periphery of the oocyte.

The nuclear membrane appears less undulating than in the previous stage. Peripheral nucleoli further increase in size, measuring 12.0μ in mean diameter.

Coinciding with the increase in yolk granule accumulation and distribution, is the dramatic increase in the mean length and height of the follicle cells which appear cuboidal and measure 21.5μ and 8.7μ respectively. (Fig. 48). Mean oocyte diameter further increases to 1536.5μ while the mean diameter of the nucleus reaches 467.5μ. The thickness of the zona pellucida also increases to 2.6μ.

Stage D:

At this stage, most of the yolk granules have migrated away from the ooplasmic transformational region, resulting in a broader ring of uniformly increased granule accumulation (Fig. 49). The persistence of some areas of the ooplasmic transformational region may suggest that active yolk formation and accumulation is still occurring within this site. The cortical yolk granules increase further to a mean diameter of 4.8μ while those at the outer nuclear periphery

measure only 2.3μ-2.6μ in mean diameter (Fig. 50).

The "lampbrush" chromosomes are undergoing some sort of activity whereby deeply basophilic, spherical nucleoli appear to be formed. These appear "solid" and do not possess the duplex structure or nature of the larger, spherical or irregularly shaped nucleoli that still persist in these oocytes. The "solid", uniformly basophilic nucleoli migrate to the inner nuclear membrane and may measure as much as 7.1μ in mean diameter (Fig. 50).

Dimensions of the follicle cells are decreased i.e. the mean length is reduced to 18.5μ while the mean height is reduced to 3.4μ. Oocytes at this maturation stage grow further to a mean cell diameter of 1387.0μ and mean nuclear diameter of 493.5μ. The height of the zona pellucida ranges between 2.86μ and 3.0μ.

Stage E:

Yolk granule accumulation has increased almost throughout the ooplasm except for the ooplasmic transformation region where the tiny yolk granules of mean diameter range of 2.3μ-2.6μ have begun to migrate away from this site of initial accumulation (Fig. 51). The cortical yolk granules increase further to a mean diameter of 8.6μ. Since granules of intermediate size

(4.5μ - 6.0μ) lie in the mid-region of yolk granule accumulation (i.e. halfway between the outer nuclear membrance and the oolemma) (Fig. 52), it suggests that they grow whilst migrating from the ooplasmic transformational region toward the oocyte periphery i.e. growth and migration of the yolk granules in the oocytes of this stage, occur simultaneously.

The mean length of follicle cells reduces even further to 16.5μ while the mean height increases to 4.3μ. The zona pellucida also increases in height that ranges between 2.9μ and 3.5μ. Peripheral nucleoli are as in the previous stage. Mean oocyte diameter reaches a large size of 1561.1μ while mean nuclear diameter decreases to 481.3μ.

Stage F:

Yolk granule accumulation in the ooplasmic transformational region has by this stage totally eased out, such that no density differential areas in the ooplasm occurs (Fig. 53). However, in the clear ooplasmic region between the peripheral boundary of yolk distribution and the oolemma, minute yolk granules occur, which are even smaller than those of mean diameter 1.4μ which initially accumulate in the ooplasmic transformational region, and therefore not accurately measurable (Fig. 54). This seems to suggest that the

extreme cortical ooplasmic zone is the second region of yolk granule formation and accumulation, after these processes have slowed down or ceased in the ooplasmic transformational region. These minute yolk granules grow while simultaneously migrating inwards from the cortical ooplasmic region. With a further increase in growth, the large yolk granules of ooplasmic transformational origin, which are now below the region of the minute yolk granules, measure upto 10.5μ in mean diameter while those around the nucleus have a mean diameter of 3.2μ. Mid-way in the yolk granule distribution, the granules range from 6.1 to 7.3μ in diameter.

The mean length of the follicle cells remains at about 16.5μ but the mean height increases even further to 4.9μ. In oocytes of this maturation stage, which attain a large mean cell diameter of 1760.0μ, the zone pellucida is reduced in height to 2.8μ. Furthermore, mean nuclear diameter is also decreased to 341.0μ.

Stage G:

The accumulation and mean diameter of the minute yolk granules in the extreme cortical ooplasmic region, appear to have increased i.e. granules here measure 2.5μ (Figs. 55 and 56).

In these large oocytes of mean diameter 2575.5μ, the large, previously cortical yolk granules of mean diameter 10.5μ, have migrated back towards the peri-nuclear region. A simultaneous migration and growth has also occurred of the smaller yolk granules from the ooplasmic transformational region towards the oocyte periphery, where they measure 4.8μ in mean diameter (Fig. 56). The large yolk granules in the peri-nuclear region tend to adopt an oval-shape instead of spherical and have a mean diameter of 11.1μ (Fig. 57). Since a few, larger and somewhat spherical yolk granules of mean diameter 19.7μ are present in the cortical and middle regions of the yolk distribution, but not where the ovoid granules are (Fig. 56), this may suggest that the inward migration of the large cortical yolk granules is accompanied by further growth and by the time they reach the peri-nuclear region, some sort of condensation of their yolk content occurs. Therefore, in oocytes of this maturation stage, there are granule size differential areas from the extreme cortical region toward the peri-nuclear region i.e. mean diameter of yolk granules increases centripetally from 2.5μ to 11.1μ.

With further oocyte maturation, there is growth and inward migration of the extreme cortical yolk granules or growth alone when migration ceases, resulting in large granules occurring almost throughout the ooplasm.

The follicle cells appear longer, having a mean length of 23.4μ and narrower - mean height of 4.1μ, and are not so closely adjacent to each other. The zona pellucida has also further decreased to a height of 2.0μ.

Stage H:

Towards peak maturation of the oocyte, the nucleus migrates from its central location to the zona pellucida, thereby becoming polar in position (Fig. 58). The yolk granules in the wide central region and extreme cortical region, aggregate into dense clumps. The latter region still accumulates yolk granules, some of which grow to a mean diameter of 8.4μ. The oval-shaped granules in the central region still maintain their mean diameter of 11.1μ and the largest spherical ones that are present between the cortical and central regions, do not exceed 13.5μ in mean diameter. These spherical granules may represent those of the extreme cortical region which have migrated inwards and increased in size. Condensation of their yolk content and adoption of an oval shape may also occur.

From Stage G to Stage H, further growth of the oocyte is slight i.e. from a mean diameter of 2575.5μ to a mean diameter of 2615.8μ. Mean nuclear diameter remains at 340.1μ. The large duplex nucleoli and the

smaller basophilic ones are still present in the nucleoplasm which is slightly acidophilic.

Stage I: oocyte just prior to ovulation

The polar nucleus pushes against the zona pellucida forming a slight protruberance on the surface of the oocyte. Yolk granules migrate away from the nuclear vicinity and also away from the zona pellucida, on the neighbouring lateral sides, forming a clear ooplasmic polar region (Fig. 59). It is suggested that this phenomenom occurs to remove obstructing yolk granules in the nuclear vicinity and facilitate sperm entry into the nucleus.

Mean oocyte diameter further increases to 3213.0μ at this stage. Furthermore, the nucleus also increases from a mean diameter of 340.1μ to 500.3μ, suggesting that a spurt of growth does occur in both the oocytes and their nuclei just prior to ovulation. The peripheral nucleoli resemble those of the protoplasmic oocytes, being spherical, deeply basophilic and lacking the duplex structure (Fig. 60). They measure upto 8.5μ in mean diameter. The follicle cells are also similar to those of early protoplasmic oocytes in being widely separated from one another and appearing as narrow and elongated spindle-shaped fibroblasts. Their mean length and height measure 25.6μ and 3.9μ respectively.

The zona pellucida is very thin and measures only 2.1μ thick.

Stage J: oocytes ovulated into the body cavity

Clumping of the yolk granules occurs throughout the oocyte and the oval-shaped granules decrease even further to a mean diameter of 10.2μ, suggesting that further condensation of the yolk granules has taken place at this stage. Yolk granules retain their discrete individual structure and do not transform or coalesce into a single homogeneous mass (Fig. 61).

Oocytes ovulated into the body cavity, usually have a ruptured follicular epithelium which can be easily removed to reveal a thick layer of jelly-like material beneath i.e. above the zona pellucida. This jelly-like layer has a wide extent ranging from 142.4μ to 184.8μ and includes a thickening at its outer periphery, which may measure as much as 18.1μ thick. The zona pellucida (or vitelline membrane) becomes thicker i.e. 4.0μ and is clearly visible.

Atresia of Oocytes:

Atresia was noted to occur in late protoplasmic and vitellogenic oocytes or in ovaries that contained mostly intact dark protoplasmic oocytes and some light

staining ones (Fig. 62). These ovaries are likely to be at the post-spawning or spent stage where the unspawned and less developed oocytes become atretic. In both the atretic late protoplasmic and vitellogenic oocytes, the zona pellucida appears to be phagocytosed by the follicle cells, which invade the oocytes and further phagocytose the ooplasm and nucleus of the late protoplasmic oocytes or the cortical yolk granules of vitellogenic oocytes. The nuclei of the follicle cells apear small, having a mean diameter of 10.3μ. During this degeneration process, the oocyte loses its regular spherical or oval shape and in vitellogenic oocytes, there also appears to be liquification of the nucleus and the surrounding yolk granule area which are probably reabsorbed by the follicle cells.

HISTOLOGY OF THE OVARY

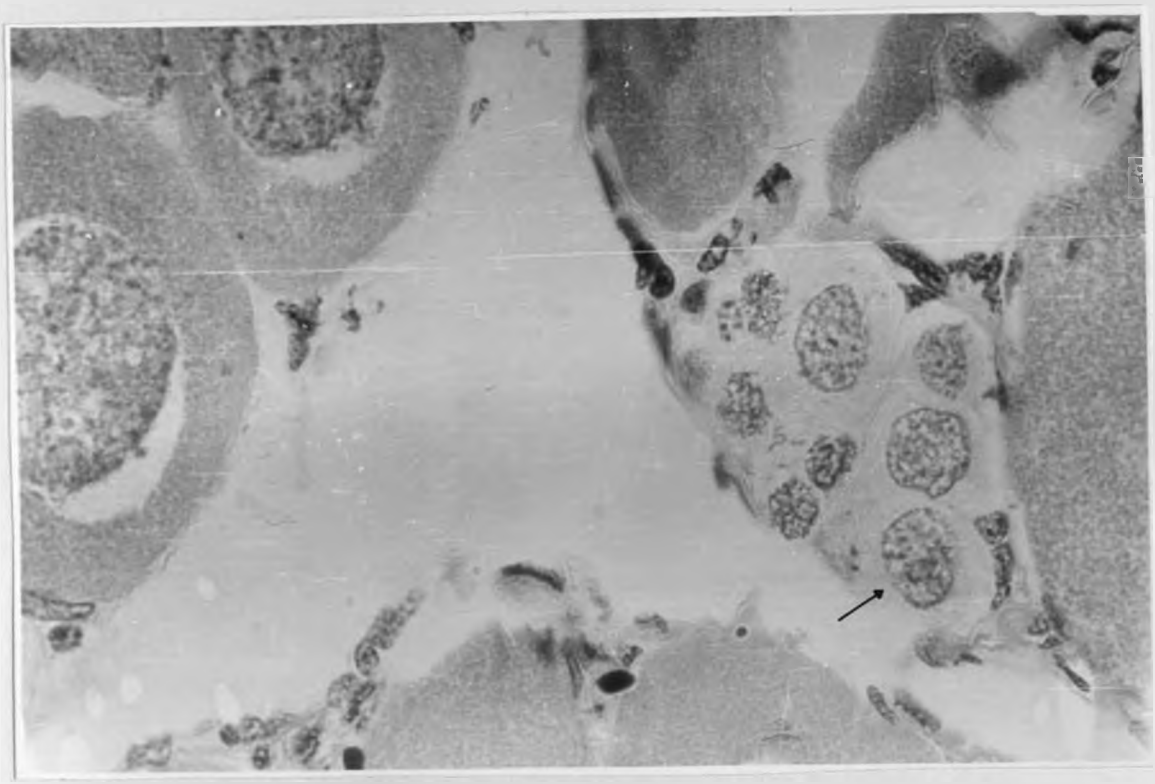
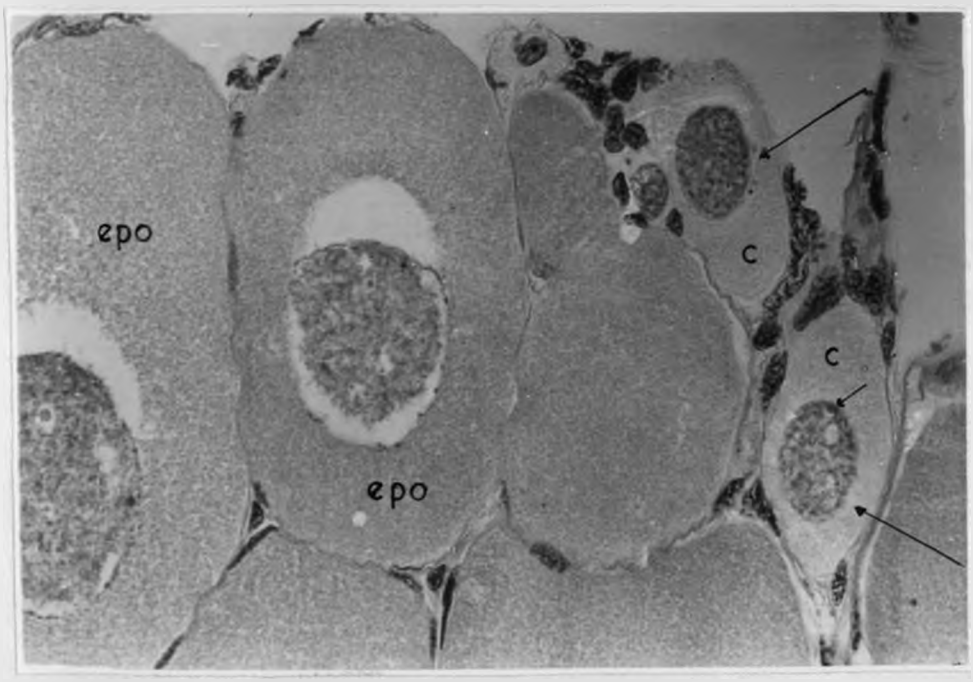


Fig.33: A group of pre-protoplasmic oocytes (arrow). Mag.X 1200.

Fig.34: Pre-protoplasmic oocytes (long arrows). Nucleolus (short arrow) is positioned at the inner nuclear membrane. Note pale, non-staining cytoplasm (c). epo: early protoplasmic oovytes. Mag.X 900



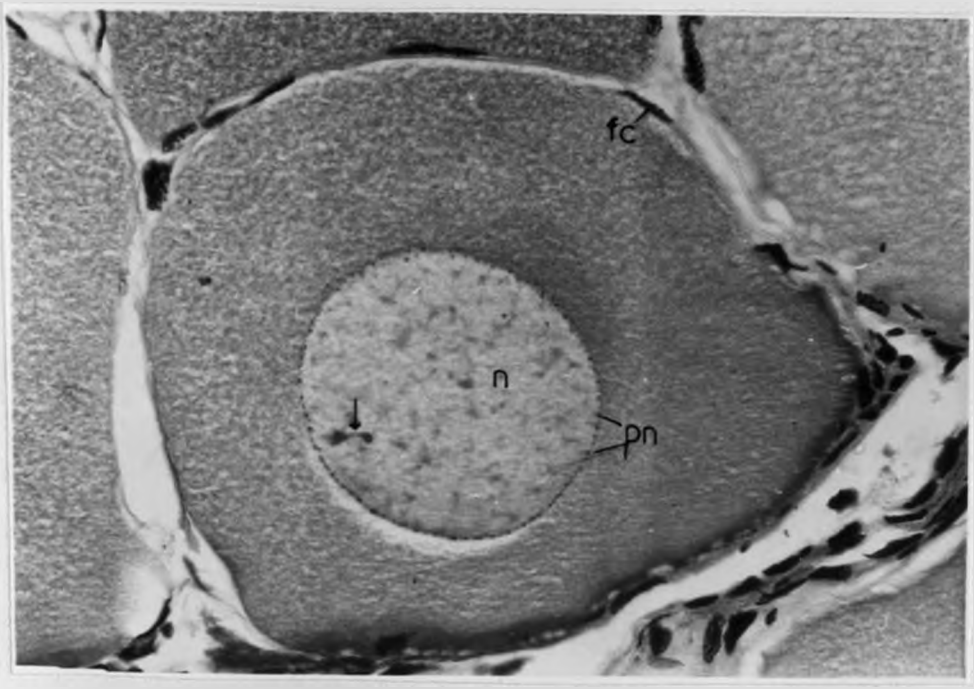
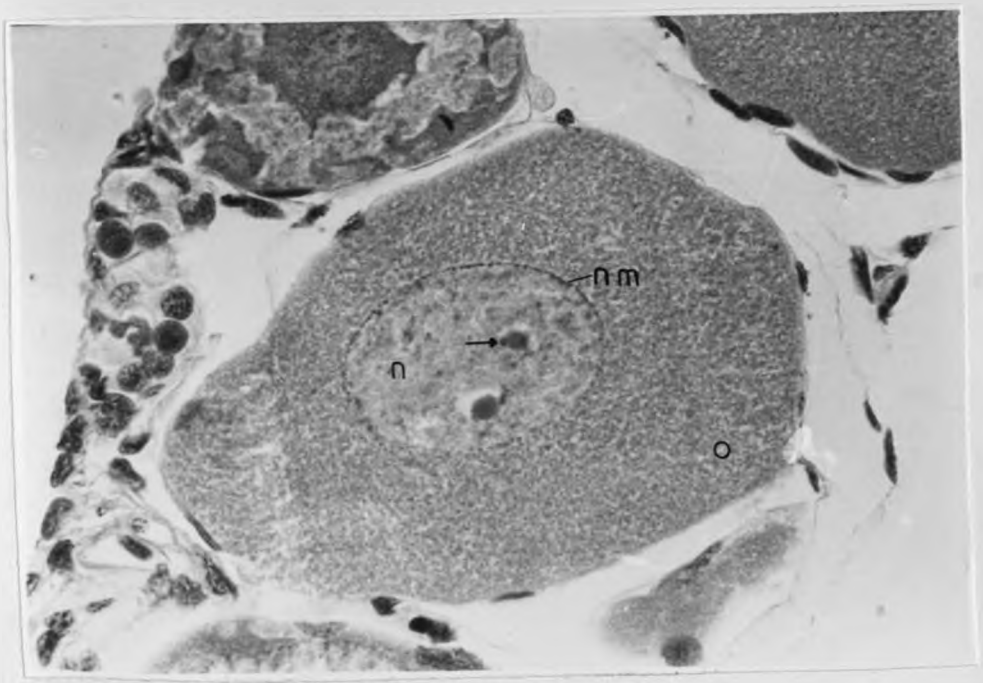


Fig.35: Early protoplasmic oocyte with a fragmenting nucleolus (arrow). Several, tiny peripheral nucleoli (pn) oocur at the periphery of the nuclear membrane. fc: follicle cell; n: nucleus. Mag.X 900.

Fig.36: Nucleus (n) of an early protoplasmic oocyte showing a sub-nucleolus (arrow) within a larger nucleolus. nm: nuclear membrane; o: ooplasm. Mag.X925.



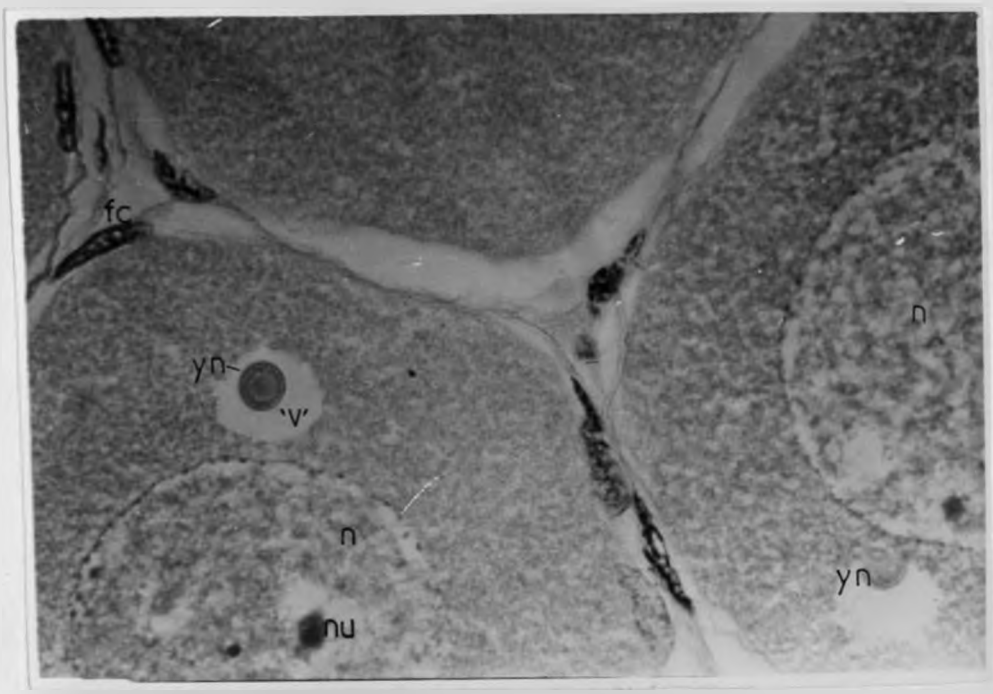
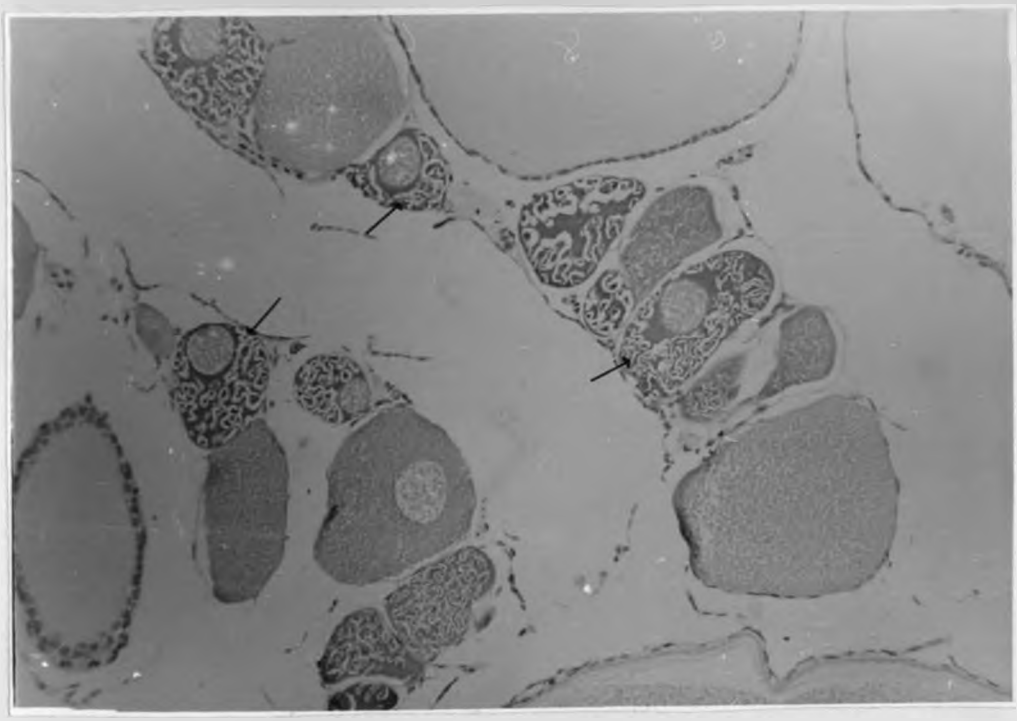


Fig.37: Yolk nucleus (yn) in the ooplasm of early protoplasmic oocytes. Note duplex nature of this organelle. fc: follicle cell; n: nucleus; nu: nucleolus; 'v': vacuole. Mag.X 1480.

Fig.38: Early protoplasmic oocytes at the mottled vacuolar stage (arrows). Mag.X 239



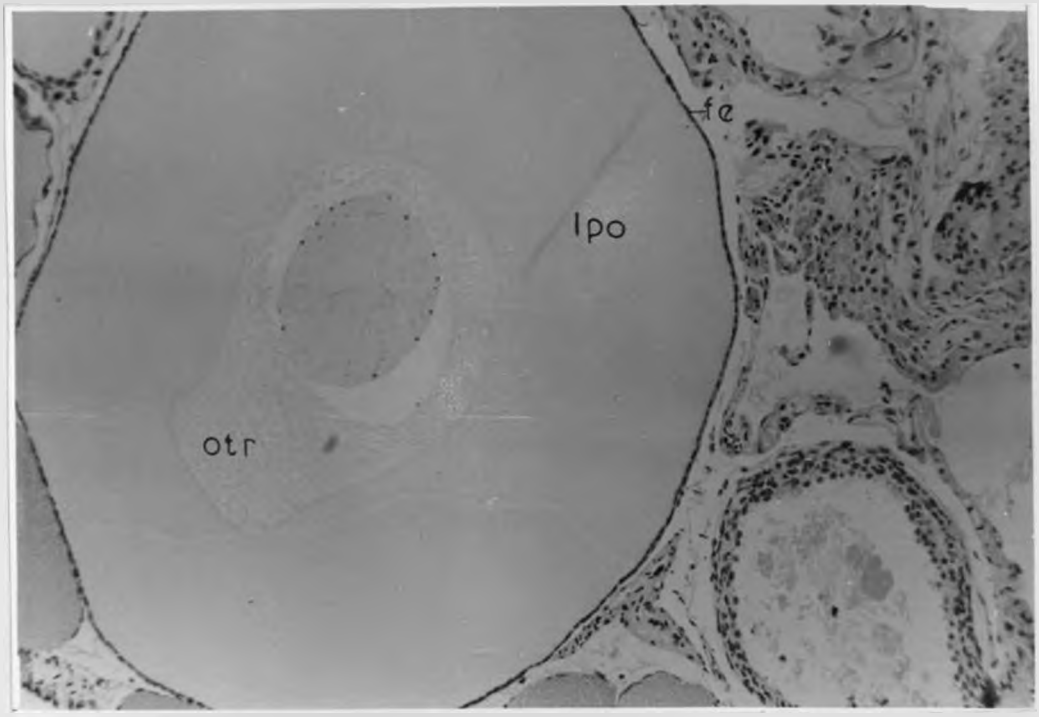
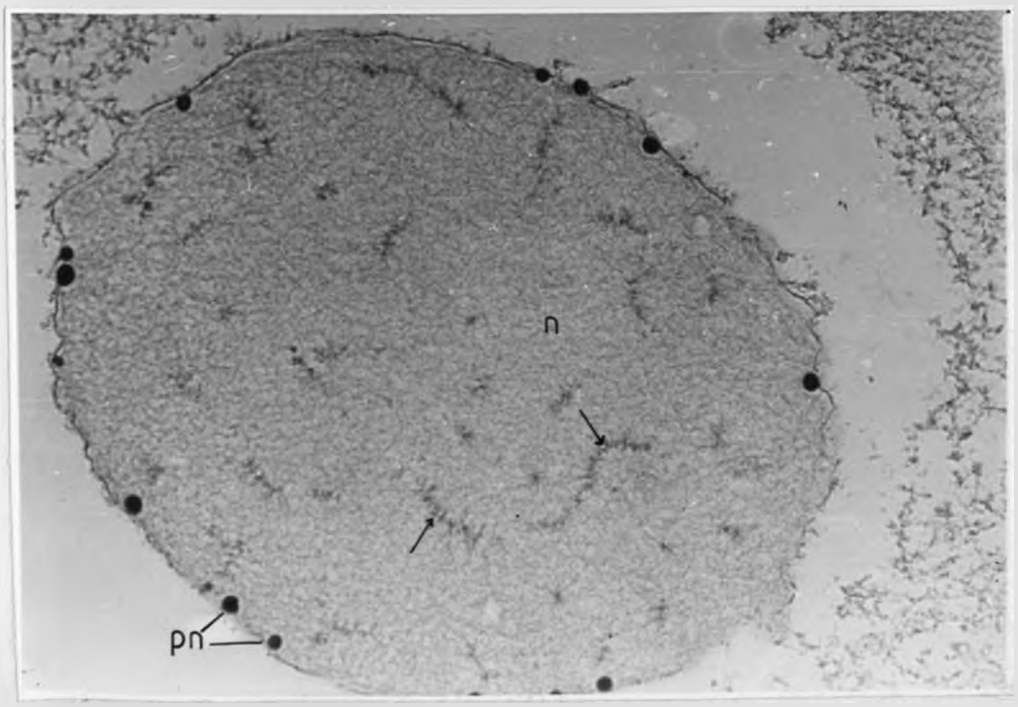


Fig.39: Light-staining (late) protoplasmic oocyte (lpo).fe: follicular epithelium; otr: ooplasmic transformational region. Mag.X 239

Fig.40: "Lampbrush" chromosomes (arrows) within the nucleus (n) of a light-staining protoplasmic oocyte. Note the presence of fewer but larger peripheral nucleoli (pn). Mag.X 1480.



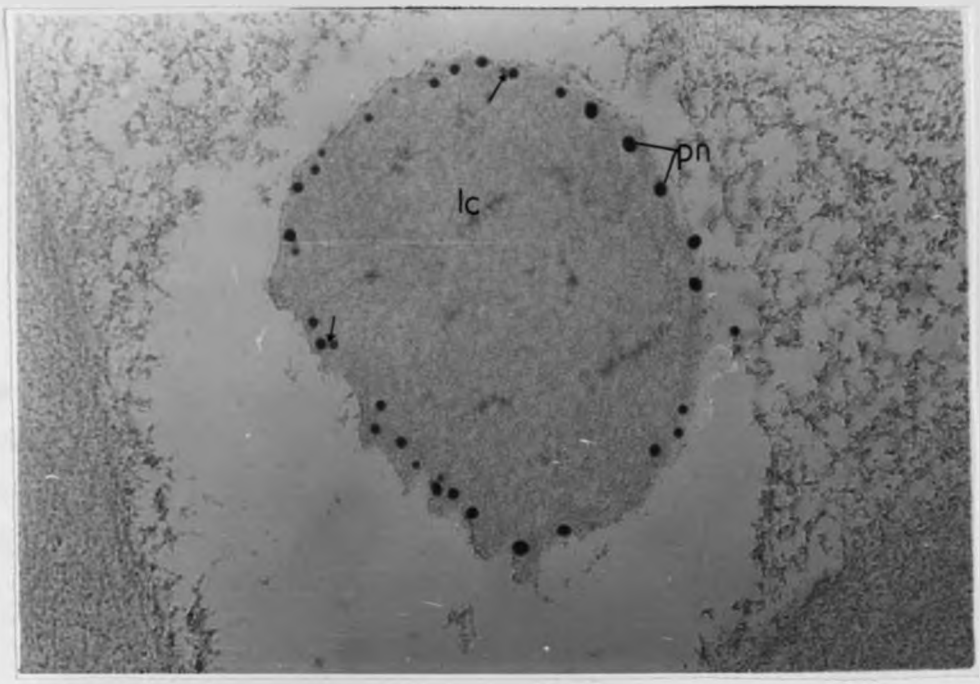
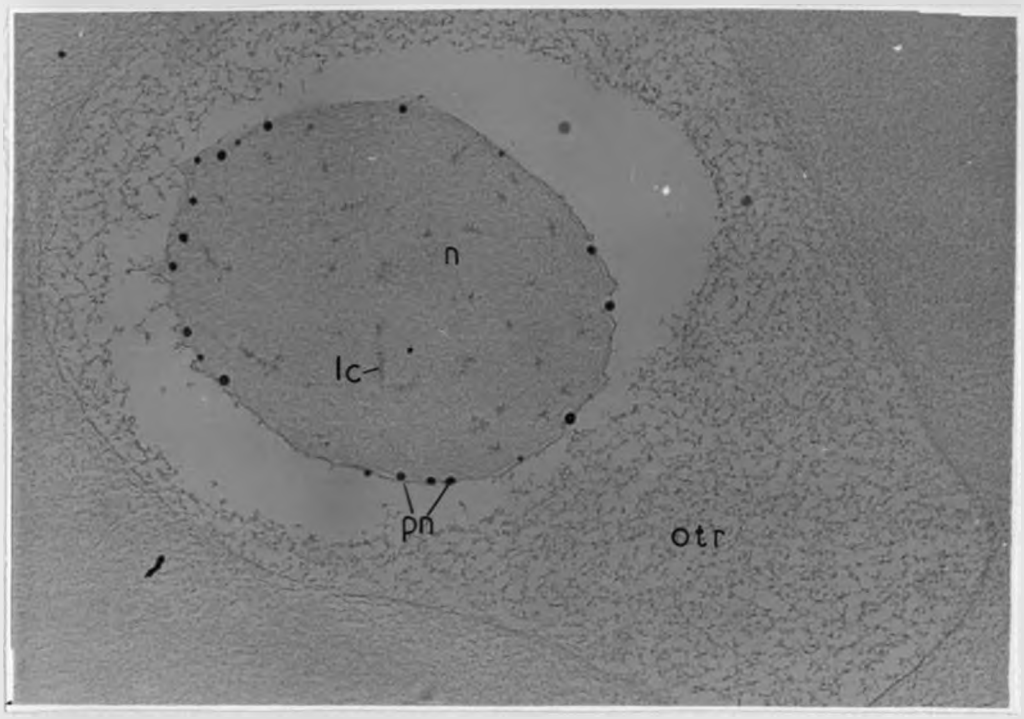


Fig.41: Nucleus of a late protoplasmic oocyte showing smaller peripheral nucleoli (arrows) aggregating into larger ones (pn) lc: lampbrush chromosomes. Mag.X 925.

Fig.42: Ooplasmic transformational region (otr) around the nucleus of a light-staining protoplasmic oocyte. lc: lampbrush chromosomes; n: nucleus; pn: peripheral nucleoli. Mag.X 608.



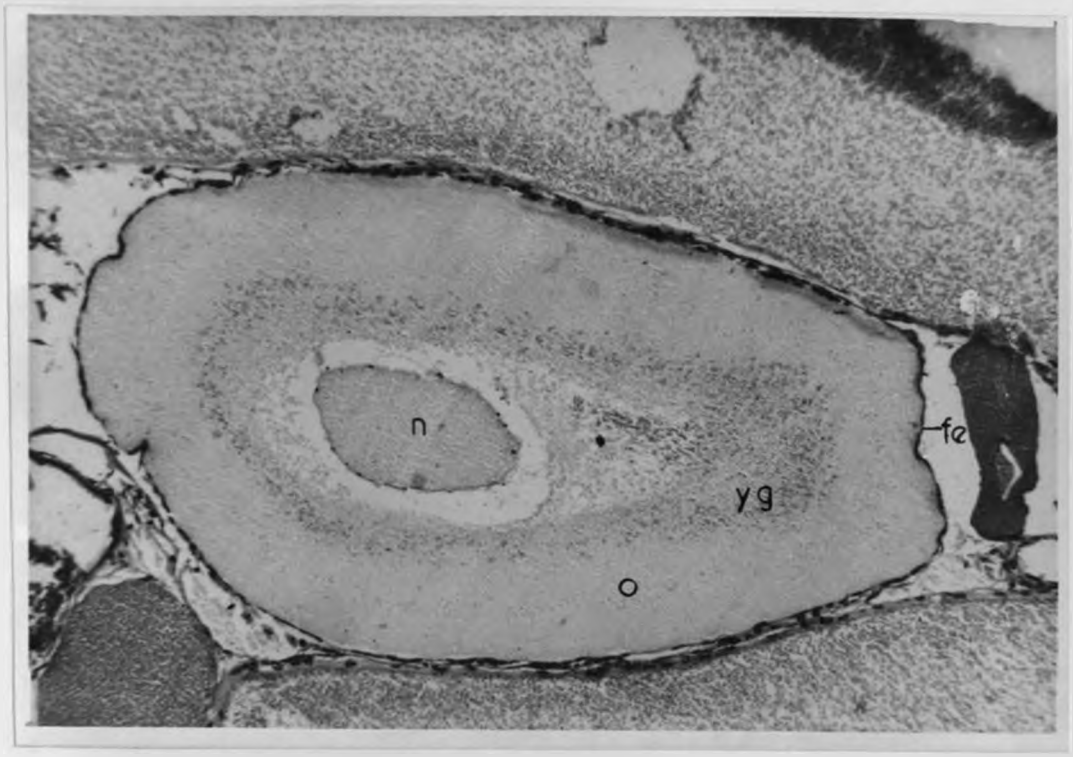
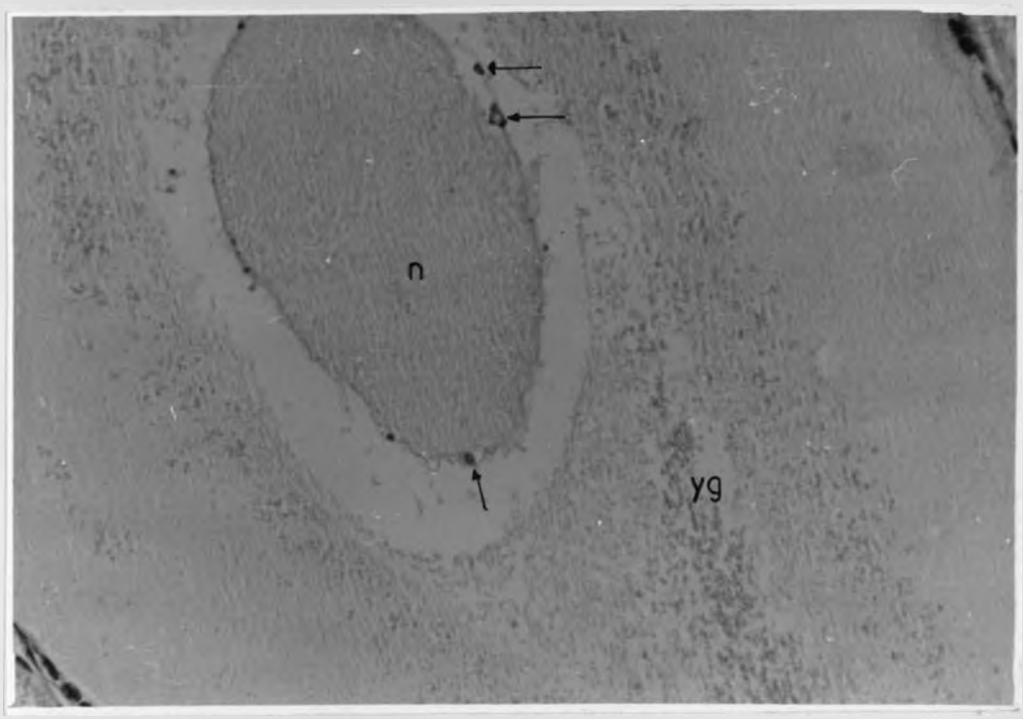


Fig.43: Stage A - initial accumulation of yolk granules (yg) in the ooplasmic transformational region. fe: follicular epithelium; n: nucleus. Mag.X 245.

Fig.44: Stage A. Peripheral nucleoli with a duplex structure (arrows). Yolk granules (yg) around the nucleus (n) form clumps. Mag.X 925.



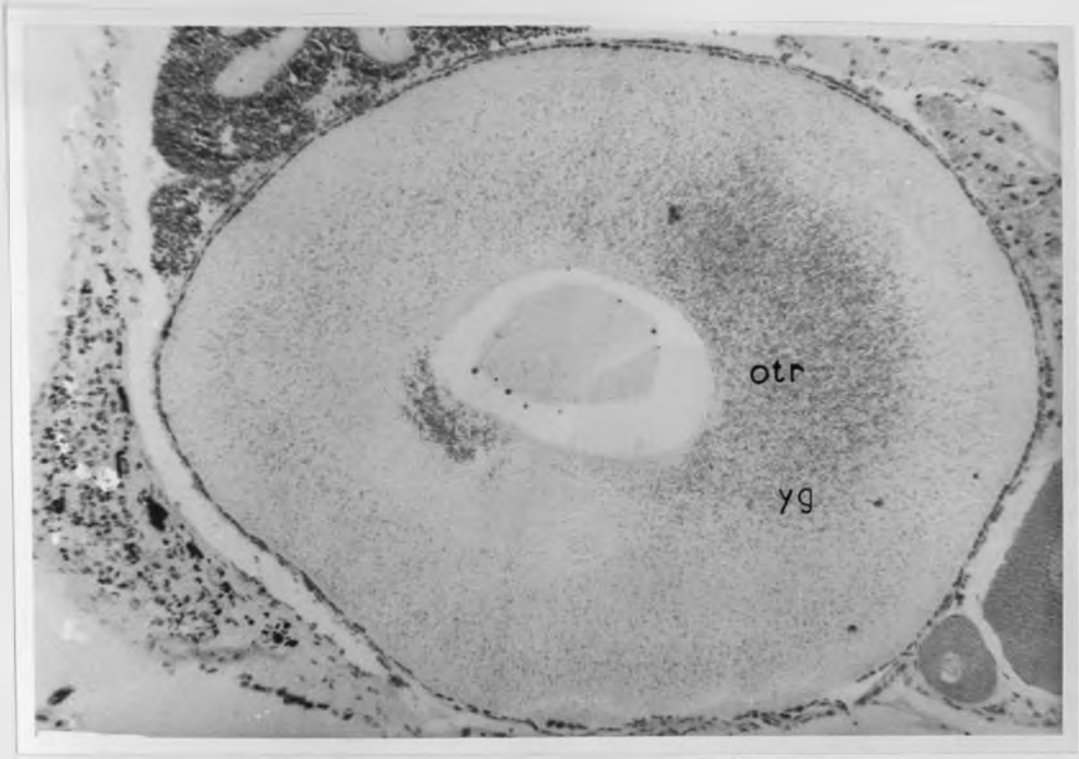
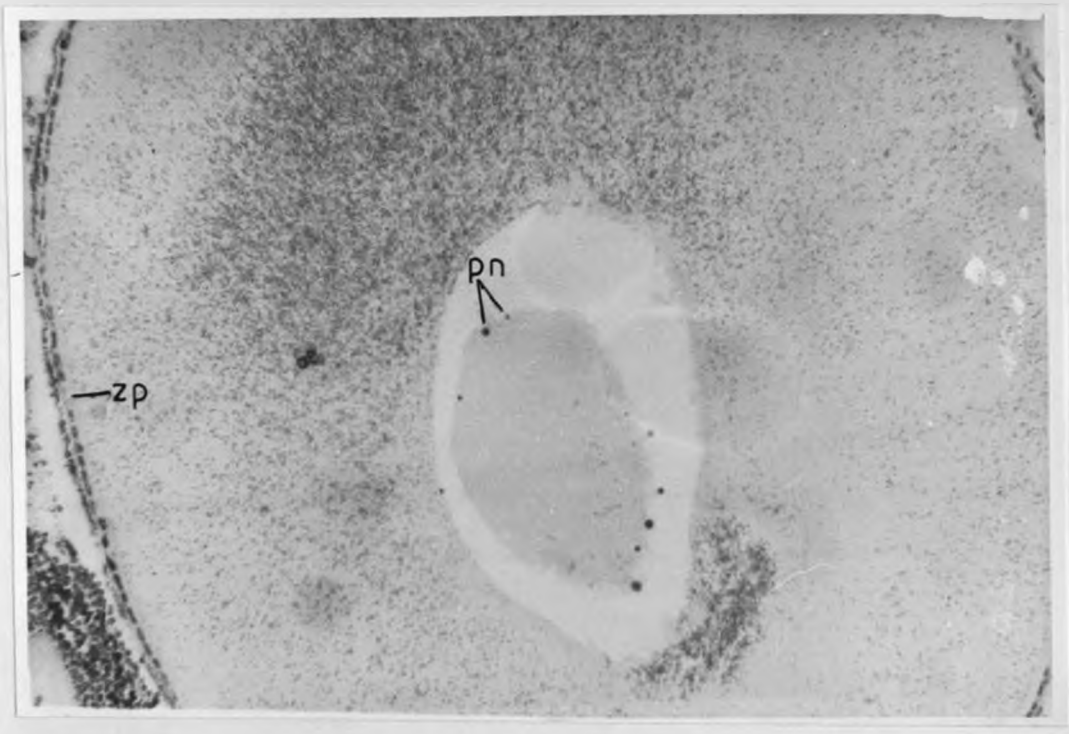


Fig.45: Stage B. Distribution of yolk granules (yg) has extended to almost all areas of the ooplasm. Ooplasmic transformational region (otr) however, has a greater yolk accumulation. Mag.X 245.

Fig.46: Stage B. Close-up of Fig.45. pn: peripheral nucleoli (of a duplex nature); zp: zona pellucida. Mag. X 388.



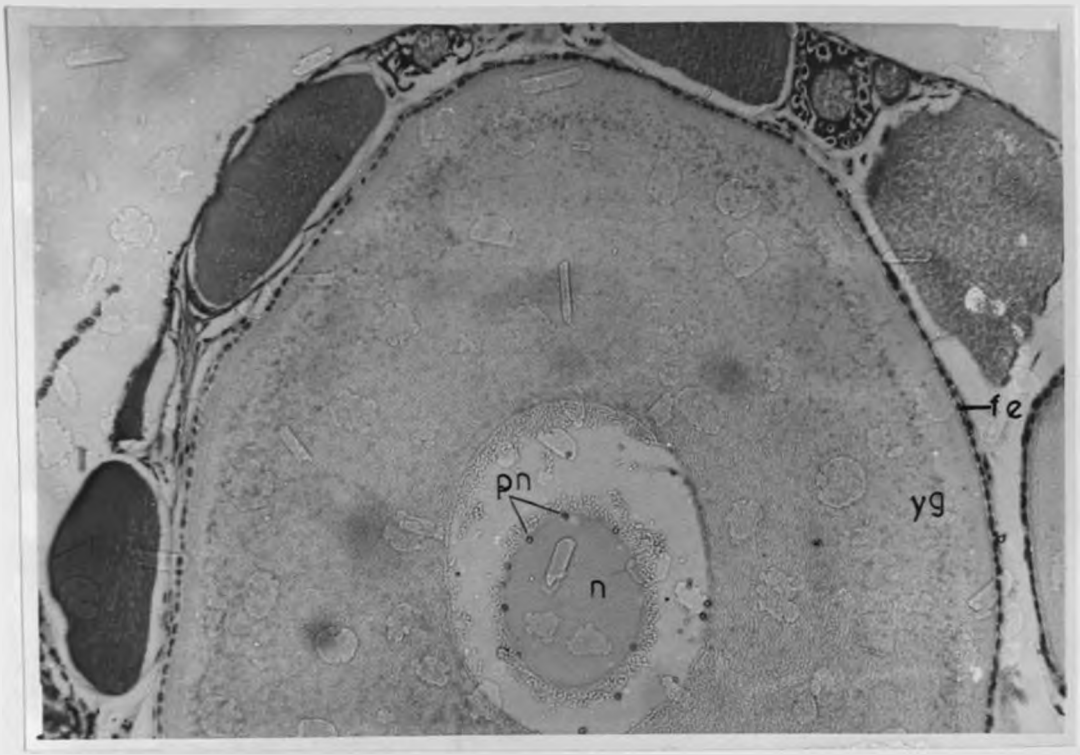
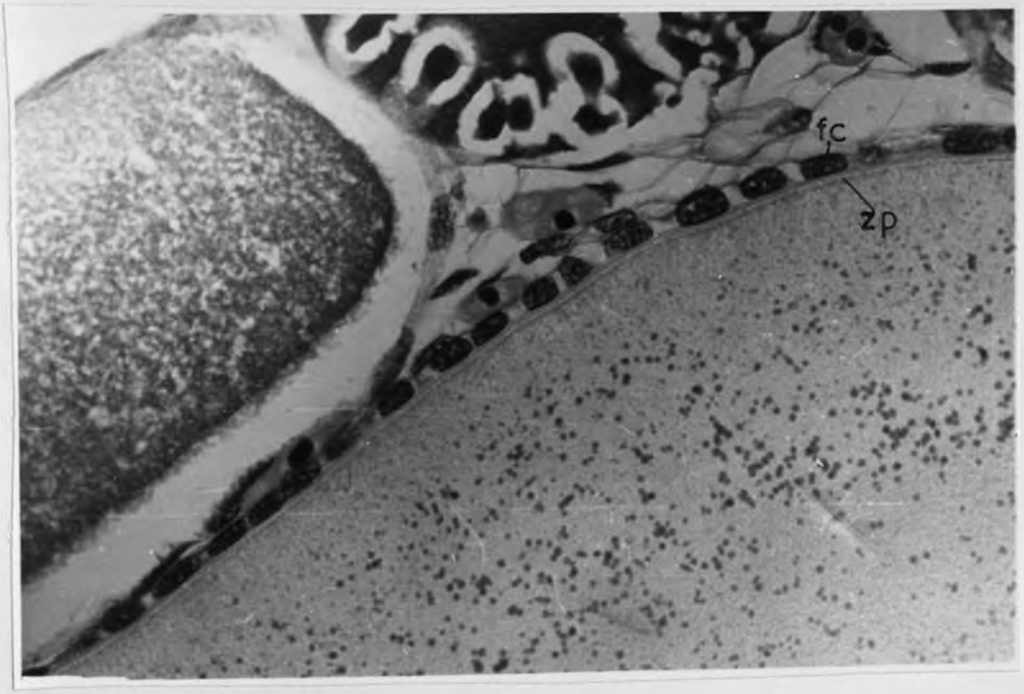


Fig.47: Stage C.Distribution or amount of yolk granules (yg) in the ooplasm has increased. fe: follicular epithelium; n: nucleus; pn: peripheral nucleoli. Mag.X 245

Fig.48: Stage C. Peripheral yolk granules are larger than those in the rest of the ooplasm and their accumulation is also slightly greater than the accumulation in the rest of the ooplasm. fc: follicle cell; zp: zona pellucida. Mag.X 925.



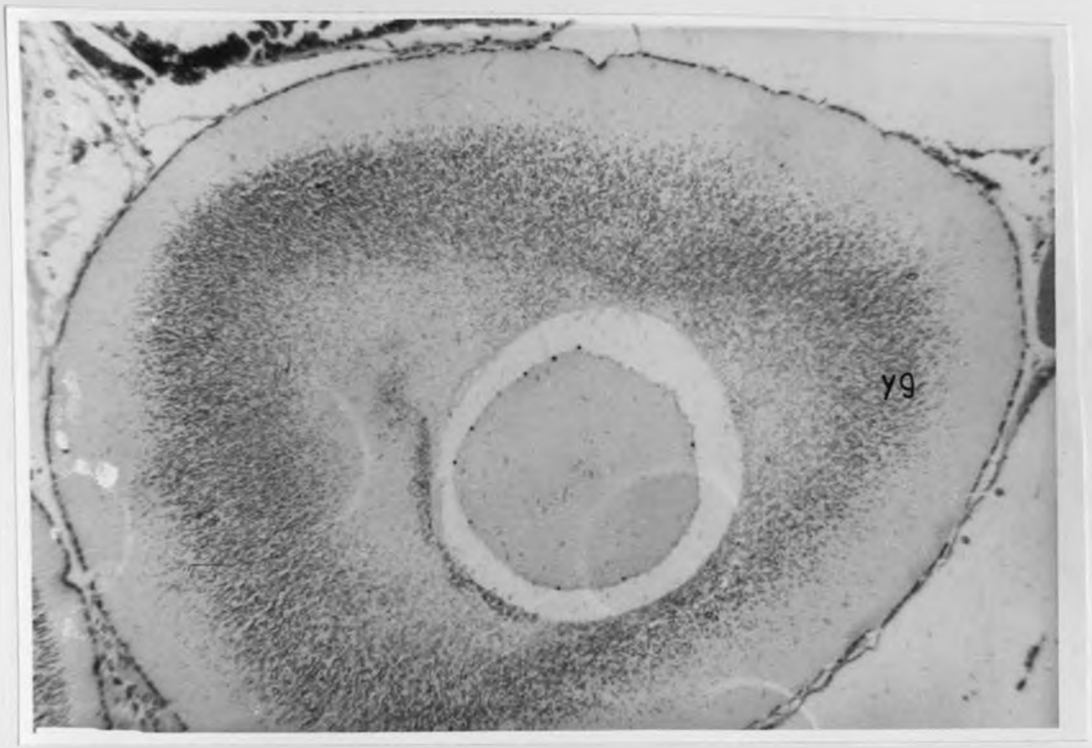


Fig.49: Stage D. Yolk granules (yg) have formed a broad ring of somewhat even accumulation. Mag.X 245.

Fig.50: "Lampbrush"chromosomes (lc) undergoing some sort of activity whereby tiny, basophilic nucleoli are formed (arrows). pn: peripheral nucleoli; yg: yolk granules; Mag.X 383.

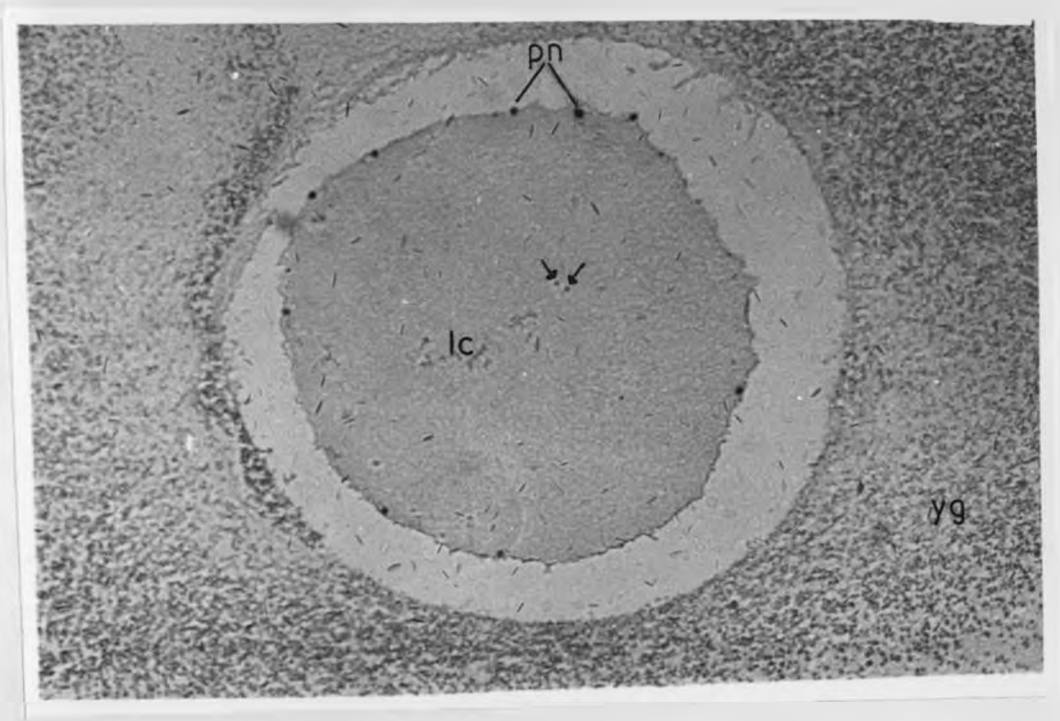
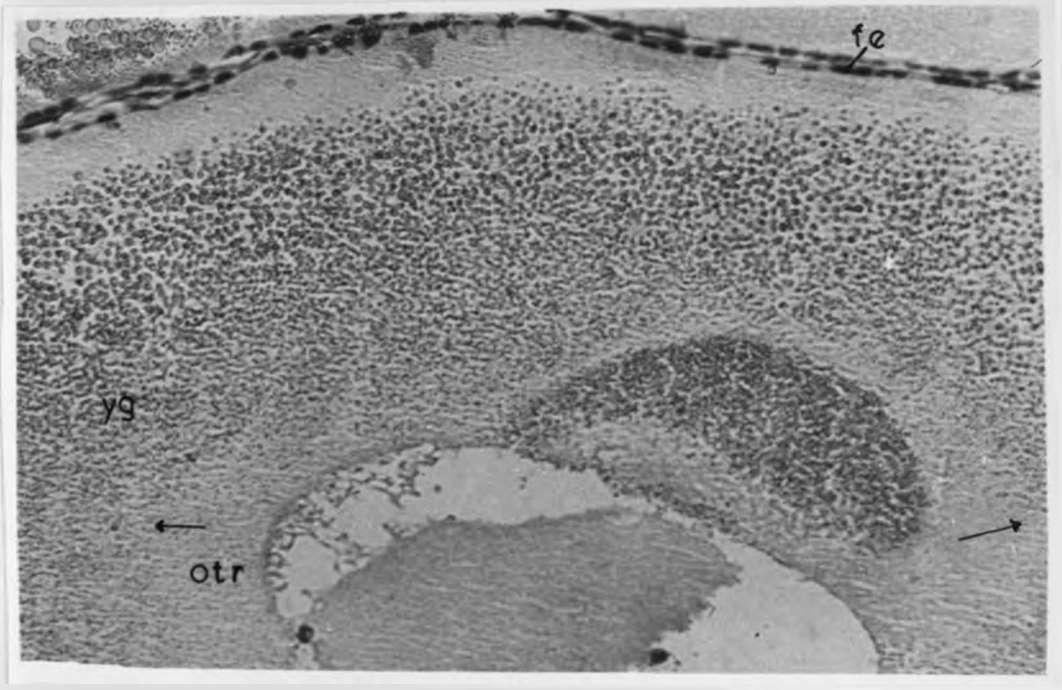


Fig.51: Stage E. yolk granules (yg) occur throughout the ooplasm except for most of the ooplasmic transformational region and the extreme cortical or peripheral region (c). Mag.X 128.

Fig.52: Stage E. migration of yolk granules away from the ooplasmic transformational region (arrows). Granules increase in size as they move from the latter region towards the oocyte periphery.
fe: follicular epithelium. Mag.X 383.



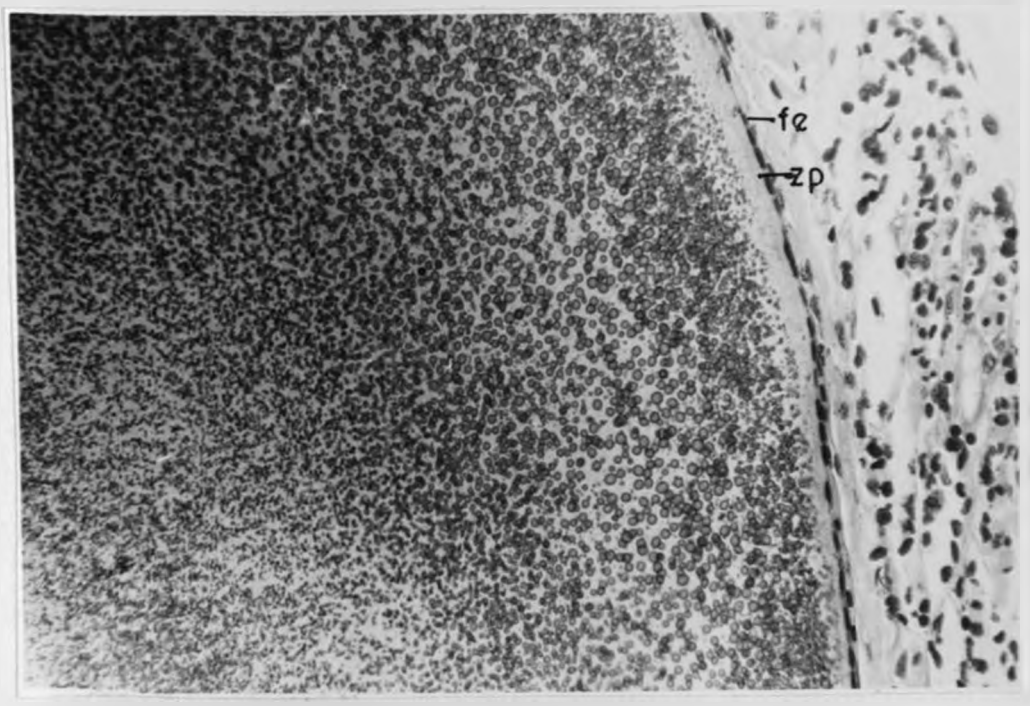


Fig.53: Stage F. Apparent occurrence of the yolk granules into size-differential zones i.e. granule size increases from the ooplasmic transformational region towards the oocyte periphery. Nucleus is towards lower left-hand corner. fe: follicular epithelium; zp: zona pellucida. Mag.X 390.

Fig.54: Stage F. Accumulation of minute yolk granules (arrows) in the extreme cortical ooplasm. fc: follicle cell; zp: zona pellucida. Mag.X 1480.

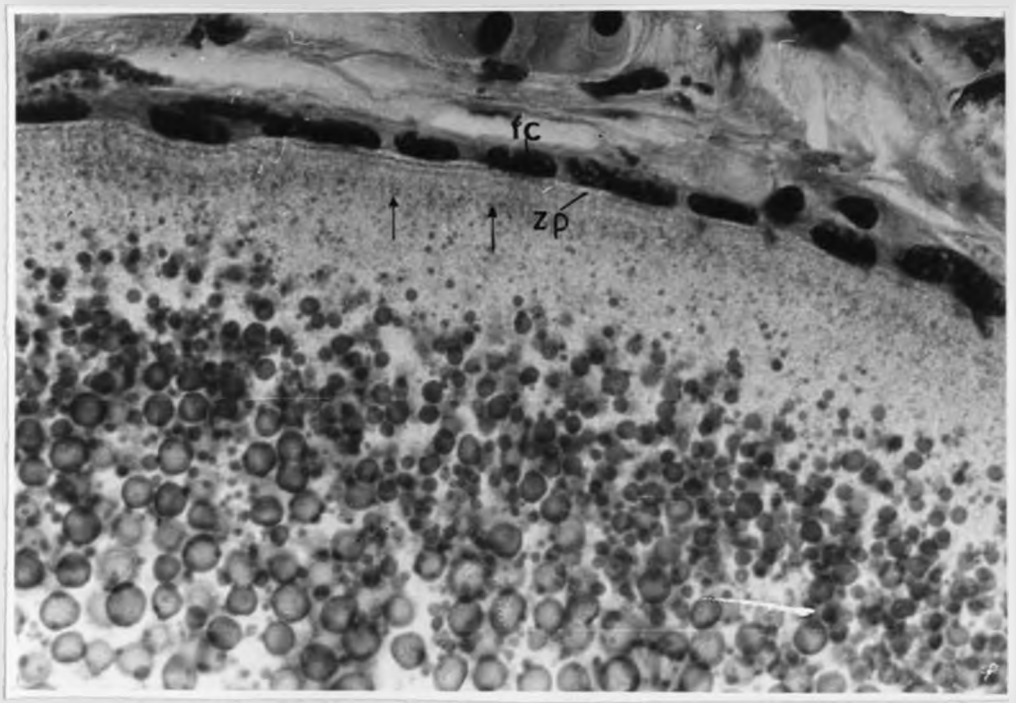
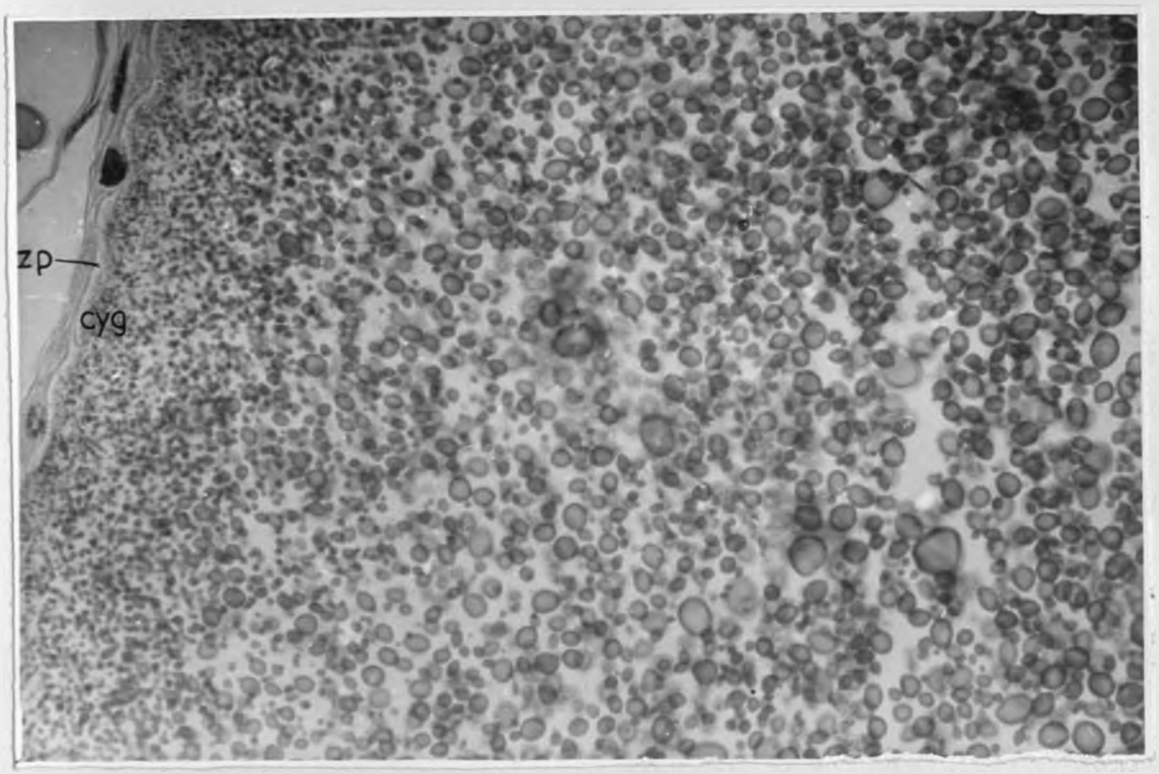


Fig.55: Stage G. Further increase in yolk granule accumulation. Nucleus is towards lower right-hand corner. Mag.X 420

Fig.56: Stage G. The extreme cortical yolk granules (cyg) have increased. The large yolk granules (of ooplasmic transformational origin) have begun to migrate back towards the ooplasmic transformational region or peri-nuclear region. zp: zona pellucida. Mag.X 1050



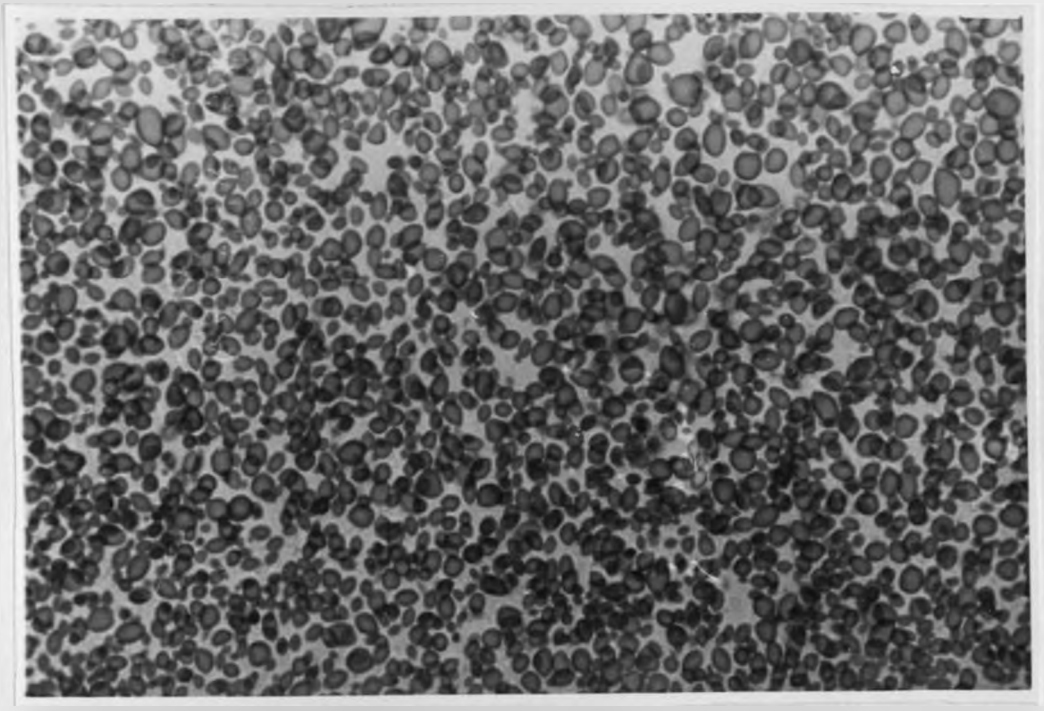
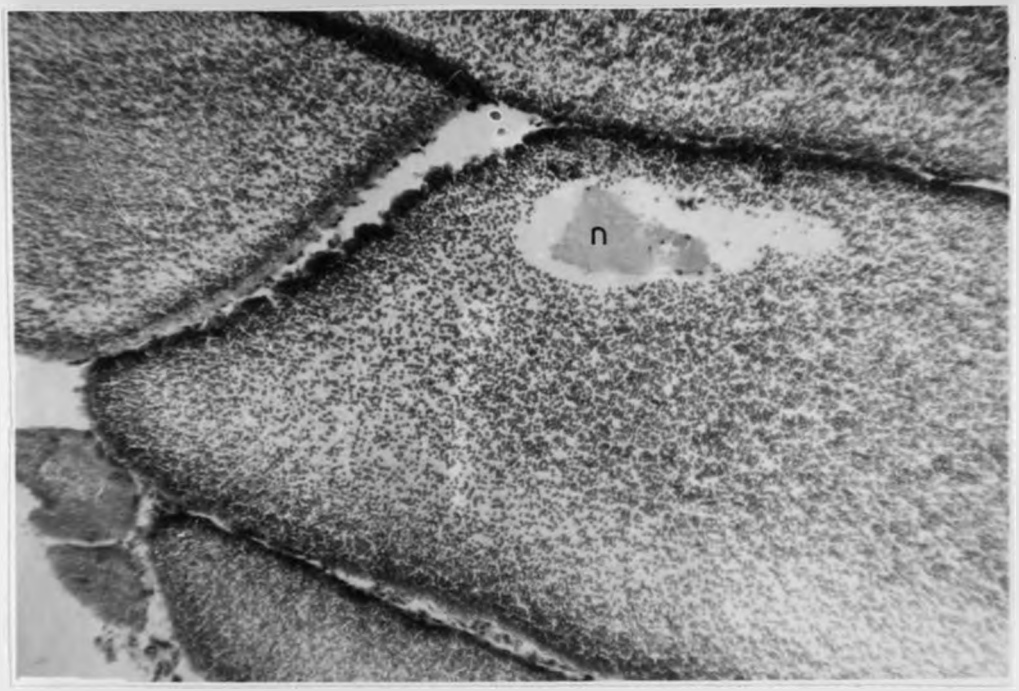


Fig.57: Stage G. A predominance of ovoid yolk granules in the peri-nuclear region. Nucleus is towards lower right-hand corner. Mag.X 925.

Fig.58: Stage H. Migration of the nucleus (n) towards the oocyte periphery. Mag.X130.



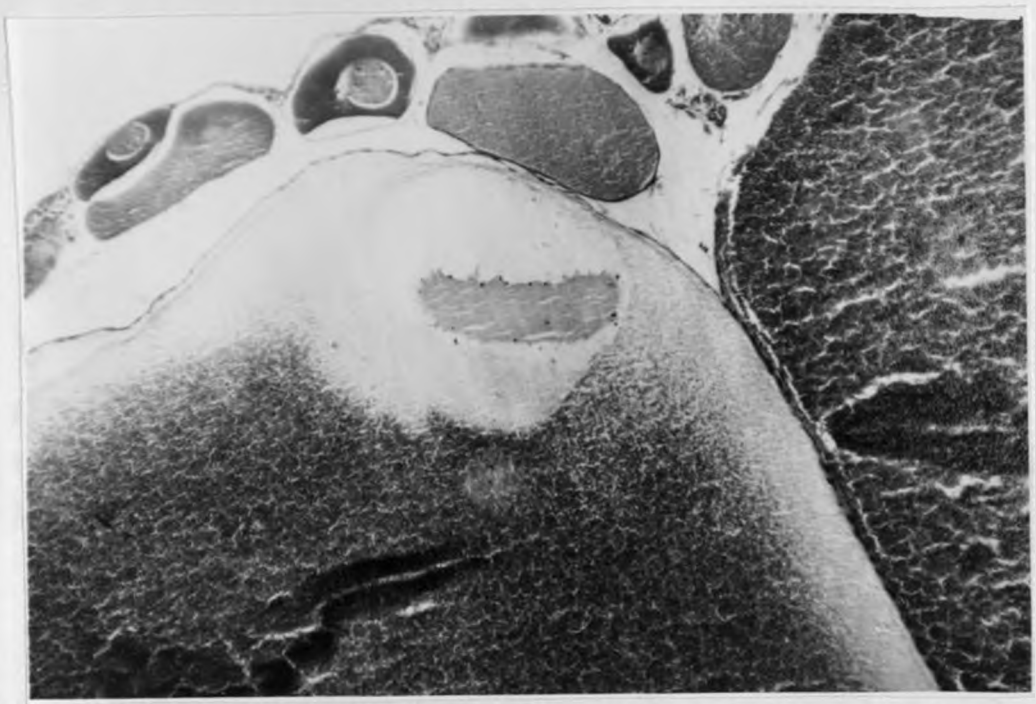
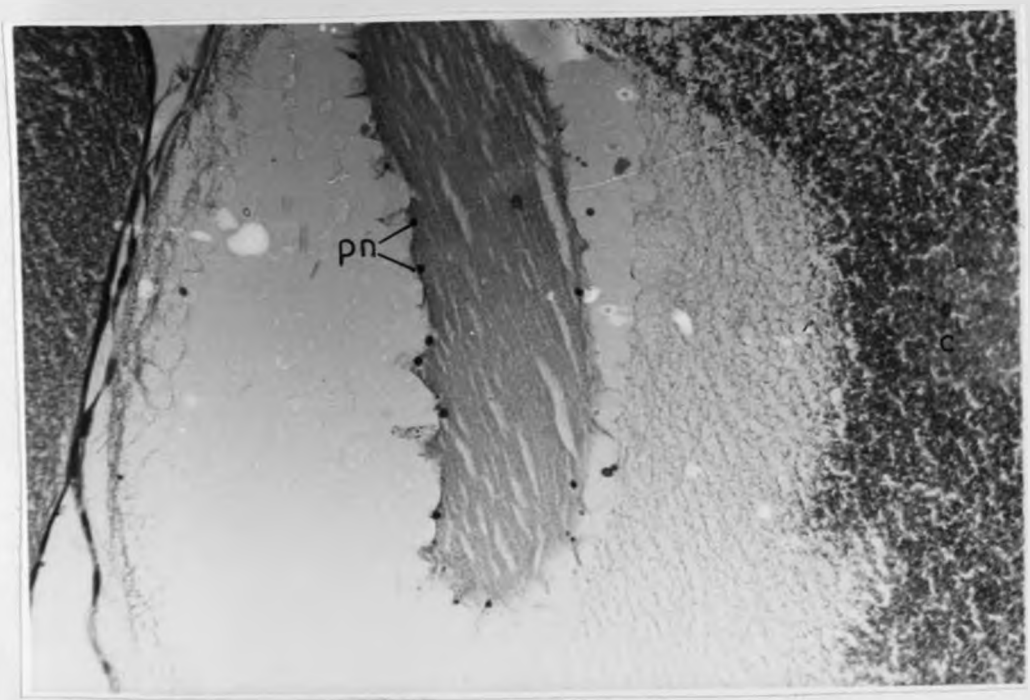


Fig.59: Stage I. Polar nucleus pushes against the surface of the oocyte forming a protruberance. Yolk granules have migrated away from the nuclear vicinity and from the zona pellucida on the neighbouring sides. Mag.X 126.

Fig.60: Stage I. peripheral nucleoli (pn) are as those in protoplasmic oocytes i.e. tiny and uniformly basophilic. c: clumps of yolk granules. Mag.X 390.



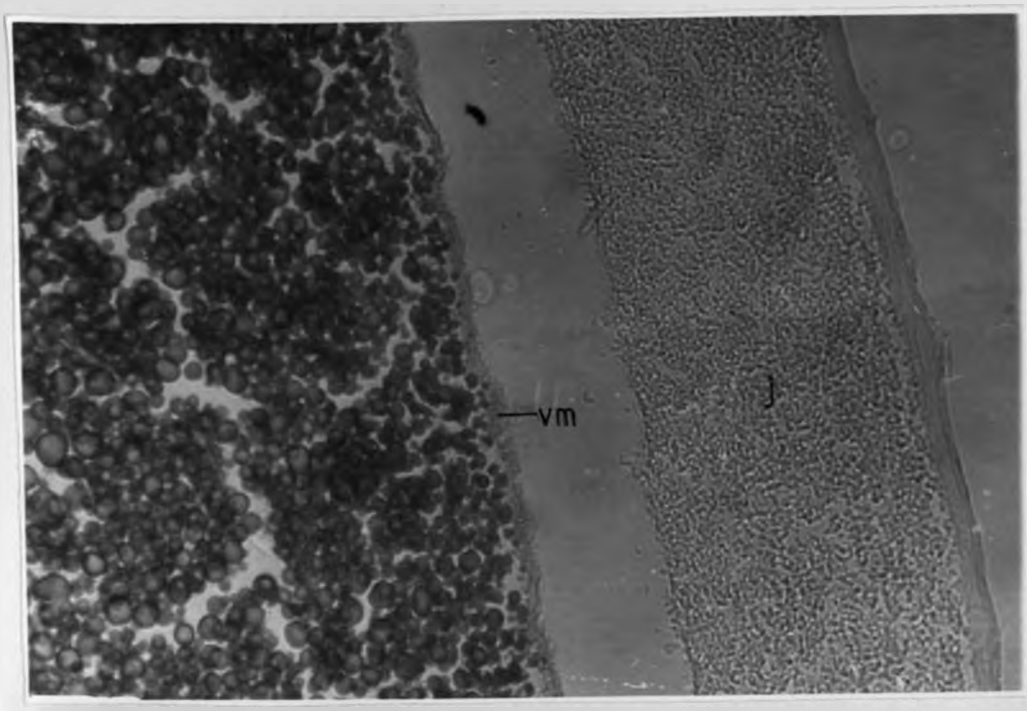
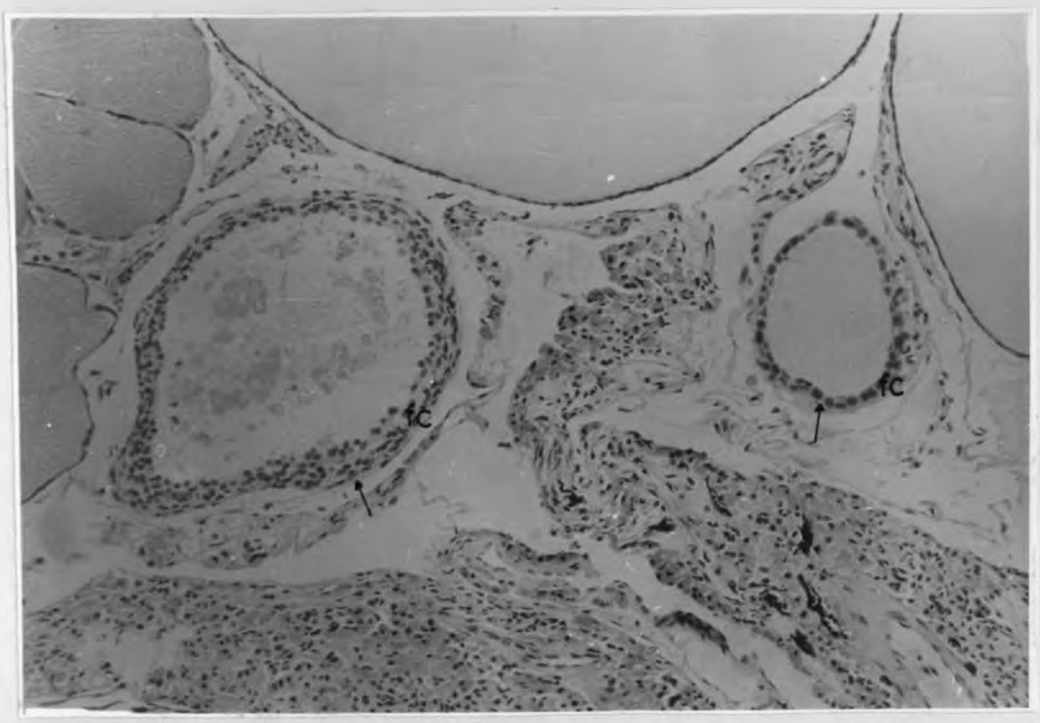


Fig.61: Stage J. Ovulated oocyte with surrounding jelly-like layer (j). vm: vitelline membrane. Mag.X 925.

Fig.62: Atresia of oocytes (arrows). Note invading follicle cells (fc). Mag.X 239.



4.2.2. Ultrastructure of the oocytes of the African lungfish P. aethiopicus

Mature oocytes were not included in this study since their large size prevented rapid and satisfactory penetration and therefore fixation by the E.M. fixative.

i). Follicular layer

The nuclei of the follicle cells of early protoplasmic oocytes are narrow and spindle-shaped and contain an uneven distribution of coarse electron dense granules (Fig. 63). The continuity of the nuclear membrane is interrupted along several places by the presence of nuclear pores which are filled with a seemingly agranular material. The immediate inner areas of the nuclear pores, however, either lack the nuclear granules or contain very few (Fig. 64).

Strands of fine fibril-like material occur in several areas of the cytoplasm (Fig. 65), the latter also containing coarse granules (ribosomes?) similar to those in the nucleus (Figs. 64 and 65); several mitochondria (Fig. 64) and some fine granular material (Fig. 65). Along the dorsal areas of the cytoplasm i.e. below the prominent and homogeneous basement membrane, are vacuoles which may represent lipid droplets (Fig. 65).

Formation of the zona pellucida begins as a highly irregular layer of electron luscent, homogeneous material deposited between the plasma membrane of the oocyte (oolemma) and the follicle cell layer. The oolemma develops sinuous microvilli which traverse the substance of the zona pellucida and establish contact with the plasma membrane of the follicle cells (Figs. 63 - 65). Within longitudinal portions of the microvilli, fine filaments or fibrils can be discerned which run parallel to or closely follow the shape of the microvilli, thereby appearing as minute granular inclusions in regular cross-sections of these microvilli (Fig. 64). The material of the zona pellucida appears to be formed in the ooplasm and probably diffuses through the oolemma where it accumulates between this plasma membrane and the follicle cell layer.

Areas of cell membrane attachment (desmosomes) occur where the oolemma and the plasma membrane of the follicle cells meet (Fig. 65), prior to their separation by the accumulation of the zona material.

In early vitellogenic oocytes, the thickness of the zona pellucida is greatly increased, with a dark homogeneous substance pervading through it except for the extreme apical and basal regions. The small pores in the basal region represent the very sinuous micro-

villi of the oolemma, while the larger, wider pores throughout the rest of the zona pellucida are the sinuous cytoplasmic pedicels of the follicle cells, these also penetrating the zona material as far as its basal region (Fig. 66). It is uncertain whether or not there is continuity of the follicular pedicels with the microvilli of the oolemma or the ooplasm, and to this end, improved fixation techniques would be of great importance. The apical surface of the zona pellucida appears undulating and the nuclei of the follicle cells have adopted a short and broad oval shape. The slightly undulating nuclear membranes probably suggest a morphological instability of the follicle cells as a whole due to active physiological processes occurring within these cells.

A noticeable feature of the zona pellucida of later vitellogenic oocytes is the uniform level of its apical surface and its reduction in height. The substance of medium electron density occurs almost throughout this layer. The follicle cells appear cuboidal and the nuclei are ovoid with non-undulating nuclear membranes. Both the microvilli and the cytoplasmic pedicels of the follicle cells at this stage alter from a sinuous to a more radial form (Fig. 67). Although there is no clear structural evidence that the microvilli reach the apical surface of the zona pellucida and even further into the follicular cyto-

plasm, some of them do seem to merge with the cytoplasmic pedicels, thereby probably providing a means of continuity with the ooplasm and the cytoplasm of the follicle cells.

It has been noted that oocytes released from the ovary into the body cavity, have a ruptured follicular epithelium which can be easily peeled off. This is probably due to loss of attachment or adhesion between the follicular layer and the zona pellucida of the oocyte, provided by the cytoplasmic pedicels of the follicle cells. Towards ovulation, these pedicels probably regress, thereby releasing the follicle cell layer from the rest of the oocyte. Rupture of this layer may be due to the pressure exerted by the jelly-like layer that is produced between the follicular epithelium and the zona pellucida or to the increased pressure of the follicle cells against each other caused by the regressed cytoplasmic pedicels.

Cytoplasmic organelles, inclusions, etc. are more prominent and well developed in the follicle cells of vitellogenic oocytes (Fig. 68). Numerous membrane-bound mitochondria with wide tubular cristae occur in the granular cytoplasm (Figs. 68 and 69). The coarse granules (ribosomes?) of the rough endoplasmic reticulum closely resemble the nuclear granules. Only some portions of Golgi lamellae are evident, while the rest

of the lamellae have pinched off their dilated tips into vesicles such that certain areas of the cytoplasm consist mainly of such vesicles (Fig. 70). Some of these vesicles are filled with a fine, granular material of medium electron density, while in the majority, the material is limited to the vesicle membranes.

The presence of membrane-bound vesicles, some empty, others partially filled with the coarse, electron dense granules and a few containing a mixture of fine and coarse granules (together with some non-granular material) (Fig. 68), may suggest a synthesizing and secretory activity of the follicle cells. Furthermore, the area below the undulating nuclear membrane, consisting of such vesicles or vacuoles may be the phsiologically active site of such synthesis and secretion of probably lipids and proteins.

ii). Thecal layer

The theca is composed of the collagen fibres of connective tissue and their associated spindle-shaped fibroblasts or thecal cells (Fig. 68) and forms several discontinuous layers in vitellogenic oocytes, while immature protoplasmic oocytes usually have a single layer. Ultrastructurally, the nuclei of thecal cells appear identical to those of the follicle cells i.e. both containing the uneven distribution of the

coarse electron granules. Most thecal cells contain very little cytoplasm which lacks recognizable organelles or features. Where the cytoplasm is present in adequate amounts, mitochondria can be faintly discerned which appear undeveloped and have narrow tubular cristae (Fig. 72). Other organelles in the granular cytoplasm include Golgi complexes, the lamellae of which are narrow and the pinched off vesicles are small and few. As in the follicle cells, the Golgi complexes in thecal cells are associated with a fine granular material (Fig. 71). No areas of typically granular or agranular endoplasmic reticulum were observed. Since the thecal cells appear less developed than the follicle cells, the theca may serve mainly as a supportive fibrous meshwork for the maturing oocytes.

The follicle cells which are established as early as in the pre-protoplasmic oocytes, seem to originate from the fibroblasts. These eventually become developed and modified during the later oocyte stages, especially during vitellogenesis. Their dramatic increase in number from the early dark staining protoplasmic oocytes to the late light staining protoplasmic oocytes and the maturation stages from thereon, appears to be due to framentation, this process also occurring in the fibroblasts or thecal cells. Figure 68 shows a fragmenting nucleus of a thecal cell.

Although the follicle cells lack ultrastructural evidence of typical steroidogenic activity (i.e. namely extensive areas of agranular, vesicular endoplasmic reticulum), in the seeming absence of a developed or functional theca, the possibility that the follicle cells have some steroidogenic capacity, cannot be entirely ruled out.

ULTRASTRUCTURE OF THE OOCYTES

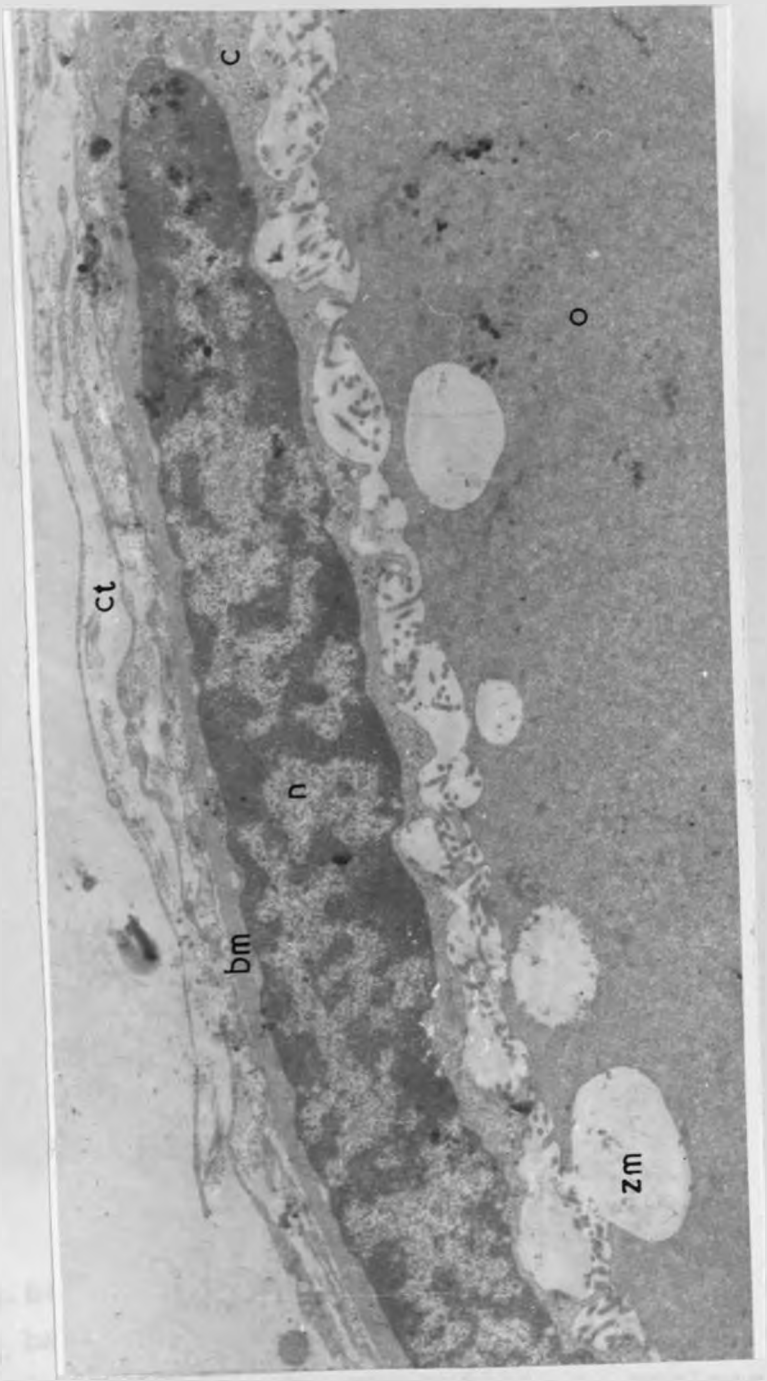


Fig.63: Follicle cell of an early protoplasmic oocyte. bm: basement membrane; c: cytoplasm; ct: fibres of connective tissue; n: nucleus; o: ooplasm; zm: zona material. Mag.X 7,750.

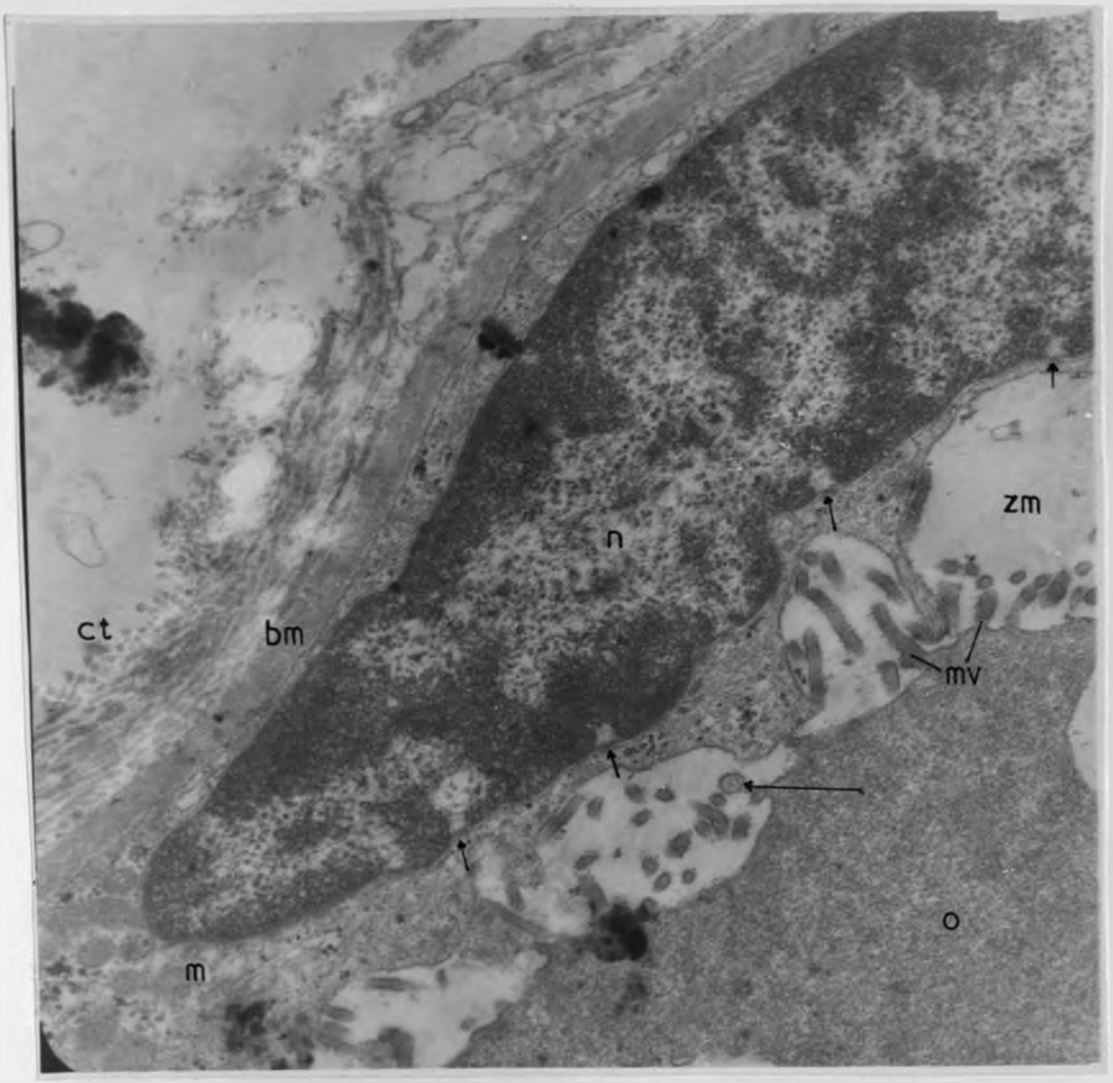


Fig.64: Follicle cell of an early protoplasmic oocyte. bm: basement membrane; ct: fibres of connective tissue; m: mitochondria; mv: microvilli; n: nucleus; o: ooplasm; zm: zona material; short arrows: nuclear pores; long arrow: transverse section of a microvillus, showing fibrils within. Mag.X 26,000.

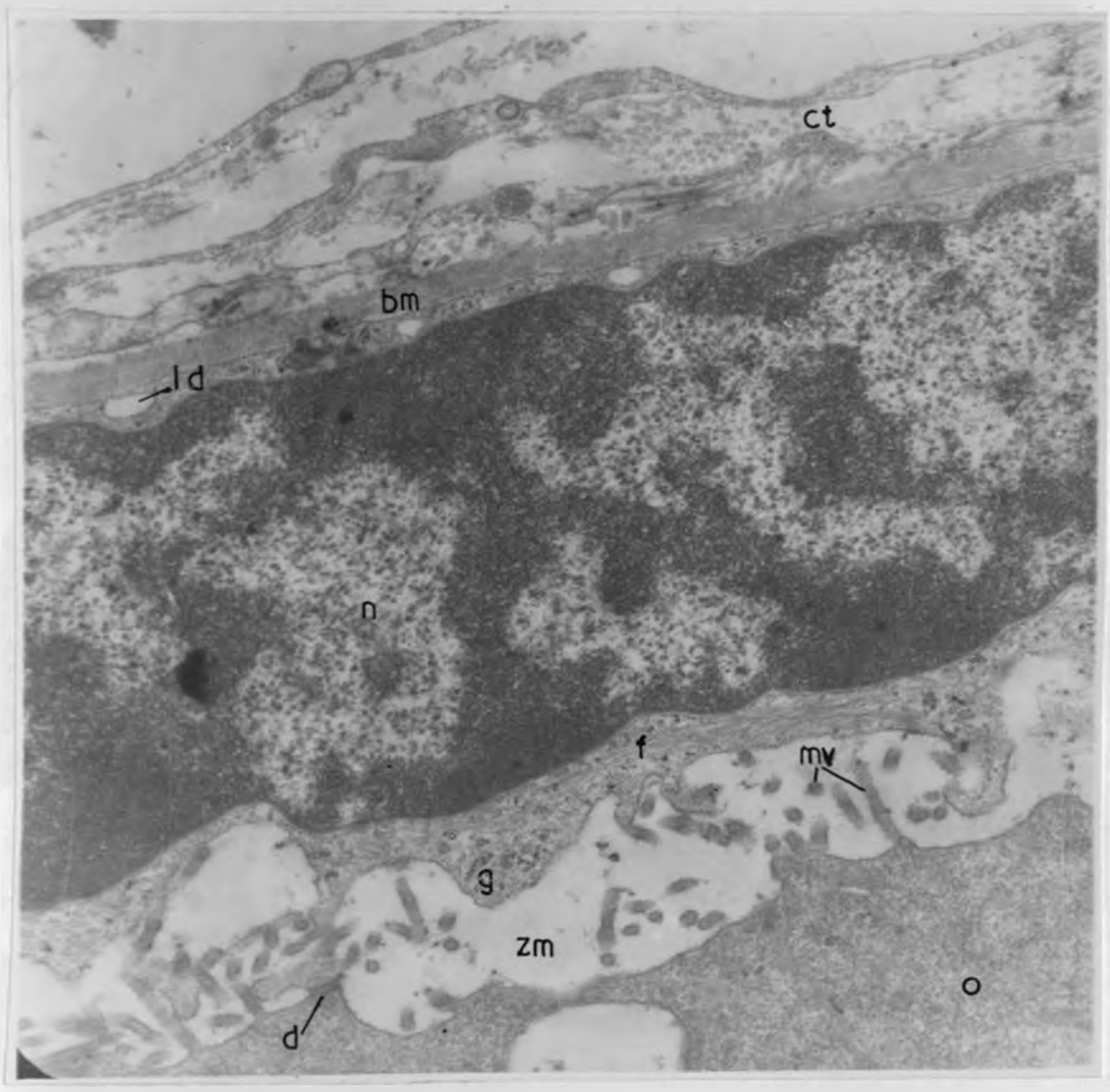


Fig.65: Follicle cell of an early protoplasmic oocyte. bm: basement membrane; ct: fibres of connective tissue; d: desmosomes; f: fibrils; g: granules; ld: lipid droplets; mv: microvilli; n: nucleus; o: ooplasm; zm: zona material. Mag.X 26,000.

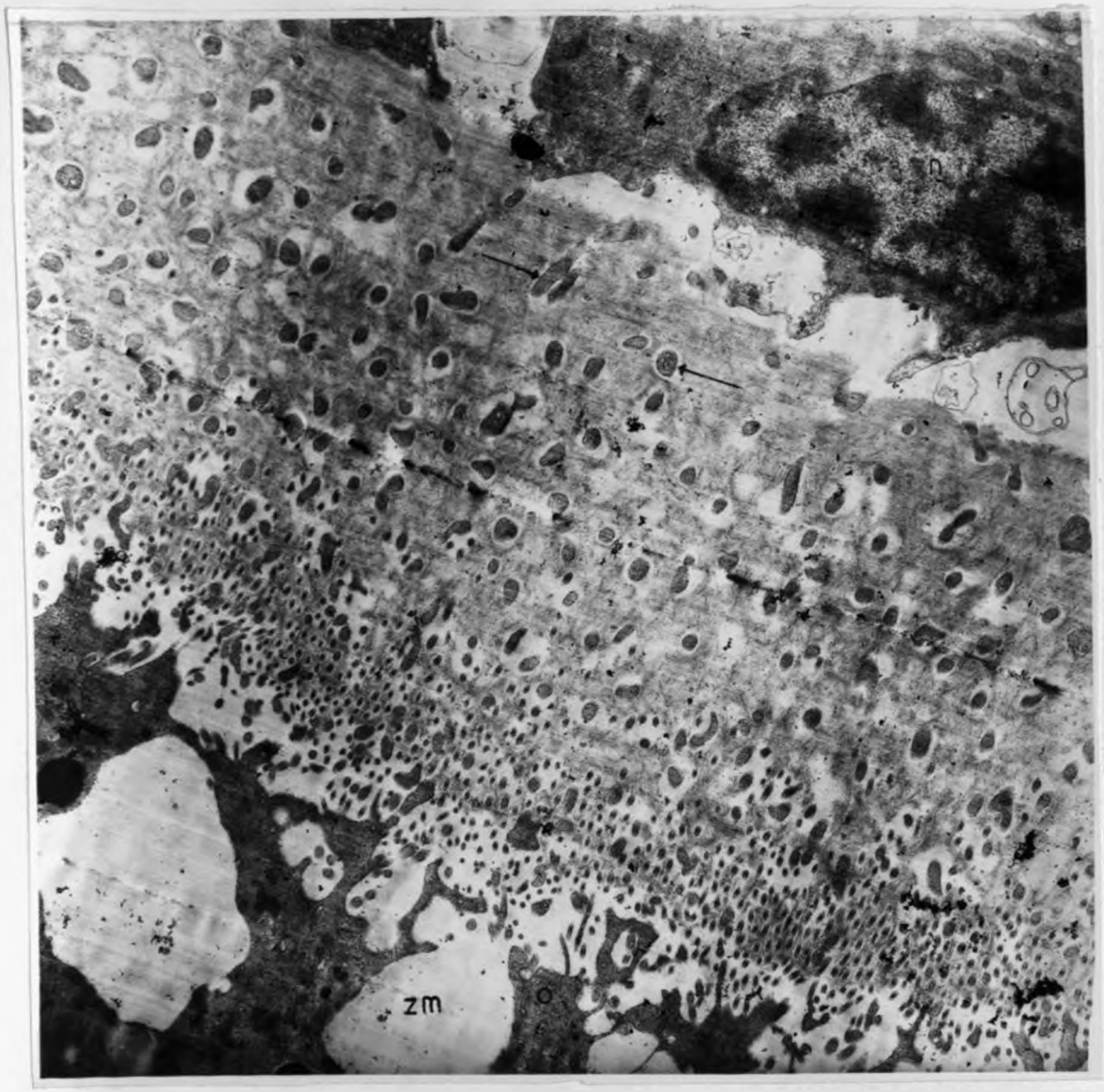


Fig.66: Follicular layer of an early vitellogenic oocyte. n: nucleus of follicle cell; o: ooplasm; zm: zona material; arrows: cytoplasmic pedicels or macrovilli. Note very sinuous micro- and macrovilli and undulating surface of the zona pellucida. Mag.X 6,800

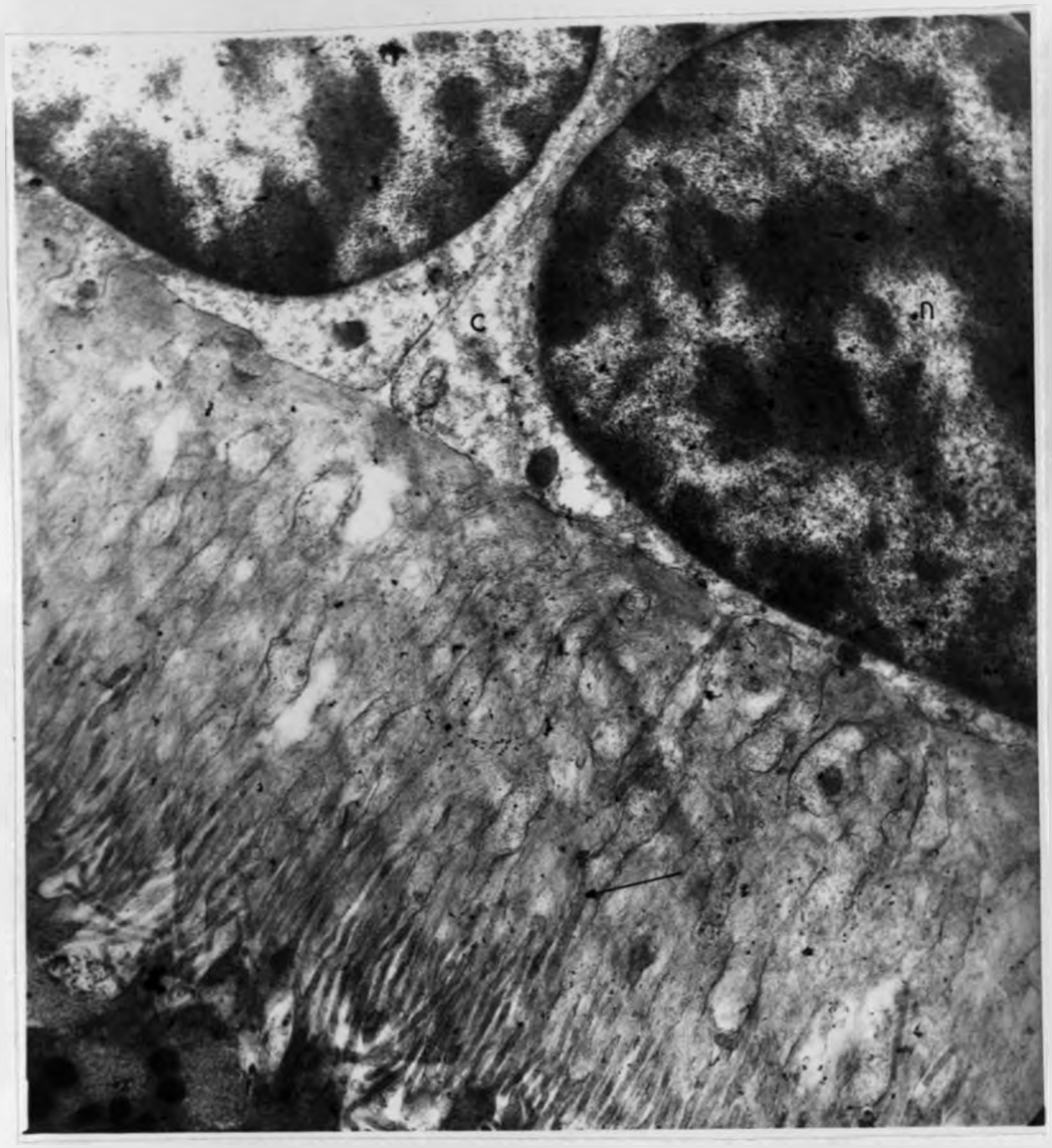


Fig.67: Follicular layer of a later vitellogenic oocyte. Note that both the micro- and macrovilli are radial in form instead of sinuous, and that the apical surface of the zona pellucida is uniform or even. c: cytoplasm; n: nucleus; arrow indicates where a microvillus appears to merge with a macrovillus. Mag.X 9,760

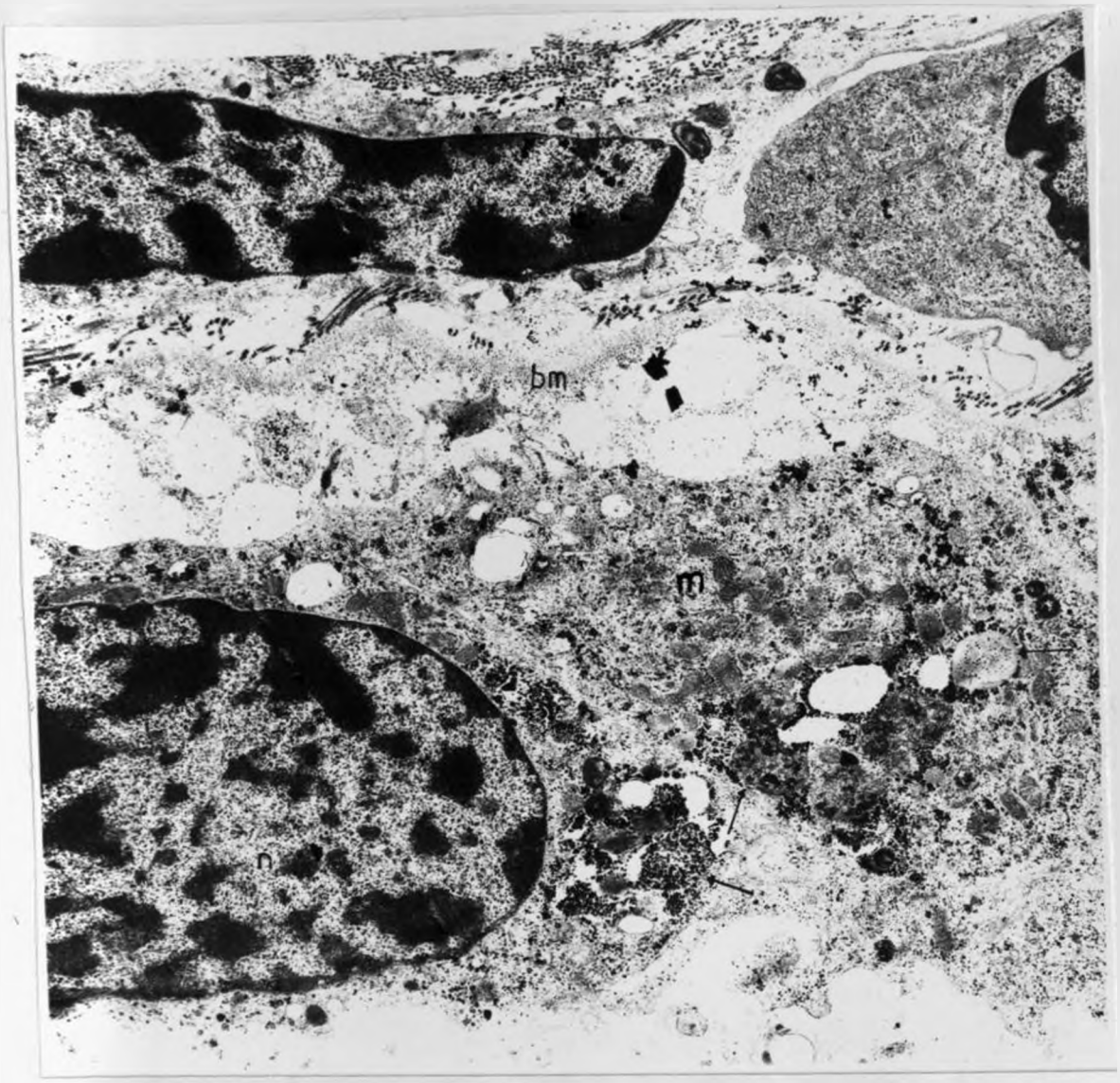


Fig.68: Cytoplasmic organells in the follicle cells of vitellogenic oocytes. These include mitochondria (m) and vesicles, either partially filled with coarse granules or together with non-granular material (arrows). bm: basement membrane; n: nucleus; t: thecal cell. Mag.X 3,750

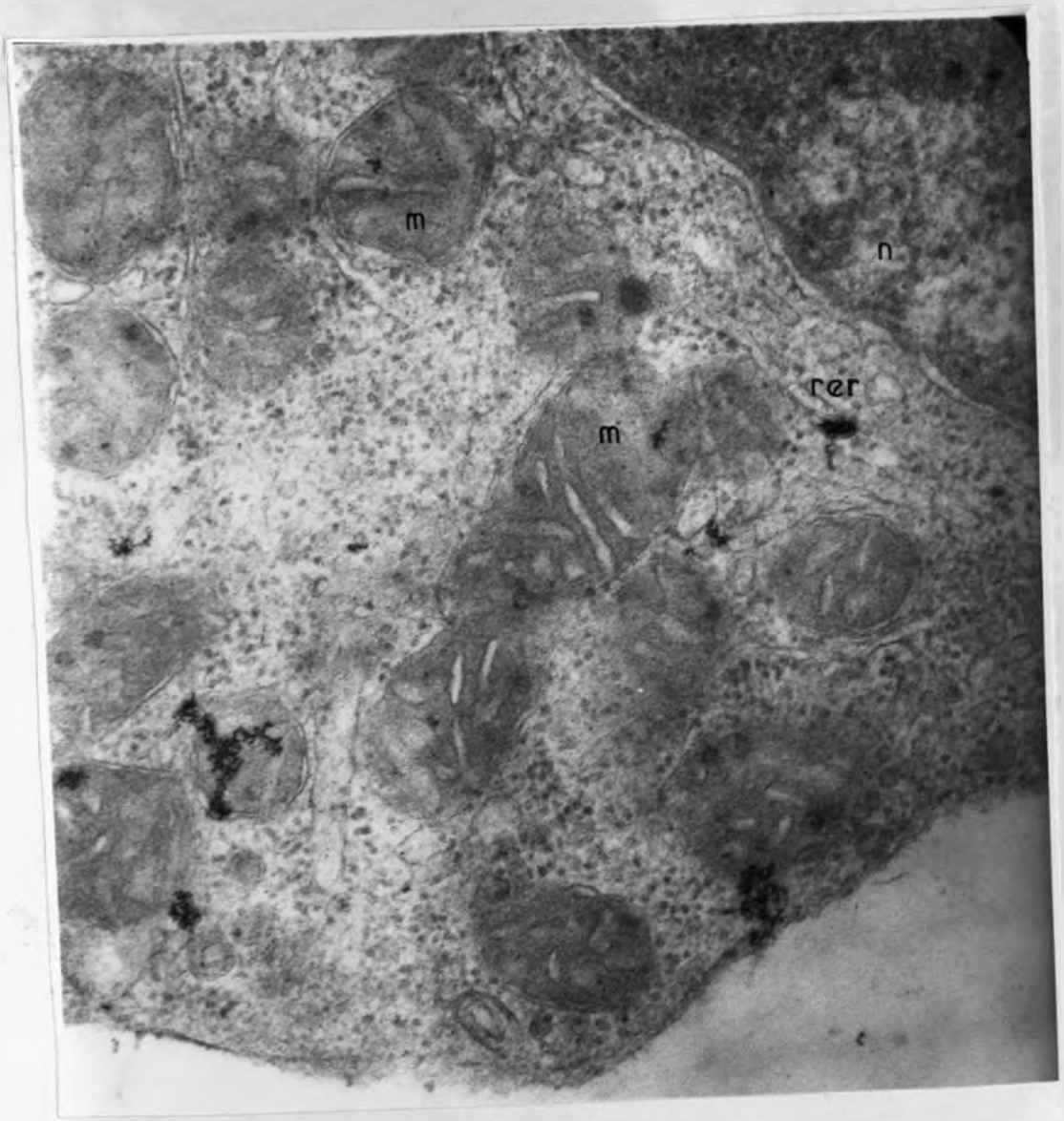


Fig.69: Mitochondria (m) in the granular cytoplasm of follicle cells of vitellogenic oocytes. These have developed tubular cristae. Endoplamic reticulum is of the rough or granular variety (rer). n: nucleus. Mag.X 63,400

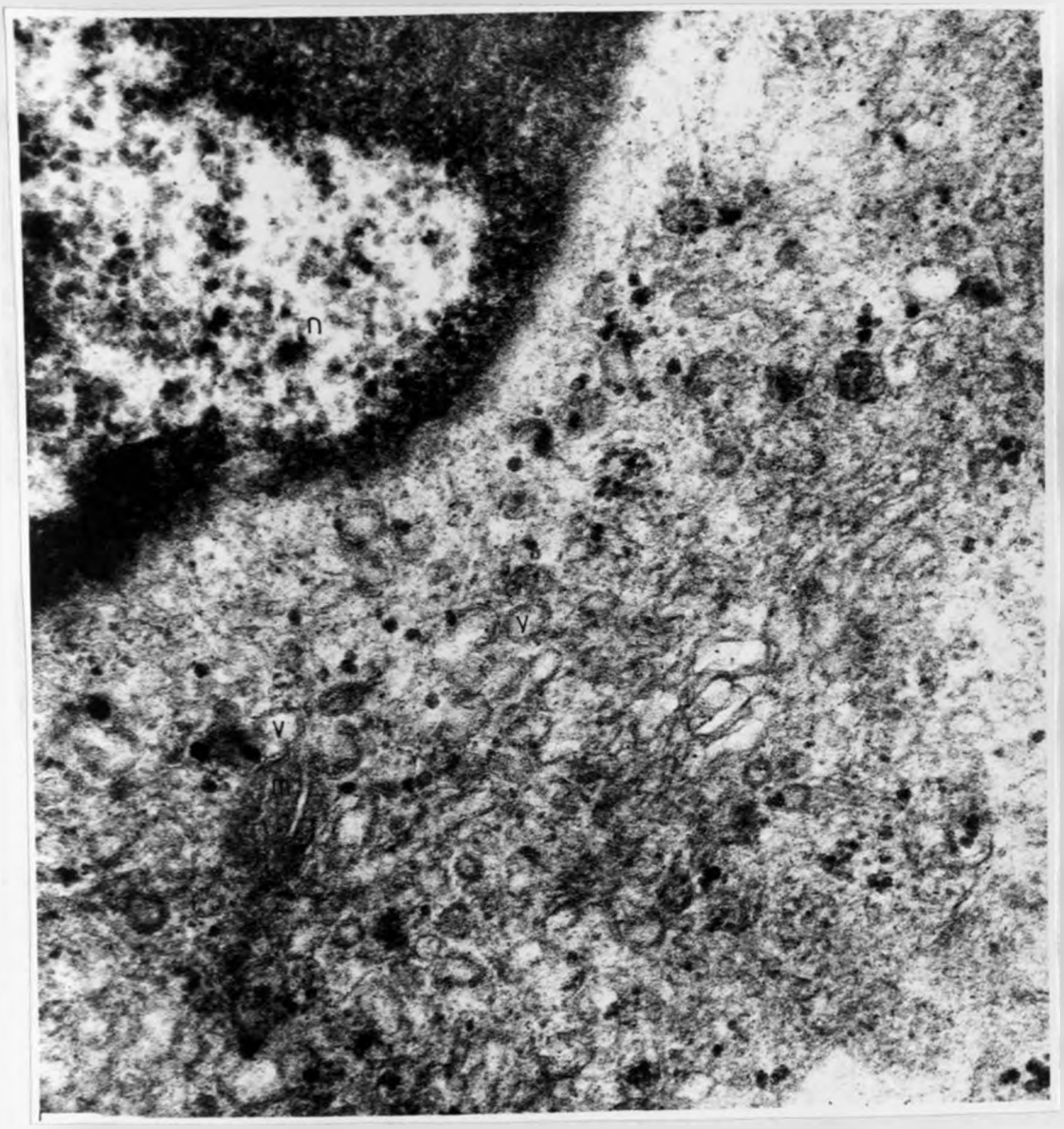


Fig.70: Remnants of a Golgi complex (Gc) and its vesicles (v) in the follicular cytoplasm of a vitellogenic oocyte. Vesicles are associated with a fine, granular material. m: mitochondria; n: nucleus. Mag.X 77,500

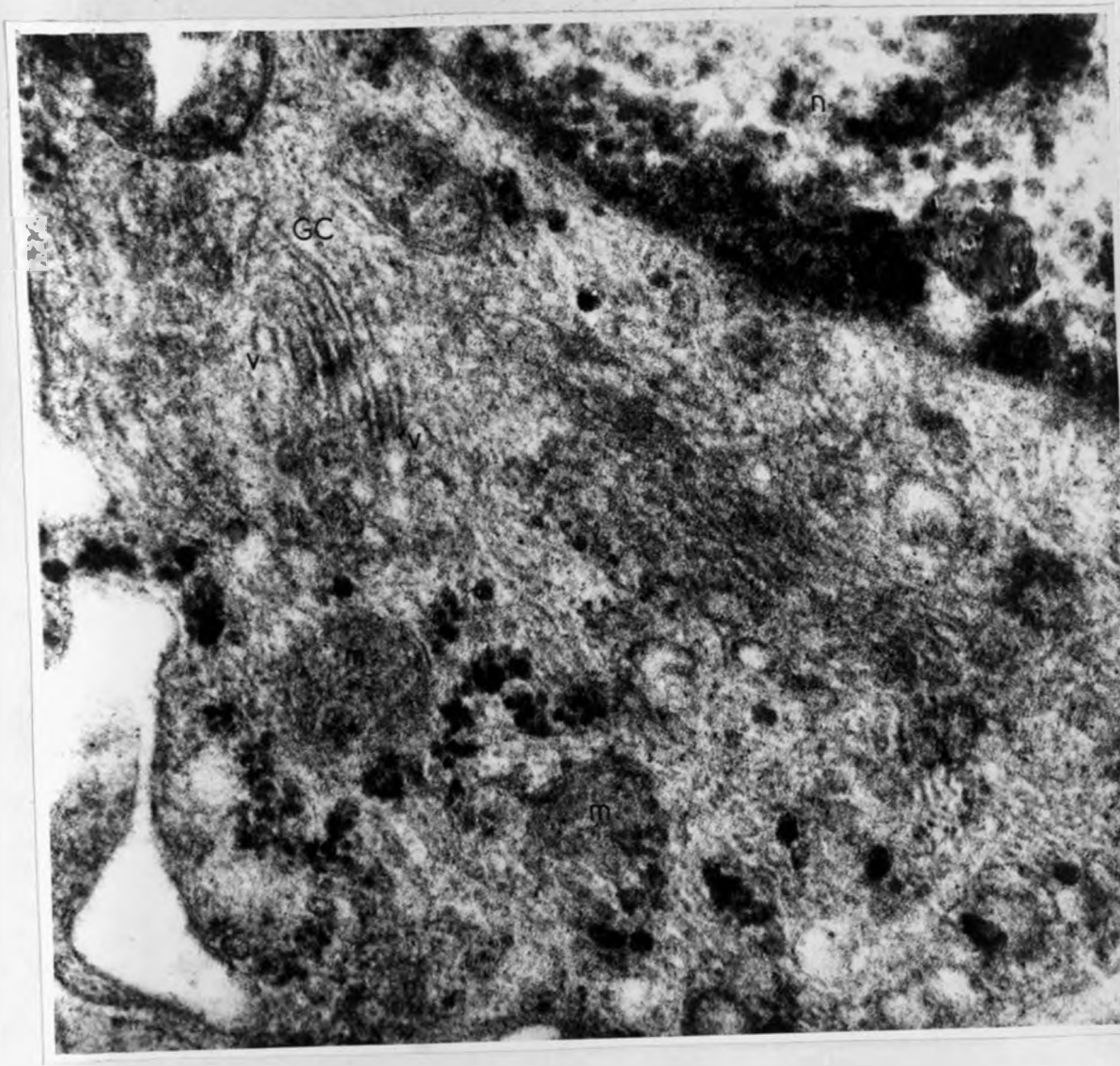


Fig.71: Cytoplasm of a thecal cell. Note ill-developed or undifferentiated mitochondria (m) and Golgi complex (Gc) with narrow lamellae and a few, small vesicles (v). Endoplasmic reticulum, either granular or agranular, is lacking. n: nucleus. Mag.X 64,600.

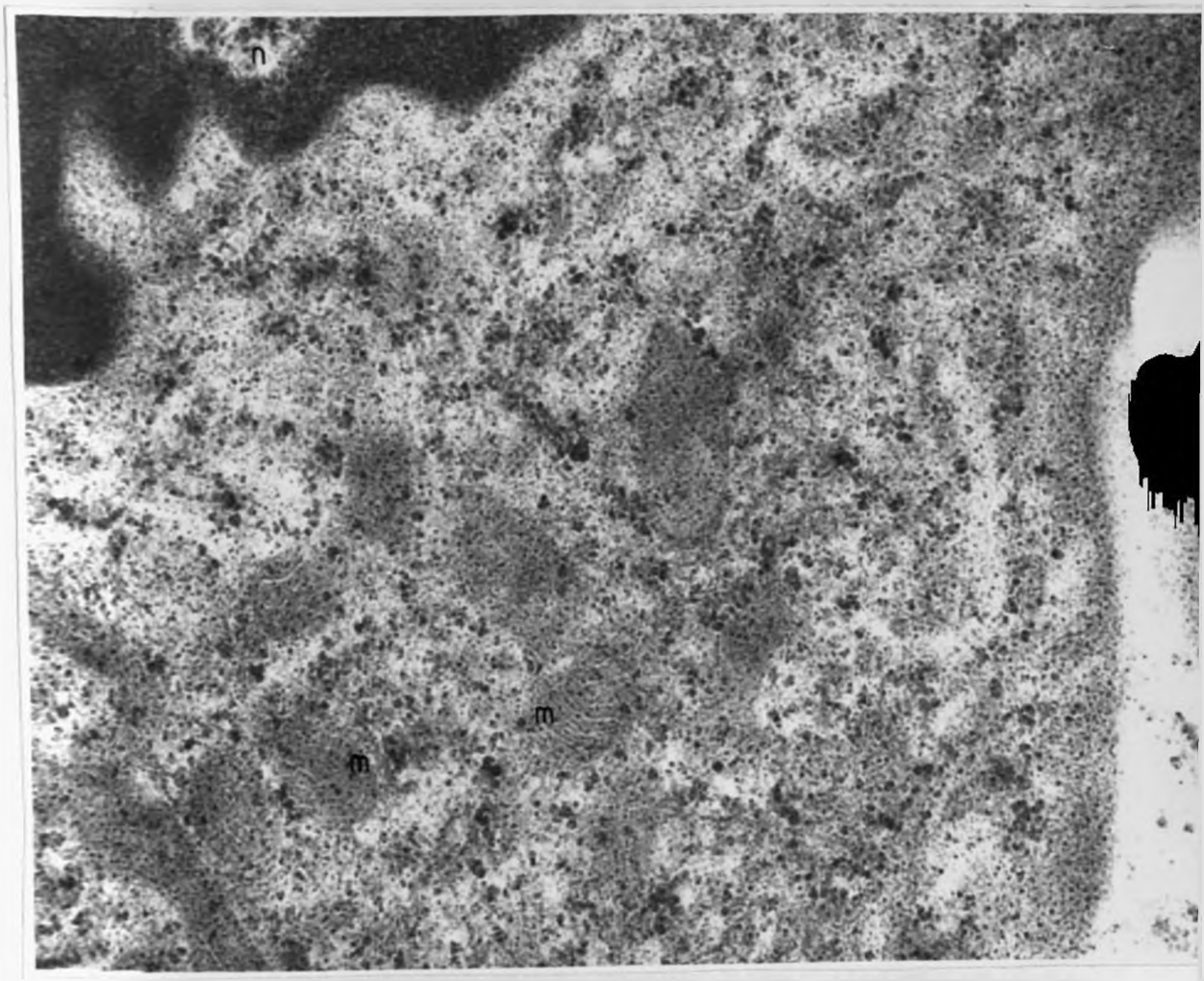


Fig.72: Cytoplasm of a thecal cell with undifferentiated mitochondria (m) having narrow, tubular cristae. n: nucleus. Mag.X 50,000.

4.3 Correlation of the state of the pituitary pars distalis of P. aethiopicus with the stage of gonadal maturation.

The structure of the lungfish pituitary has been described in the literature review (CH. 2.3.5., Fig. 7). Previously, by relating or correlating the size of the lungfish P. aethiopicus with the state of the pars distalis, specifically with variations in the three types of basophils, Kerr and van Oordt (1966) tentatively regarded the types 1, 2 and 3 basophils as the TSH, FSH and LH producing cells respectively. Recently however, the technique of immunohistochemistry identified the type 3 basophils in the South American lungfish L. paradoxa as being the ACTH-producing cells (Hansen et al, 1980). Furthermore, in the amphibians, which are phylogenetically related to the lungfishes, the type 3 basophils were also demonstrated by immunohistochemistry, to be the ACTH-producing cells (Doerr-Schott, 1976), On this evidence, there is perhaps no reason to disregard the type 3 basophils in P. aethiopicus as also being the ACTH-producing cells.

The pituitary generally reflects functional changes in its target organs (i.e.thyroid; adrenals; gonads) by structural variation in certain of its cell types. By correlating different stages of gonadal maturation with the state of the basophils types (namely

types 1 and 2) in the pars distalis, we hope to identify those basophils which, with some degree of accuracy, can be regarded as the gonadotropin-producing cells.

Sagittal sections of P. aethiopicus pituitaries were stained with (AB-PAS-OG), in accordance with the staining method employed by Kerr (1965), and nomenclature of the basophil types was retained. In all the pituitaries examined, the type 2 basophils were absent, and it were the type 1 basophils that exhibited noticeable variation with different stages of gonadal maturation. Body length of these lungfish ranged from 18.5cm to 66.5cm.

The pituitary of female P. aethiopicus with immature or early maturing ovaries (i.e. ovaries either containing all protoplasmic oocytes or a few early vitellogenic ones within a majority of protoplasmic oocytes) resemble each other. Their pars distalis contain numerous and very distinct type 1 basophils which occur almost throughout this region except for the extreme posterior region and the rostral (anterior) pars distalis, the latter being occupied by the violet type 3 basophils (Fig. 73). Basophils type 1 occur almost all the way to the hypophysial cavity and their chromophilic substance is prominent and deeply stained. Most of these cells are dominated by the presence of vacuoles whereas some are filled solely with AB-PAS

positive granules (Fig. 74). A similar situation exists in the pars distalis of an immature male P. aethiopicus having testes that are at either Stage I or II of maturation.

The type 1 basophils in the pars distalis of a mature female (i.e. ovaries that contain mainly mature, vitellogenic oocytes) appear very reduced in number or distribution together with a reduction in staining intensity and extent of their chromophilic substance. Such basophils are almost absent in the posterior or proximal half of the pars distalis (Fig. 75). There are also almost no discernible granules within these cells which appear to be reverting the chromophobic condition (Fig. 76).

The pars distalis of maturing males closely resembles that of the immature and early maturing female P. aethiopicus, except that there appears to be more type 1 basophils in the proximal pars distalis (Fig. 77). These basophils are again dominated by several vacuoles and a few granules, prominent and deeply stained chromophilic substance, and only some are partially filled with the AB-PAS positive granules (Fig. 78). This may suggest an increase in the secretory activity of these basophils.

In mature male P. aethiopicus, the discernible

type 1 basophils are restricted to the anterior one-third of the pars distalis (Fig. 79) and there also appears to be a general decrease in the extent of the chromophilic substance of most cells. Only a few type 1 basophils have a sparse accumulation of the AB-PAS positive granules, while vacuoles predominate in the rest. Faint remnants of the chromophilic substance occur throughout the rest of the pars distalis.

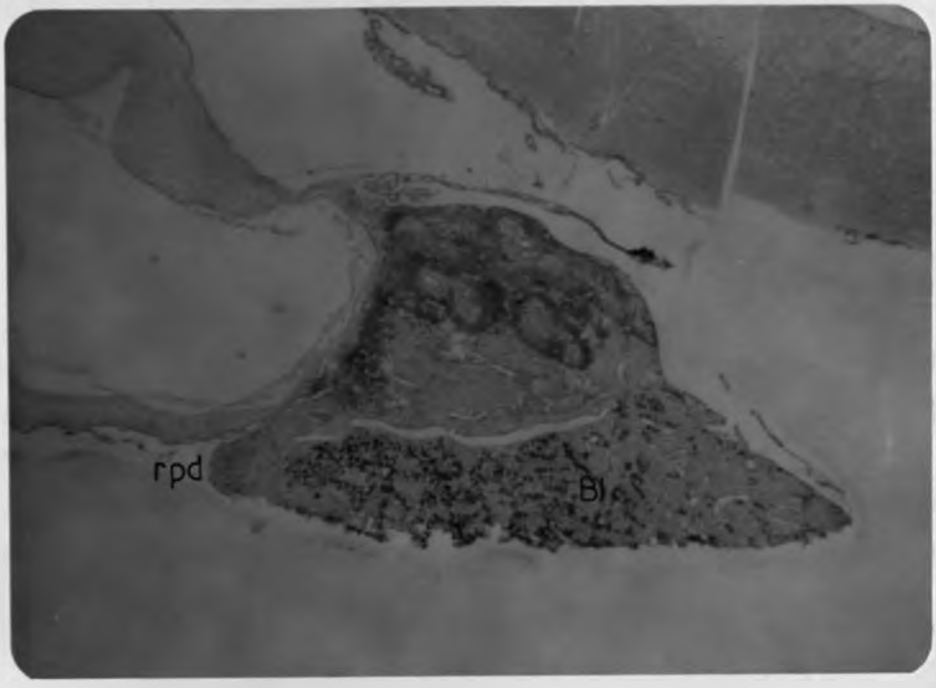
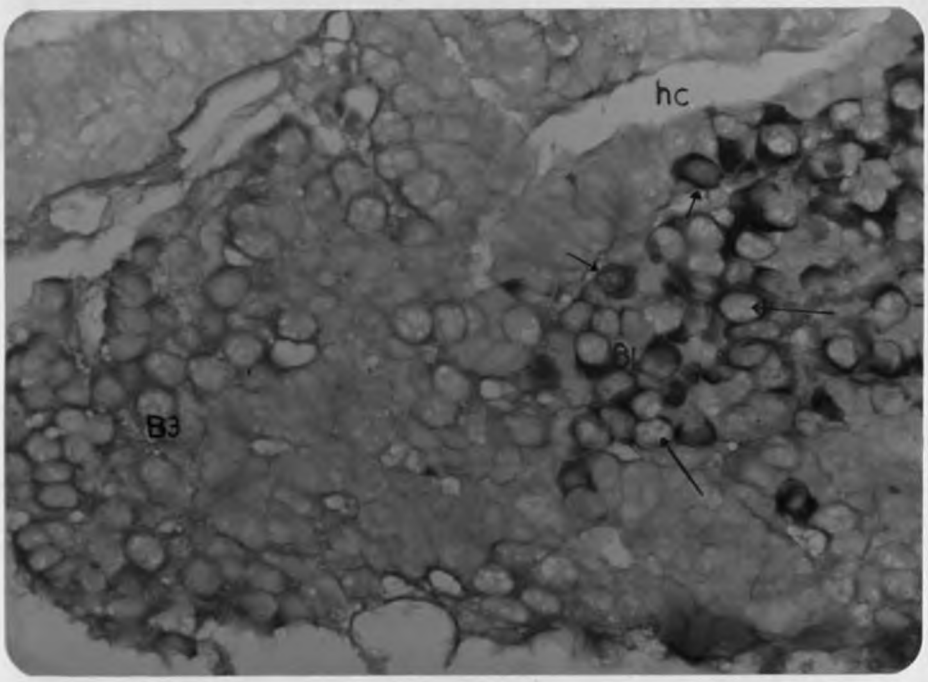


Fig.73: Low power view of the pituitary of an immature or maturing female P. aethiopicus. Type 1 basophils (Bl) occur almost throughout the pars distalis except for the rostral (anterior) pars distalis (rpd) which is occupied by the violet type 3 basophils, and the extreme proximal (posterior) pars distalis. Mag.X130.

Fig.74: Close-up of Fig.73. Type i basophils (B1) have prominent and deeply staining chromophilic substance. Most of them contain vacuoles (long arrows) while others are filled with AB-PAS positive granules (short arrows). B3: basophils type 3; hc: hypophysial cavity. Mag.X 925.



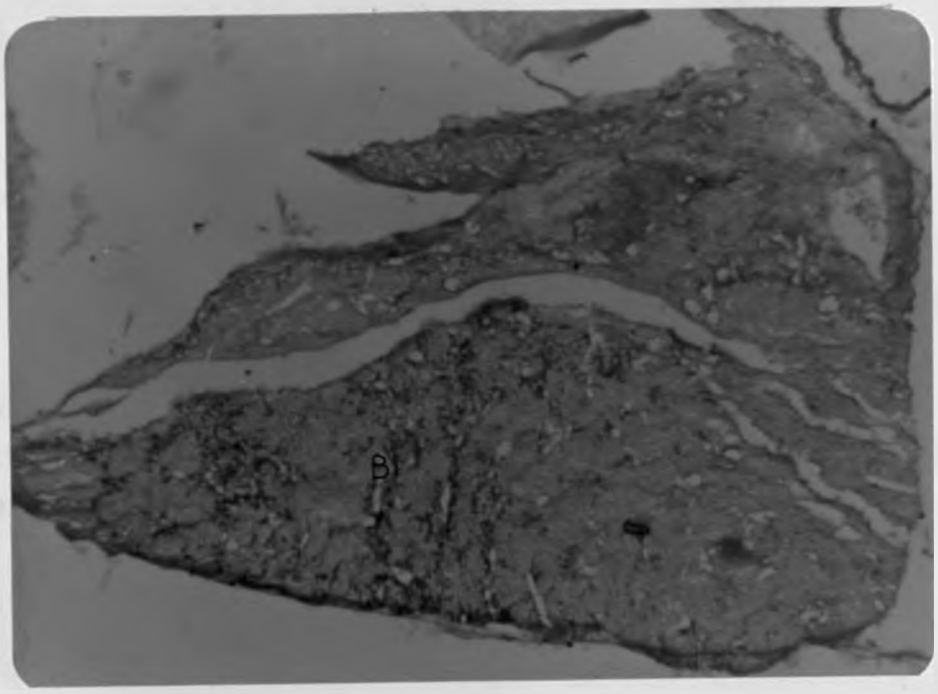


Fig.75: Low power view of the pituitary of a mature female P. aethiopicus. Type 1 basophils (Bl) are faint and their distribution in the pars distalis has lessened. Mag.X 233.

Fig.76: Close-up of Fig.75. Extent and staining intensity of the chromophilic substance of the type 1 basophils (B1) are reduced. Mag.X 925.

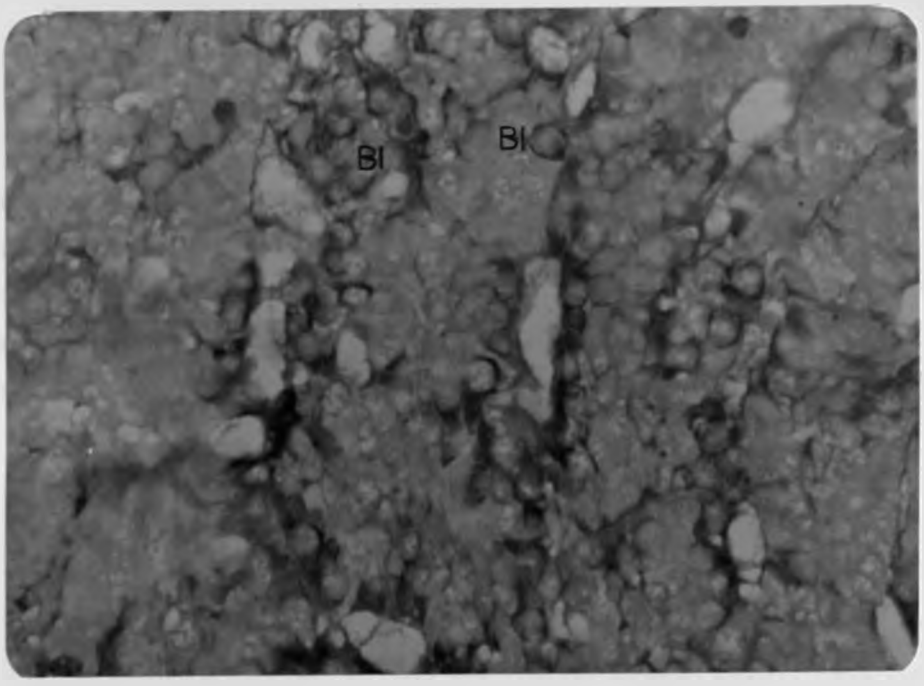


Fig.77: Low power view of the pituitary of a maturing male P. aethiopicus. The rostral pars distalis (rpd) is occupied by the violet type 3 basophils (B3). Type 1 basophils (B1) occur throughout the rest of the pars distalis, including the proximal region. Mag.X 130.

Fig.78: Close-up of Fig.77. Most of the type 1 basophils (B1) are dominated by the presence of vacuoles. Chromophilic substance is prominent and intensely stained. Mag.X 592.

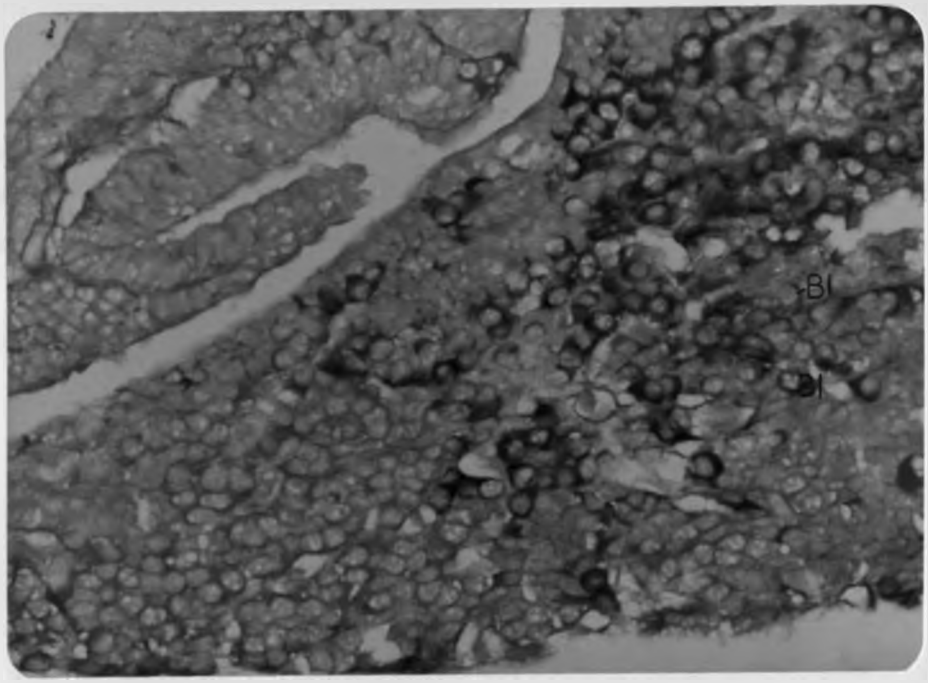
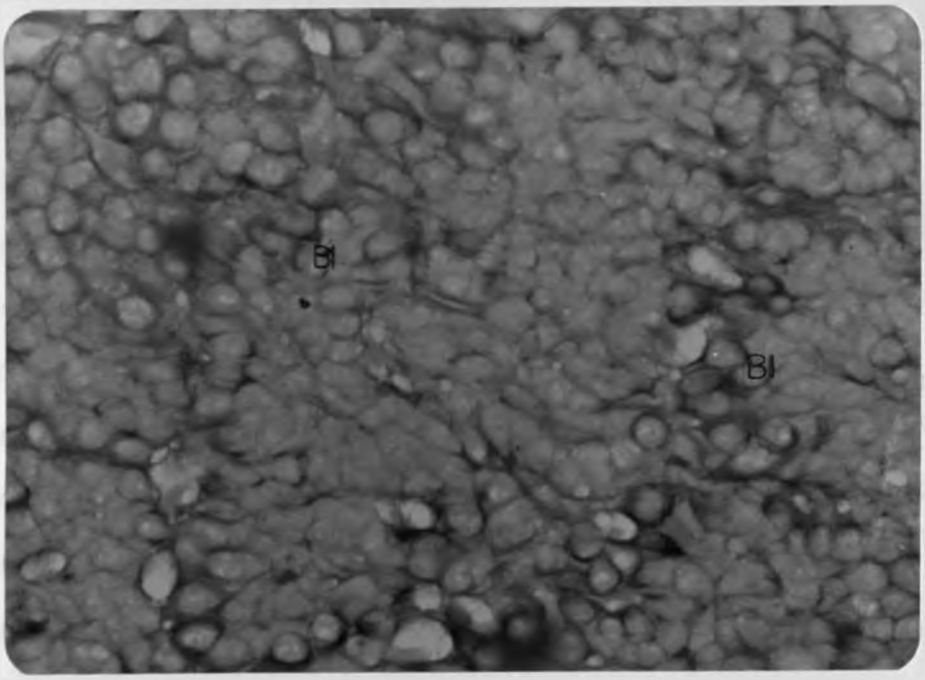


Fig.79: Low power view of the pituitary of a mature male P. aethiopicus. Basophils type 1 (B1) are limited to the anterior one-third of the pars distalis.
B3: basophils type 3 of the rostral pars distalis.
Mag.X 130.

Fig.80: Close-up of Fig.79. Type 1 basophils (B1) appear faint since extent and staining intensity of their chromophilic substance is reduced. Mag.X 925.



5. DISCUSSION

5.1. Testis

As in all the teleosts and amphibians cited in the literature review, spermatogenesis in P. aethiopicus also appears to be cystic, this view being supported by the apparent observation of the spermatogenetic generations occurring in clusters or groups, although surrounding cyst membranes are not readily evident, and the presence of visible cysts of late secondary spermatocytes in the testis of Stage V. Spermatogenesis in the cyclostomes and elasmobranchs may also be regarded as cystic since each follicle or ampulla only contains a single generation of germ cells (as in the teleosts and amphibians) which all develop synchronously (Dodd, 1960; Walvig, 1963; Hoar, 1969; Hardisty et al, 1967; Callard et al, 1978). Cystic spermatogenesis involves several multiplications of the spermatogonial generations and enables the testis to produce copious quantities of spermatozoa for spermiation during the breeding season. Such a pattern is said to be mainly suitable for a reproductive cycle where the spawning or breeding season is short but intense and where fertilization is external, as in the anamniotes (Lofts, 1968). In P. aethiopicus, breeding or spawning is suggested to occur during the rainy seasons and also during the unseasonal wet

periods (Greenwood, 1957). Even after spermiation in most of the tubules (Stage V), there was already spermatogenetic recovery in some of the tubules. This may reflect the capability of P. aethiopicus undergoing several spawnings within a single rainy season.

The testis of teleosts lacks a permanent germinal epithelium (Lofts, 1968; Hoar, 1969; Callard et al, 1978), whereas such occurs in the testis of the cyclostomes (Dodd, 1960; Walvig, 1963; Tsuneki and Gorbman, 1977a) and the anuran amphibians (Lofts, 1968; Callard et al, 1978). The elasmobranchs too possess a "germ-line" or "tubulogenic zone" which runs along the medio-ventral surface of the testis and proliferates follicles or ampullae of germ cells (Dodd, 1960; Hoar, 1969). With the use of autoradiography, Ruby and McMillan (1972) showed that the interstitium of E. inconstans was the intermediate source of germ cells and implicated the vascular stroma as being the original source. The presence of spermatogonia and chromosomal or mitotic figures in the testicular stroma of P. aethiopicus, suggests that this area may be the source of germ cell proliferation and spermatogonia, though not necessarily the original source of the germ cells.

Seasonal or cyclical histological changes have been observed in the interstitial cells (Leydig cell

homologues) of the lamprey L. fluviatilis (Hardisty et al, 1967), the elasmobranchs (Hoar, 1969), the teleosts (Lagios, 1965; Rai, 1965; Chan and Phillips; 1967; de Vlaming, 1972; Lofts et al, 1966) and the amphibians (Mathews and Marshall, 1956; Lofts and Boswell, 1960; Lofts, 1964; 1968; Lofts et al, 1972, Callard et al, 1978). Generally, during active spermatogenesis, the nuclei of the Leydig cells were large and prominent and round or oval in shape. After spermiation, these Leydig cells atrophied, with their nuclei becoming shrunken or pycnotic (Lofts and Boswell, 1960; Lagios, 1965; Chan and Phillips, 1967; Lofts et al, 1966; 1972; de Vlaming, 1972). In the immature testis of L. fluviatilis, the interstitial tissue consisted only of a few isolated connective tissue cells but during the later stages of maturation, these cells increased in number and formed compact acinar groups (Hardisty et al, 1967). Similarly, the interstitial tissue of R. nigromaculata was reported to occur in the greatest amount during the breeding season and least during active spermatogenesis (Mathews and Marshall, 1956). Interstitial cells were previously reported as being lacking from the testis of E. lucius (Marshall and Lofts, 1956), S. fontinalis (Henderson, 1962) and S. salar (O'Halloran and Idler, 1970). However, a very recent study on the cellular organization of the testis of teleosts, states that intersitial cells do occur in all teleosts and are a

typical component of the interstitium, and that earlier reports stating that these cells were absent in certain teleosts, are incorrect (Grier, 1981). It is possible that previously, these interstitial cells could have gone unobserved or undetected simply because they may not have revealed distinct and evident histomorphological variation with changes in testicular maturation. On the other hand, the testis of B. belone did possess interstitial cells but these revealed no morphological changes that could suggest a steroidogenic function (Upadhyay and Guraya, 1971). In P. aethiopicus, only some of the intersitial spaces in an actively spermatogenetic testis are occupied by amorphous cells and spindle-shaped fibroblasts. With later maturational stages, there appears to be no evident increase in the amount of these cells and in a testis that has undergone spermiation, what appears to be large interstitial masses, are in fact the surface sections of the tubules, the latter being collapsed and lying very close to each other. Furthermore, no distinct morphological changes with different maturational stages were observed in these cells occupying the interstial areas. Since such cells were not of a consistent morphology, any change in them with varying testicular maturation would not have been evident. However, these observations do not conclusively prove that P. aethiopicus lacks functional interstitial cells.

Histochemical evidence of steroidogenesis i.e. the presence of lipids and/or cholesterol-positive droplets, were demonstrated in the interstitial cells of L. fluviatilis (Hardisty et al, 1967), M. albus (Chan and Phillips, 1967), R. temporaria and R.esculenta (Callard et al, 1978) and in the lobule boundary cells (Leydig cell homologues) of the urodele T. hongkongenesis (Tso and Lofts, 1977a). Generally, lipid accumulation in the intersitial cells increased with advanced testicular maturation (Hardisty et al, 1967; Lofts, 1964; 1968; Chan and Phillips, 1967; Tso and Lofts, 1977a). However, just prior to or during breeding in M. albus, there was a sudden depletion of the interstitial lipids (Chan and Phillips, 1967). The evacuated tubules of T. hongkongenesis were similarly devoid of lipid droplets (Tso and Lofts, 1977a). In R. esculenta and R. temporaria, interstitial lipids reached a maximum after spawning, but diminished with the resurgence of spermatogenesis (Lofts, 1964; 1968). The high accumulation of interstitial lipids (such that generally occurs towards peak testicular maturation and after spermiation), indicates a cessation of the steroid hormone (androgen) output, resulting in a build-up of the lipid precursor material. On the other hand, the presence of a few or lack of lipid droplets in the interstitium during resurgence of spermatogenesis, is said to be due to androgen synthesis and secretion, resulting in none or a few droplets of the

lipid material (Lofts and Boswell, 1960; Callard et al, 1978).

The activity of the enzyme involved in the synthesis of steroids i.e. 3β-HSD, has been demonstrated in the interstitial cells of L. fluviatilis (Barnes and Hardisty, 1972), E. lucius (Grier, 1981), the elasmobranchs T. marmorata, S. stellaria (Callard et al, 1978), the urodeles A. mexicanum (Lazard, 1979), T. hongkongenesis (Tso and Lofts, 1977b) and in the anurans R. temporaria, R. esculenta (Callard et al, 1978) and R. cyanophlyctis (Saidapur and Nadkarni, 1973). In L. fluviatilis, the intensity of 3β-HSD activity reached a maximum just prior to the development of the secondary sex characters i.e. prior to breeding, and correlated with an abundance of lipid in the interstitial cells (Barnes and Hardisty,1972). Likewise, this enzyme activity in the interstital cells of A. mexicanum was restricted to the breeding period and active secretion of androgens by the glandular tissue which in turn was related to the stimulation of the secondary sex characters (Lazard, 1979). In R. temporaria and R. esculenta, 3β-HSD activity in the interstitial cells correlated with the lipid cycle: thus during the winter non-breeding season, interstitial cells lacked cholesterol-positive droplets, lipids and 3β-HSD activity. With active spermatogenesis, there was the appearance of

lipids, cholesterol and 3β-HSD activity in the interstitial cells. After spawning, there was greater accumulation of the cholesterol-positive lipid content but the activity of 3β-HSD decreased. This enzyme activity in the testis of the amphibians correlates with the appearance of the secondary sex characters, suggesting that the interstitial Leydig cells secrete the androgens (Callard, et al, 1978). The further demonstration of glucose 6-phosphate dehydrogenase activity in the interstitial cells of R. cyanophlyctis, is said to indicate further but indirect evidence of steroidogenic activity, since this enzyme is said to be the principal one that provides NADPH employed in hydroxylations during steroidogenesis (Saidapur and Nadkarni, 1973). Since histochemical tests have not been carried out on the testis of P. aethiopicus, such tests employing lipid-detecting stains or reagents, 3β-HSD and G6-PDH with various testicular maturational stages, may reveal a lipid cycle in the cells of the interstitium and further suggest whether such cells possess steroidogenic functions. However, if such tests do prove to be negative, the interstitial cells of P. aethiopicus should not be conclusively regarded as being devoid of steroidogenic activity since previous histochemical tests have met with some inaccuracy or discrepancy. Thus, Gresik et al (1973) stressed caution in interpreting negative results when employing histochemical techniques for identifying

steroidogenic tissue, after such tests with both 3β-HSD and lipid-detecting reagents, proved negative in the interstitial cells in the testis of O. latipes but ultrastructural observations revealed that these cells did indeed possess characteristics that suggested a steroidogenic function. A positive 3β-HSD activity was later demonstrated in the interstitial cells of O. latipes by other workers (Grier, 1981).

The interstitial cells of E. stouti, L. fluviatilis and the lobule boundary cells of T. hongkongenesis possess the typical ultrastructural features of steroid-secreting cells i.e. extensive areas of well-developed, agranular, vesicular endoplasmic reticulum, mitochondria with elaborate tubular cristae and lipid inclusions (Tsuneki and Gorbman, 1977a ;Barnes and Hardisty, 1972; Tso and Lofts, 1977b). Lobule boundary cells of T. hongkongenesis were noted to be morphologically developed during maturation of spermatozoa and even more developed during spermiation, probably indicating that the steroid secretory phase of the cells begins when the spermatozoa are ready for evacuation, with the highest steroidogenic activity of the lobule boundary cells occurring when spermiation has just occurred (Tso and Lofts, 1977b). Since mature testicular stages of P. aethiopicus were not included in the present ultrastructural study, it is possible that the ultrastructural features of steroidogenesis

in the interstitial cells are only developed towards peak maturation and spermiation and therefore not evident or detectable in the interstitial cells at earlier maturational stages. Ultrastructurally, the amorphous cells or fibroblasts in the interstitium of P. aethiopicus appear to be undifferentiated or developed and contain only some recognizable mitochondria, portions of membranes and rough endoplasmic reticulum within a granular cytoplasm, and several lipid droplets - the presence of the latter probably suggesting an inactive state of these cells, leading to no steroid secretion and a subsequent build-up of the precursor lipid material. Nicholls and Graham (1972) noted the presence of very few lipid droplets within fully differentiated and apparently actively secreting Leydig cells of C. nigrofasciatum.

Large areas of well-developed, agranular, vesicular endoplasmic reticulum are regarded to be characteristic of steroid-producing interstitial cells (Barnes and Hardisty, 1972; Tso and Lofts, 1977b; Tsuneki and Gorbman, 1977a). Of interest and importance however, the developed interstitial Leydig cells of R. temporaria and B. bufo did not contain vast quantities of smooth or agranular endoplasmic reticulum despite demonstrated histochemical evidence of steroidogenesis i.e. 3β-HSD activity. Instead, these cells had a predominance of rough, cisternal endoplas-

mic reticulum prior to spermiation. It has been suggested that the predominance of rough, cisternal endoplasmic reticulum could be due to the fact that the high rate of synthesis of the protein enzyme that is required for steroid synthesis, requires rough and not smooth endoplasmic reticulum (Unsicker, 1975). Extensive areas of rough endoplasmic reticulum were also reported in the Leydig cells of O. latipes and again regarded as being compatible with a steroidogenic function (Gresik et al, 1973). The finely flocculent material within the cisterns of the rough endoplasmic reticulum is suggested to represent storage of steroid precursors or synthesized products (Nicholls and Graham, 1972). Therefore, on this evidence, should later ultrastructural studies of more mature testicular stages of P. aethiopicus reveal an absence of smooth endoplasmic reticulum in the interstitial cells and instead reveals rough endoplasmic reticulum, a steroidogenic function on the interstitial cells should not be overlooked.

When first described, the lobule boundary cells of E. lucius were regarded to be homologous with interstitial Leydig cells after revealing the presence of cholesterol-positive lipids (Lofts and Marshall, 1957). Similar cells were later described in S. fontinalis (Henderson, 1962), S. salar (O'Halloran and Idler, 1970), B. belone (Upadhyay and Guraya, 1971),

B. tor (Rai, 1965) and in C. nigrofasciatum (Nicholls and Graham, 1972). However, ultrastructural studies of the lobule boundary cells of C. nigrofasciatum (Nicholls and Graham, 1972) and E. lucius (Grier and Linton, 1977) revealed that these cells were in fact intralobular homologs of Sertoli cells, such cells in E. lucius exhibiting identifying criteria such as phagocytosis of residual spermatid bodies and the occasional enveloping of sperm by their cytoplasm (Grier and Linton, 1977). These cells in C. nigrofasciatum also exhibited similar ultrastructural characteristics (Nicholls and Graham, 1972). According to Grier (1981), lobule boundary cells previously described in teleosts are in fact intralobular (intratubular) Sertoli cells. These cells in S. salar, B. belone, the lobule boundary cells (probably Sertoli cells) of L. fluviatilis and the Sertoli cells of T. hongkongenesis, R. esculenta and R. temporaria, were also histochemically demonstrated to contain cholesterol-positive lipids and hence regarded as steroidogenic (O'Halloran and Idler, 1970; Upadhyay and Guraya, 1971; Hardisty et al, 1967; Tso and Lofts, 1977b; Lofts, 1964; Lofts and Boswell, 1960).

Sertoli cells of S. canicula, S. acanthias (Hoar, 1969), S. salar (O'Halloran and Idler, 1970), A. mexicanum (Lazard, 1979), R. temporaria (van Oordt and Brands, 1970) and R. cyanophlyctis (Saidapur and

Nadkarni, 1973) have also demonstrated the activity of 3β-HSD. Towards the breeding season in R. temporaria and R. esculenta, Sertoli cells became glandular in appearance and accumulated lipid droplets (Lofts, 1964; Lofts and Boswell, 1960). Where changes in the interstitial cells have led to parallel changes in the Sertoli cells morphologically and histochemically, have been reported: thus in the testis of L. fluviatilis , during the later stages of maturation, both the interstitial and the lobule boundary cells (Sertoli cells) accumulated extensive lipid droplets and appeared morphologically developed (Hardisty et al, 1967). In the testis of B. tor, cytoplasmic vacuolization occurred in both the interstitial cells and the Sertoli cells during spermiation (Rai, 1965). Furthermore, the Sertoli cells of A. mexicanum revealed intense 3β-HSD activity only during regeneration of the tubules after spawning, while this enzyme activity diminished with the onset of spermatogonial mitosis and the greater part of spermatogenesis. However, in the Leydig cells, 3β-HSD activity occurred only during the final steps of spermiogenesis i.e. during spermiation and the active secretion of androgens related to the development of the secondary sex characters. This delayed correlation between the Leydig and Sertoli cells has suggested that the stimulation of enzyme activity in the Sertoli cells may depend to a certain extent on a steroid factor synthe-

sized by the Leydig cells (Lazard, 1979). Histochemical tests - use of lipid-detecting reagents, 3β-HSD and G6-PDH on the testis of P. aethiopicus may suggest or indicate whether the Sertoli cells of this lungfish possess a steroidogenic capacity and in turn suggest whether correlative changes can be discerned between the Sertoli cells and the cells in the interstitium and with spermatogenesis itself.

Typical ultrastructural features of steroidogenesis occurred in the lobule boundary cells (Sertoli cells) of L. fluviatilis (Barnes and Hardisty, 1972) and in the Sertoli cells of the anurans (Callard et al, 1978). Although the Sertoli cells of E. stouti also contained such organelles as mitochondria, some with tubular cristae, rough and smooth endoplasmic reticulum, glycogen particles, lipid droplets and lysosomes, they were not well developed and such cells in this hagfish were not implicated with a steroidogenic function (Tsuneki and Gorbman, 1977a). Nevertheless it is possible that the Sertoli cells, like the lobule boundary cells of T. hongkongenesis (Tso and Lofts, 1977b) may undergo ultrastructural developmental stages that could correlate with different maturational stages of the testis. The developed Sertoli cells of P. aethiopicus revealed no areas of typically vesicular, agranular endoplasmic reticulum. Instead, cisternal, granular or rough endoplasmic reticulum,

with a finely flocculent material within the cisterns, predominated the cytoplasm of the cells, together with a few lipid droplets, numerous large mitochondria with tubular cristae and well developed Golgi complexes. As in the steroidogenic Leydig cells of R. temporaria, B. bufo (Unsicker, 1975) and O. latipes (Gresik et al, 1973), which lacked vast amounts of smooth, vesicular endoplasmic reticulm and instead possessed cisternal rough endoplasmic reticulum, the Sertoli cells of P. aethiopicus cannot be denied as possessing a steroidogenic capacity since this variety of endoplasmic reticulum is said to be necessary for the synthesis of the protein enzyme that is needed for steroid synthesis (Unsicker, 1975). Furthermore, the finely flocculent material within the cisterns of the rough endoplasmic reticulum is said to indicate storage of steroid precursors or synthesized products (Nicholls and Graham, 1972). The presence of granules in dilated portions of the intermembranal spaces of the Sertoli cells of P. aethiopicus and pinocytotic features at the plasma membrane, may perhaps suggest a nutritive or secretory involvement. Cytological features suggesting secretion of electron dense granules at the plasma membrane were also observed in the Leydig cells of O. latipes, and regarded to be of a proteinaceous nature (Gresik et al, 1973). However, Nicholls and Graham (1972) regarded electron dense granules to be glycogen deposits.

The association of Sertoli cells with spermatogenetic cells or their cysts has been reported in the cyclostomes (Walvig, 1963; Tsuneki and Gorbman, 1977a), elasmobranchs (Dodd, 1960; Lofts, 1968; Hoar, 1969), teleosts (Lagios, 1965; Lofts et al, 1966; Chan and Phillips, 1967; Ruby and McMillan, 1972) and in the amphibians (Mathews and Marshall, 1956; Dodd, 1960; Lofts, 1964; 1968; Lofts et al, 1972). As in E. stouti, M. glutinosa, E. jacksoni, E. inconstans and M. albus, Sertoli cells in the tubules of P. aethiopicus closely ensheath a spermatogonium. Due to the non-staining nature of the cytoplasm and cell membranes in the present study, no association of Sertoli cells with later spermatogenetic stages could be observed at the light microscopy level. 3β-HSD activity or steroidogenic activity within Sertoli cells of amphibians is said to correlate with spermatogonial cell division which may take place when the peripheral plasma androgen level is low or absent. These cells are also said to possibly secrete steroids and stimulate spermatogenesis, without affecting the secondary sex characters (Callard et al, 1978). In P. aethiopicus, the association of Sertoli cells with spermatogonia was frequently observed in a very immature testis where these two cell types predominated and in testes that had undergone spermiation and resurgence of spermatogenesis was already taking place in some tubules - these facts seemingly supporting the influ-

ence of Sertoli cells on spermatogonial mitosis and on spermatogenesis itself.

Where the amount or level of plasma androgen may reflect the nature or amount of the interstitial Leydig cells, has been reported in E. stouti: thus the low plasma testosterone level in E. stouti is attributed to the fact that this hagfish possesses a low number of interstitial cells in its testis and lesser developed steroidogenic organelles within these cells as compared with those in the Leydig cells of higher vertebrates (Tsuneki and Gorbman, 1977a).Since the testis of P. aethiopicus also possesses a very limited amount of interstitial cells that reveal no elaborate changes or variation in histomorphology and distribution with varying testicular maturational stages, it will be of interest to see how the level of plasma androgens correlates or compares with the nature of the interstitial cells.

The plasma levels of testosterone in the two major amphibian groups are not consistent i.e. urodeles have a higher range of the levels of this androgen (40-200 ng/ml) while anurans have low plasma testosterone levels ranging from 0.1 ng/ml to 25 ng/ml (Callard et al, 1978). Furthermore, in anurans, the major metabolite of testosterone appears to be a high

yield of dihydrotestosterone while usually in the urodeles, testosterone can be converted to 11-keto-testosterone (Callard et al, 1978; Kime, 1980). Dihydrotestosterone does not appear to be a usual or normal occurrence in the testis of the urodeles (Callard et al, 1978), but recently, Specker and Moore (1980) measured dihydrotestosterone plus testosterone in the plasma of T. granulosa which ranged from less than 5ng/ml to 54.7± 7.9ng/ml. The determination and establishment of the major androgens and their plasma levels in P. aethiopicus may perhaps reveal whether the lungfishes share a close phylogenetic relationship with either one of the amphibian groups and may further clarify our understanding of the evolution of the androgens in the tetrapods.

5.2. Ovary

Oocyte development in P. aethiopicus is clearly asynchronous. This is especially evident in a mature ovary where the oocytes within a lobe are at different maturational stages, ranging from the immature protoplasmic oocytes to the large "near-ovulation" oocytes. However, there is synchrony among the lobes, such that any one lobe represents the overall maturational stage of the ovary. Asynchronism in oocyte development also occurs in G. mirabilis (de Vlaming, 1972), M. tengara (Guraya et al, 1975) and in X. laevis

(Dumont, 1972), and is said to indicate a mode of reproduction with a prolonged spawning season, and with several or multiple spawnings within the season (Guraya, et al, 1975).

The oogonia of P. aethiopicus closely resemble those of the amphibian X. laevis (Al-Mukhtar and Webb, 1971) in that they both possess large nuclei which are highly lobed. Another ovarian characteristic that P. aethiopicus shares with X. laevis, are the dense "subnucleoli" of the larger nucleoli within the nucleus of dark staining protoplasmic oocytes. Dumont (1972) described these subnucleoli as being dense protruberances of the larger nucleoli. Such an oocyte characteristic has not been reported in the cyclostomes, elasmobranchs and teleosts.

Peripheral nucleoli (peri-nuclear nucleoli) are initially discernible in the early, dark staining protoplasmic oocytes. Previous ultrastructural observations of the immature oocytes of P. aethiopicus revealed that the peripheral nucleoli are formed by fragmentation of the existing larger nucleoli (Scharrer and Wurzelmann, 1969). Peripheral nucleoli are also initially visible in the immature oocytes of the teleosts G. qiuris (Rajalakshmi, 1966), S. fuscus (Anderson, 1968), P. reticulata (Lambert, 1970), G. mirabilis (de Vlaming, 1972), M. tengara (Guraya

et al, 1975) and in the anuran X. laevis (Al-Mukhtar and Webb, 1971; Dumont, 1972). Light microscopy observations suggesting nucleoli fragmentation, have also been reported to occur in the immature oocytes of X. laevis (Al-Mukhtar and Webb, 1971) and M. tengara (Guraya et al, 1975).

"Lampbrush" chromosomes appear in the nucleus of light-staining protoplasmic oocytes of P. aethiopicus and likewise, are a common feature of the diplotene stage or light staining oocytes of the lampreys L. planeri (Hardisty, 1965a) and P. marinus (Tokarz, 1978), the teleosts E. inconstans, Clarias batrachus, Brachydanio rerio (Tokarz, 1978) and the amphibians X. laevis (Dumont, 1972) and T. cristatus (Tokarz, 1978). In the present study, spherical, basophilic nucleoli, smaller than the existing peripheral ones, appear to be produced by the lampbrush chromosomes of the Stage D vitellogenic oocytes. Dumont (1972) also reported the association of nucleoli with the lampbrush chromosomes in X. laevis. It has been suggested that nucleoli originate from certain heterochromatic regions of these chromosomes known as nucleolus organizers, although a non-heterochromatic region also forms extra nucleoli (Bara, 1965). At the start of yolk granule accumulation into the oocytes of P. aethiopicus, the peripheral nucleoli adopt a duplex nature, which persists until the final oocyte matura-

tion stage. Duplex nucleoli were also observed in the oocytes of M. tengara at the beginning of the maturation phase when yolk vesicles are forming in the ooplasm (Guraya et al, 1975) and in the young oocytes of S. fuscus (Anderson, 1968). Their ultrastructure in the latter teleost revealed a dark cortical ribosomal region together with some granular material and a fine filamentous substance in the centre. Scharrer and Wurzelmann (1969) suggested that the pale, filamentous region of duplex nucleoli of P. aethiopicus consists of a proteinaceous material. From a survey of the literature, it was agreed that nucleoli are the sites of ribonucleic acid (RNA) synthesis and that the purpose of nucleolar extrusions or nucleolar products observed in the juxtanuclear ooplasm of immature oocytes of P. aethiopicus (Scharrer and Wurzelmann, 1969), S.fuscus, F. heteroclitus (Anderson, 1968) and M. tengara (Guraya et al, 1975), is to provide ribosomes for the ooplasm.

In the early protoplasmic oocytes, a spherical body (yolk nucleus) with a dark staining cortex and light medulla, differentiates in the cytoplasm. This yolk nucleus presumably disintegrates towards the beginning of the late protoplasmic stage since it has not been observed in oocytes beyond the early protoplasmic stage. A yolk nucleus with a homogeneous structure, also occurred in the immature oocytes of

M. tengara (Guraya et al, 1975). Ultrastructurally, this cytoplasmic organelle in the young oocytes of S. fuscus (Anderson, 1968) and in the germ cells, oogonia and oocytes of X. laevis (Al-Mukhtar and Webb, 1971; Dumont, 1972) consisted of an aggregation of mitochondria, whereas the yolk nucleus (or the so called Balbiani body) in the cytoplasm of pre-vitellogenic oocytes of the trout S. gairdneri, was composed of protein and RNA and unrelated to mitochondria (Beams and Kessel, 1973). Overall, the cytoplasmic yolk nucleus was regarded as being important for oocyte growth and vitellogenesis, providing the ooplasm with either mitochondria or with RNA (Al-Mukhtar and Webb, 1971; Beams and Kessel, 1973).

The follicle cells of the oocytes of the cyclostomes and teleosts are derived from coelomic epithelial cells (Tokarz, 1978), whereas the follicle cells of P. aethiopicus appear to originate from the fibroblasts of the connective tissue. The pre-protoplasmic and early, dark protoplasmic oocytes are surrounded by follicle cells which are histomorphologically and ultrastructurally similar if not identical to the fibroblasts. A similar situation exists in the amphibians B. stomaticus, R. pipiens and T. viridescens, where the follicle cells of immature oocytes are also fibroblast-like (Guraya, 1978). As in P. aethiopicus, the follicle cells of the oocytes of the elasmobranch

S. sorrakowah, the teleosts and the amphibians, undergo morphological variation with growth or maturation of the oocyte, changing from a flattened squamous or spindle shape and gradually becoming cuboidal or columnar. In large, mature oocytes, the follicular epithelium is stretched, becoming thin, and the follicle cells are reduced in size (Guraya, 1978). Towards peak maturation or ovulation, the follicle cells of P. aethiopicus resume their original flattened and elongated spindle shape as those of the early protoplasmic oocytes. From light and ultrastructural observations, these morphological changes appear to be related to the formation of the zona pellucida, vitellogenesis and growth of the oocyte. However, in the lamprey L. planeri, it is the cells of the theca and not the follicle cells that undergo morphological and ultrastructural changes with oocyte maturation (Guraya, 1978).

Ultrastructurally, some of the features or characteristics of the follicular epithelium and the oolemma during the formation of the zona pellucida and growth of the oocytes of P. aethiopicus, are shared by the oocytes of O. latipes (Hirose, 1972), B. pholis (Shackley and King, 1977), T. viridescens (Hope et al, 1963) and other amphibians (Guraya, 1978) i.e. the oocytes of all these species revealed formation of microvilli or pore canals from the oolemma and

their penetration into the zona material; formation of follicle cell processes (macrovilli or cytoplasmic pedicels) which also penetrate the zona pellucida; and the presence of microfilamentous material in the microvilli. The oocytes of the lamprey L. planeri exhibit all such features - radial microvilli of the oolemma, formation of a zona pellucida (zona radiata) (Guraya, 1978) and fine fibres or microfilaments in the microvilli, the latter characteristic also occurring in the oocytes of L. fluviatilis (Afzelius, Nicander and Sjoden, 1968). Of exception however, the follicle cells of the lampreys do not develop cytoplasmic pedicels (Guraya, 1978).

Towards peak maturation of the oocytes in O. latipes (Hirose, 1972), B. pholis (Shackley and King, 1977) and T. viridescens (Hope et al, 1963), the cytoplasmic pedicels either lost continuity with the oocyte, or like the microvilli, regressed from the zona pellucida. The latter then becomes the vitelline membrane of envelope. Although very mature oocytes of P. aethiopicus were not included in the ultrastructural study, the apparent loss of structural or cellular adhesion between the follicular layer and the zona pellucida of ovulated oocytes, perhaps suggests a similar occurrence i.e. regression of the cytoplasmic pedicels of the follicle cells and the subsequent formation of a well-defined vitelline

membrane (Stage J). It has been suggested that the follicle cells and their pedicels are involved in the process of vitellogenesis by transporting or manufacturing and transporting nutritive material or yolk precursors to the oocyte, while the microvilli of the oolemma function in the absorption of this nutritive material (Hope et al, 1963; Hirose, 1972; Shackley and King, 1977).

Employing histochemical methods i.e. mainly the use of labelled enzymes that are involved in steroid synthesis, the possible sites of steroidogenesis were demonstrated in the thecal cells of S. scomber (Bara, 1965), in the follicle cells of L. fluviatilis (Hardisty and Barnes, 1968), the elasmobranch S. acanthias (Lance and Callard, 1969), the teleosts A. terrae-sanctae (Yaron, 1971), P. reticulata (Lambert, 1970), the amphibians X. laevis (Redshaw and Nicholls, 1971), R. cyanophlyctis, R. tigrina, R. verrucosa and Cacopus systoma (Saidapur and Nadkarni, 1974) and in both the follicle and thecal cells of S. niloticus (Yaron, 1971). Despite a positive demonstration of 3β-HSD activity in the follicle cells of L. fluviatilis, ultrastructure did not reveal evidence that is considered to be typical of steroidogenic tissue, such as numerous mitochondria with well developed tubular cristae and extensive areas of agranular endoplasmic reticulum (Hardisty and Barnes,

1968). These features occur in the steroidogenic thecal cells of the dogfish S. canicula (Dodd and Dodd, 1980), the coho O. kisutch, pink salmon O. gorbuscha (Nagahama et al, 1978) and in two species of cichlids C. nigrofasciatum and H. multicolor (Nicholls and Maple, 1972). The thecal cells of L. planeri also possess numerous mitochondria, elements of agranular endoplasmic reticulum and lipid droplets, the latter two features considered to indicate secretion of steroid hormones (Guraya, 1978). Lipid droplets are further regarded as a characteristic feature of steroid-secreting cells and represent the stored precursor material for hormone or steroid synthesis (Hirose, 1972; Thornton and Evennett, 1973). The occurrence of several lipid droplets in the follicle cells of early vitellogenic oocytes of P. aethiopicus, may therefore suggest a steroidogenic capacity of these cells. Despite the absence of agranular endoplasmic reticulum in the cytoplasm of the follicle cells and in view of an undifferentiated or undeveloped thecal layer, the fibroblasts of which possess only undifferentiated mitochondria with narrow tubular cristae and some small Golgi complexes, the follicle cells may be the only cells of the oocytes possessing a steroidogenic function. Amphibians too, lack extensive areas of agranular endoplasmic reticulum and other organelles specific to steroidogenic function in the

follicle cells of their oocytes and it has been suggested that probably different pathways of steroid hormone synthesis occur in these cells, which need further investigation (Guraya, 1978).

In the early vitellogenic oocytes of P. aethiopicus, the follicle cells contain several organelles or vesicles filled with electron dense material. Such organelles, also present in the follicle cells of O. latipes at the early yolk stage, are regarded as lysosomes and suggested to have some relation with increased metabolism of the follicle cells and the oocyte during yolk formation and probably serve some function in transporting substances produced by the follicle cells (Hirose, 1972). According to Hirose (1972), cisternal, rough endoplasmic reticulum that is especially prominent in the follicle cells of O. latipes (such a feature also occurring in the follicle cells of P. aethiopicus and the thecal cells of S. canicula (Dodd and Dodd, 1980), may be a feature of secretion of certain substances which are transported to the vesicles of the Golgi complex which in turn produces the material for final secretion. The presence of fine, granular material associated with the Golgi complex and its vesicles in the follicle cells of early vitellogenic oocytes of P. aethiopicus may perhaps lend support to the concept of a secretory activity of the Golgi

complex.

Atresia has been described in the immature oocytes of L. planeri (Hardisty, 1965a) and in the yolky oocytes of the teleosts G. giuris (Rajalakshmi, 1966), A. terrae-sanctae, S. niloticus (Yaron, 1971), G. mirabilis (de Vlaming, 1972), M. tengara (Guraya et al, 1975) and the amphibian B. stomaticus (Guraya, 1969). The follicle cells of the oocytes of L. planeri were not implicated in the atretic process. In the teleosts, there was hypertrophy of the invading follicle cells as opposed to atrophy of the follicle cells of P. aethiopicus. As in A. terrae-sanctae, S. niloticus (Yaron, 1971), G. mirabilis (de Vlaming, 1972), M. tengara (Guraya et al, 1975) and B. stomaticus (Guraya, 1969), the follicle cells of atretic oocytes of P. aethiopicus appear to phagocytose the contents of the oocyte. The follicle cells of G. giuris are thought to exert an enzymatic activity in the reabsorption of atretic oocytes (Rajalakshmi, 1966). Atresia of the unspawned ripe oocytes and immature oocytes has been said to be a result of gonadotropin withdrawal from the pituitary after the spawning phase (Rajalakshmi, 1966). A low content or a lack of endogenous gonadotropin has also been said to lead to atresia of yolky oocytes (Guraya, 1969; Guraya et al, 1975).

5.3. Pituitary

A complete range of gonadal maturational stages was found within twenty-seven female P. aethiopicus ranging from 26.0 cm to 57.0 cm in total length, and within twenty-eight males of a size range from 18.5cm to 66.5 cm. In all pituitaries examined, type 2 basophils as described by Kerr and van Oordt (1966), did not occur and this observation tends to agree with Kerr and van Oordt (1966), who reported that these elongated cells with more numerous but smaller granules than those of the type 1 basophils and lacking a definite cell boundary, are absent in small (young) fish, but in adults i.e. of a studied length range of 65.0 cm to 170.0 cm, can numerically exceed the type 1 basophils.

In the present study, the type 1 basophils exhibited noticeable variation in distribution, granulation, and extent (thickness) and staining intensity of their chromophilic substance, which could be correlated with the stage of gonadal maturation. Thus the distribution, extent and staining intensity of the chromophilic substance of the type 1 basophils, appeared to be the greatest in P. aethiopicus with immature or early maturing gonads than with other maturational stages. The pars distalis of male P. aethiopicus, however, exhibited

rather more of these basophils in the posterior region than the female. Peak gonadal maturation was accompanied by a degranulation or vacuolization and regression of the chromophilic substance in most of the type 1 basophils.

Similar characteristics with variation in gonadal maturation, were noted in the gonadotropin-producing cells of the cyclostomes and teleosts: thus with either immature gonads or at the onset of gonadal maturation, the gonadotropic cells revealed variously, an increase in staining affinity, number, cell and nuclear dimensions and amount of secretory granules (van Oordt, 1968; Sundararaj, 1960; Lagios, 1965; Baker et al, 1974; Lindahl, 1980). At peak gonadal maturation or just prior to spawning, these gonadotropic cells degranulated, regressed or became chromophobic (van Oordt, 1968; Sundararaj, 1960; Lagios, 1965; Baker et al, 1974; Kaul and Vollrath, 1974; Ekengren et al, 1978a; Lindahl, 1980). Gonadal maturation, gonadal atrophy and cessation of ovulation, also correlated with the basophils in the ventral lobe of the elasmobranchs (Dodd, 1960; van Oordt, 1968). In the amphibians too, gametogenetic variation coincided with changes in the type 2 basophils, regarded therefore as the FSH-producing cells (van Oordt, 1968).

Ovariectomy in the rainbow trout S. gairdneri led to an increase in the plasma gonadotropin level and eventual degranulation of the secretory granules and globules in the gonadotropin-producing cells. The rise in plasma gonadotropin level was attributed to an increased output of this gonadotropin from the pars distalis due to the probable decrease in the level of sex steroids in the blood following ovariectomy (Peute et al, 1980). Gonadectomy in the anuran R. esculenta also led to extensive degranulation in the type 2 basophils and such postgonadectomy changes were restored to normal (i.e. cells resumed their granular form) with steroid hormone treatment (Rastogi and Chieffi, 1970). Similarly, with LH-RH injections in S. salar (Ekengren et al, 1978a) and C. auratus (Kaul and Vollrath, 1974), the gonadotropic cells appeared as those during spawning, in that they degranulated and became vacuolar. Loss of secretory granules in the gonadotropic cells has been suggested to reflect a secretory activity or release of the gonadotropin (Sundararaj, 1960; Rastogi and CHieffi, 1970; Ekengren et al, 1978a; Peute et al, 1980; Lindahl, 1980). On the other hand, the occurrence of numerous secretory granules in the gonadotropic cells is said to indicate that the cells are in the storing phase, with a possible low release rate of the gonadotropin (Lindahl, 1980).

In both male and female P. aethiopicus with mature or near-spawning gonads, it was noted that most but not all of the type 1 basophils in the pars distalis regressed, degranulated and became chromophobic. A similar observation in the pituitary of S. salar at the time of spawning, whereby not all the gonadotropic cells degranulated or vacuolated, is said to reflect a different response to the gonadotropin regulating factors which may be an adaptation to secure gonadotropin for prolonged needs (Ekengren et al, 1978a).

Where only the number of basophils or presumptive gonadotropin-producing cells in the pituitary can be correlated with the stage of gonadal maturation, was reported by Barr and Hobson (1964). In their study, the greatest proportion of basophils in the meso-adenohypophysis occurred in maturing plaice P. platessa, having the greatest rate of gonadal development, while immature and spawned fish lacked these basophils or contained very few in their pituitaries. A similar situation exists in both male and female P. aethiopicus, where the greatest amount or distribution of the type 1 basophils occur in the pars distalis of those specimens with maturing testes and ovaries, while the least distribution of these basophils occur in those lungfish with very mature gonads that are near spawning. Pituitary extracts of

maturing plaice were also reported as having the greatest gonadotropic potency while little or no activity occurred in pituitary extracts of immature and spawned fish (Barr and Hobson, 1964), thus implying that gonadotropic potency is proportional to the number of gonadotropic cells in the pituitary. In P. aethiopicus, gonadotropic potency of the pituitary may be reflected primarily by the extent and staining intensity of the chromophilic substance of the type 1 basophils. Thus these features are most developed in the pituitaries of lungfish with maturing gonads, with the basophils also having the greatest distribution. The intensely staining chromophilic substance and its wide extent may be due to a high rate of hormonogenic activity, which in turn may suggest a high gonadotropic potency or a high level of pituitary gonadotropin. Conversely, the regression and eventual chromophobia of most of the type 1 basophils in pituitaries of mature P. aethiopicus, may suggest a high rate of release of the gonadotropin, resulting in no build-up of the chromophilic substance and therefore leading to a reduced level of pituitary gonadotropin and low gonadotropic potency.

In the pituitary of the dogfish S. canicula, only one type of gonadotropic cell has been reported to occur in the ventral lobe (Firth and Vollrath, 1973), with an ultramorphologically similar cell type

also occurring in the median pars distalis. These findings suggested the presence of the same hormone in both regions (Knowles, Meurling and Vollrath quoted by Firth and Vollrath, 1973) and were confirmed by the demonstration of LH-like gonadotropic activity in both the ventral lobe and the median pars distalis (Firth and Vollrath, 1973). The occurrence also of only one type of gonadotropic cell has been demonstrated by immunofluorescence in the pars distalis of the immature male smolt, parr and precocious parr S. salar (Lindahl, 1980), in the sexually mature adult S. salar (Ekengren et al, 1978a), in M. latipinna and other Poecilinae (Goos et al, 1976), in the amphibians (Doerr-Schott, 1976), and by LH-RH administration in C. auratus (Kaul and Vollrath, 1974). It is assumed that only one gonadotropic cell type also occurs in the pars distalis of H. fossilis (Sundararaj, 1960; Baker et al, 1974), P. platessa (Barr and Hobson, 1964) and in E. jacksoni (Lagios, 1965), although such a distinction is not emphasized by the authors. Ekengren et al (1978a) have noted that depending on the phase of reproduction (or stage of gonadal maturation), the size, morphology and ultrastructure of a single gonadotropin-producing cell type can vary considerably and be easily and mistakenly interpreted as more than one gonadotropic cell type. Four ultrastructural variations, including variation in the amount of globules, granules or vesicles,

occurred in the same gonadotropic cell type in S. salar (Ekengren et al, 1978a; Lindahl, 1980). However in the roach R. rutilus, gonadotropin production has been attributed to two cell types which are cytologically different from each other (Ekengren et al, 1978b).

In this study, only basophils types 1 and 3 occurred in the pars distalis of P. aethiopicus of body length ranging from 18.5 cm to 66.5 cm. Type 2 basophils were reported to occur in larger lungfish ranging from 65 cm to 170 cm (Kerr and van Oordt, 1966). Since the type 1 basophils revealed noticeable variation with changes in gonadal maturation, and for features previously discussed in the light of previous research findings, it is tempting to suggest that they are the gonadotropin-producing cells. The type 3 basophils in both the amphibians and in the South American lungfish L. paradoxa, have been shown to be the ACTH-producing cells (Doerr-Schott, 1976; Hansen et al, 1980). The type 2 basophils therefore of larger P. aethiopicus, would appear to be the thyrotropin (TSH) producing cells. Doerr-Schott (1976) has noted that in certain amphibians i.e. Triturus marmorata, Bombina variegata, R. temporaria and Bufo vulgaris, the TSH antiserum did not identify or label the TSH-producing cells, this result said to reflect the poorly developed and scarcely visible TSH-

producing cells in the former two species and the total non-visibility of such cells in the latter two amphibians. On the other hand, X. laevis and A. mexicanum had well-developed TSH-producing cells. The amount of storage in the TSH-producing cells was suggested to determine the magnitude of the immunological reaction and since the level of thyroid function is known to vary considerably in the lower vertebrates, resulting in varying TSH production, it might explain the apparent absence of the TSH-producing cells at certain stages of the secretory cycle (Doerr-Schott, 1976). On the basis of such observations in the amphibians, it could be argued therefore, that in P. aethiopicus, the intensity of thyroid function perhaps increases with length or age of the animal, with the type 2 basophils responsible with TSH-production, only becoming well-developed and visible after the lungfish has attained a certain size. However, it could also be argued that the type 2 basophils of P. aethiopicus may be the gonadotropin-producing cells, these cells also becoming well-developed and visible in much larger lungfish due to increased gonadotropic function. On the basis of this second argument, the type 1 basophils would therefore appear to be the TSH-producing cells. Recently, the importance of the thyroid in urodele and elasmobranch reproduction, has been briefly reviewed. It has been shown that

thyroxine modulates or regulates the release of LH and FSH in Triturus pituitary in being sensitive to the rhythmic changes in serum gonadotropins and moreover that estrogens influence pituitary TSH secretion by modulating pituitary levels of thyrotropin-release hormone (TRH), such findings suggesting a synergistic or complimentary role of the thyroid with reproduction (Peyrot and Vellano, 1980). If the argument were to be accepted that the types 1 and 2 basophils are the thyrotropin and gonadotropin-producing cells respectively, the changes or variations in the type 1 basophils that appear to correlate with changes in gonadal maturation, may reflect the synergistic or complimentary action of the thyroid with the gonads. Clearly, the technique of immunohistochemistry has to be employed to ascertain the true nature of the types 1 and 2 basophils in the pituitary of P. aethiopicus. The appearance of the type 2 basophils in the pituitary of L. paradoxa of body length 35.5 cm but not in the pituitaries of P. aethiopicus below a body length of 65.0 cm, may perhaps be due to differences in the rate of growth versus gonadal or thyroidal function between these lungfish.

The nature and number of gonadotropins in the cyclostomes, P. aethiopicus and other lungfishes, have as yet not been researched into. In the dogfish

S. canicula, only one type of gonadotropin was purified, specifically from the ventral lobe and found to be structurally and biologically similar to mammalian FSH (Sumpter et al, 1978). The median pars distalis also possessed cells that were ultrastructurally similar to the gonadotropic cells of the ventral lobe, suggesting the production of the same hormone in both regions (Knowles, Meurling and Vollrath quoted by Firth and Vollrath, 1973). LH-like activity was also demonstrated in both the ventral lobe and median pars distalis (Firth and Vollrath, 1973). However, since the gonadotropin was purified from the ventral lobe only, it does not conclusively indicate that only a single gonadotropin is present in the pituitary of elasmobranchs (Sumpter et al, 1978).

At present, some controversy exists as to the number of gonadotropins present in the teleost pituitary. Two forms of gonadotropin, an LH-like and an FSH-like fraction, were fractionated from gonadotropin preparations of S. mossambicus. Two forms of gonadotropins, also obtained from the pituitaries of O. tshawytscha and O. keta, were found to possess some male and female specificity. On the other hand, a single glycoprotein gonadotropin was obtained from the pituitaries of the American plaice H. platessoides, which possessed both FSH and LH-like properties

in that it induced oocyte maturation and ovulation. Furthermore, a non-glycoprotein fraction that stimulates vitellogenesis, was obtained from the pituitary of the winter flounder P. americanus and the salmon (Peter and Crim, 1979). Concerning the amphibian gonadotropins, it has been established by the results of direct biochemical investigations on their pituitary hormones, that two separate and chemically distinct glycoprotein gonadotropins exist in both the anurans (Licht and Papkoff, 1974; Farmer et al, 1977) and in the urodeles, which appear to be homologous with the LH and FSH of mammals (Farmer et al, 1977; Licht, 1979).

The establishment as to the nature and number of gonadotropins in the pituitary of P. aethiopicus and other lungfishes, may help in the understanding and appreciation of the evolution of the gonadotropins in the lower vertebrates.

6. CONCLUSION

From the results of the present study, it appears that there are several gonadal characteristics that the lungfish P. aethiopicus shares, some perhaps solely, with the amphibians. These include an absence of smooth or agranular endoplasmic reticulum in both their follicle and interstitial cells, which are instead predominated by cisternal, rough endoplasmic reticulum; a close resemblance in the morphology of their oogonia which are large and have a highly lobed nucleus; the presence of "subnucleoli" within larger nucleoli in dark staining or early protoplasmic oocytes; and a single layer of follicle cells surrounding the oocytes. The presence of a thick, jelly-like layer surrounding the ovulated oocytes of P. aethiopicus, is also reminiscent of spawned oocytes of many amphibians. Furthermore, apart from the structural similarity in morphology and cell types of the pars distalis of both the amphibians and the lungfishes, their close phylogenetic relationship is also reflected by the sparse but direct innervation of the pars distalis in lungfishes and in tadpoles of R. temporaria. This innervation in the latter disappears at metamorphosis such that like other amphibians, the pars distalis of adult R. temporaria is not innervated (Ball, 1981). This fact reveals the evolutionary trend towards a non-innervated pars

distalis from the Sarcopterygians to the amphibians.

Since the present histological study did not reveal a dramatic or obvious histomorphological variation in the interstitial cells of P. aethiopicus with different stages of testicular maturation, indeed further experiments must be undertaken to ascertain the true nature and function of these cells i.e. administration of LH to the lungfish may bring about an elaboration of the physiological functions of the interstitial cells which may be reflected in an obvious variation in the histomorphology of these cells. A parallel ultrastructural study of these testicular cell types may further reveal changes in the nature of the cytoplasmic organelles, including the endoplasmic reticulum, and in turn reveal more accurately the nature of the physiological functions of the interstitial cells.

The Sertoli cells in the testis and the follicle cells of the oocytes of P. aethiopicus both appear to originate from fibroblasts. Furthermore, the nuclei of developed Sertoli cells and those of follicle cells, closely resemble each other histologically and ultrastructurally in possessing coarse clumps of granular chromatin material. Of interest, the nuclei of the interstitial cells of R. esculenta and R. temporaria prior to breeding, were noted to be large, rounded

and also having coarse clumps of chromatin material, this said to indicate a glandular, secretory appearance (see Literature Review 2.1.4.). Administration of gonadotropin i.e. FSH to both male and female P. aethiopicus may bring about an exaggeration or elabortion in the physiology of these cells which may reflect itself histomorphologically, quite unlike those of normal or untreated animals. Again, a joint ultrastructural and histochemical study on the gonads of FSH-treated P. aethiopicus may reveal the effect of the gonadotropin on these cell types.

Since only basophils types 1 and 3 were present in the pituitaries of all lungfish in this study, ranging from 18.5cm to 66.5cm in body length, it is questionable whether the type 1 basophils which exhibited a variation with different stages of gonadal maturation are thyrotropic or gonadotropic cells. From previous experiments on the pituitary of L. paradoxa (Hansen et al, 1980) and the amphibians (Doerr-Schott, 1976), the type 3 basophils have been accurately shown to be the ACTH-producing cells, while the types 1 and 2 basophils in the anurans have been shown to be the thyrotropic and gonadotropic cells respectively (Doerr-Schott, 1976). The uncertainty as to the function of the types 1 and 2 basophils in P. aethiopicus and other lungfishes, can perhaps be solved by employing the technique of immunohistochemistry with

the antiserum of both FSH and TSH in a wide length range of these animals, from the very small, young lungfish to the larger, older adults. The non-steroidal drug Methallibure has been shown to suppress gonadotropin production by effectively retarding spermatogenesis and vitellogenesis and causing gonadal regression in the Sarotherodon (= Tilapia) species (Hyder, 1972; Hyder, Shah, Campbell and Dadzie, 1974). This antigonadotropic effect of the drug on the pituitary of Sarotherodon mossambicus was reflected in the marked decrease in the gonadotropic cell size and in the amount of the cytoplasmic glycoprotein granules (Chiba, Honma and Lanzing, 1978). In future research, the administration of Methallibure to P. aethiopicus may indeed accurately identify the gonadotropic cells in the pituitary by causing obvious changes in (a) basophil type(s) and different from those in basophils of untreated fish.

Estrone, estriol and traces of estradiol-17B, have been claimed to have been identified in ovarian extracts of Protopterus annectens (Dean and Chester Jones, 1959 quoted by Gottfried et al, 1962). To date, nothing is known about the nature and number of pituitary gonadotropins and the nature of the androgens in male lungfish. Future research into these missing areas may perhaps shed further light into the

evolution of the tetrapodan gonadotropins, androgens and estrogens. Furthermore, determination of plasma levels of gonadotropin(s), gonadal steroids and thyrotropin, and coupled with the immunofluorescence study aimed at determining both the gonadotropic and thyrotropic cells in the pars distalis and the magnitude of the immunoreactivity in lungfishes at various gonadal maturational stages and body sizes, may perhaps reveal more about the pituitary-gonadal axis and the pituitary-thyroidal axis in the lungfish.

This unique animal, which fortunately for us, has survived virtually unchanged for some 400 million years, clearly deserves more attention and study especially concerning its life in its natural environment i.e. studies on the effect of temperature, rainfall, availability of food, etc. on reproduction, that would eventually ensure its efficient and successful propagation and continuing survival in the future.

7. REFERENCES

ADAM, H. (1959): Hypophyse und hypothalamo-neurohypophysares Neurosekretsystem bei den Cyclostomen Myxine glutinosa und Bdellostoma stouti. Verh. Deut. Zool. Ges. (Munster) 157-171.

AFZELIUS, B.A., NICANDER, L. and SJODEN, I. (1968): Fine structure of egg envelopes and the activation changes of cortical alveoli in the river lamprey Lampetra fluviatilis. J. Embryol. Exp. Morphol. 19: 311-318.

AL-MUKHTAR, K.A.K. and WEBB, A.C. (1971): An ultrastructural study of primordial germ cells, oogonia and early oocytes in Xenopus laevis. J. Embryol. Exp. Morphol. 26: 195-217.

ANDERSON, E. (1968): Cortical alveoli formation and Vitellogenesis during oocyte differentiation in the pipefish Syngnathus fuscus and the killifish Fundulus heteroclitus. J. Morph. 125: 23-60.

BAKER, B.I., KESHAVANATH, P. and SUNDARARAJ, B.I. (1974): The cell-types in the adenohypophysis of the Indian catfish Heteropneustes fossilis (Bloch). Cell Tiss. Res. 148: 347-358.

BALL, J.N(1981): Review. Hypothalamic control of the pars distalis in FIshes, Amphibians and Reptiles. Gen. Comp. Endocrinol. 44: 135-170.

BALL, J.N. and BAKER, B.I. (1969): The pituitary gland. Anatomy and histophysiology. In "Fish Physiology" (W.S. Hoar and D.J. Randall, eds.), Vol. 2, pp. 1-110. (Academic Press, New York).

BARA, G. (1965): Histochemical localization of 3B-Hydroxysteroid dehydrogenase in the ovaries of a Teleost fish, Scomber scomber L. Gen. Comp. Endocrinol. 5: 284-296.

BARNES, K. and HARDISTY, M.W. (1972): Ultrastructural and histochemical studies on the testis of the river lamprey Lampetra fluviatilis (L). J. Endocrinol. 53:59-69.

BARR, W.A. (1968): Patterns of ovarian activity. In "Perspectives in Endocrinology", Hormones in the lives of lower vertebrates. (Ed. Barrington, E.J.W. and Jorgensen, G.B.) Ch.3. pp.164-237. Academic Press London and New York.

BARR, W.A. and HOBSON, B.M. (1964): Endocrine control of the sexual cycle in the plaice Pleuronectes platessa L. IV. Gonadotropic activity in the pituitary gland. Gen. Comp. Endocrinol. 4: 608-613.

BARRINGTON, E.J.W. (1968): Phylogenetic perspectives in vertebrate endocrinology. In "Perspectives in Endocrinology", Hormones in the lives of lower vertebrates. (Ed. Barrington, E.J.W. and Jorgensen, G.B.) Ch.1. pp. 1-46. Academic press London and New York.

BEAMS, H.W. and KESSEL, R.G. (1973): Oocyte structure and early vitellogenesis in the trout Salmo gairdneri. Am. J. Anat. 136: 105-122.

BERN, H.A., NISHIOKA, R.S. and NAGAHAMA, Y. (1974): The relationship between nerve fibres and pituitary cell types in the cichlid teleost Sarotherodon mossambicus (Tilapia mossambica).

In. L. Arvy ed., Recherches biologiques contemporaines. Manfred Gabe Memorial Vol. Vagner, Nancy.

CALLARD, I.P., CALLARD, G.V., LANCE, V., BOLAFFI, J.L. and ROSSET, J.S. (1978): Testicular regulation in nonmammalian vertebrates. Biol. of Reprod. 18, 16-43.

CHAN, S.T.H. and PHILLIPS, J.G. (1967): Seasonal changes in the distribution of gonadal lipids and spermatogenetic tissue in the male phase of Monopterus albus (Pisces: Teleostei). J. Zool. Lond. 152, 31-41.

CHIBA, A., YOSHIHARU, H. and LANZING, W.J.R. (1978): Effect of Methallibure on the Hypophysis and Gonadal development of the Cichlid fish, Tilapia mossambica. Japanese Journal of Ichthyology. Vol.25, No.2. 107-114.

COLBERT, E.H. (1969): Evolution of the vertebrates. 2nd Edition. John Wiley and Sons, Inc. A history of the backboned animals through time.

CRIM, J.W., URANO, A. and GORBMAN, A. (1979a): Immunochemical studies of Luteinizing hormone-releasing hormone in brains of Agnathan fishes. I. Comparisons of adult Pacific lamprey (Entosphenus tridentata) and the Pacific hagfish (Eptatretus stouti). Gen. Comp. Endocrinol. 37: 295-305.

CRIM, J.W., URANO, A. and GORBMAN, A. (1979b): Immunocytochemical studies of Luteinizing hormone-releasing hormone in brains of Agnathan fishes. II. Patterns of immunocytochemistry in larval and maturing western brook lamprey (Lampetra

richardsoni). Gen. Comp. Endocrinol. 38:290-299.

DODD, J.M. (1960): Gonadal and gonadotrophic hormones in lower vertebrates. In " Marshall's Physiology of Reproduction" (A.S. Parkes, ed), 3rd. ed., Vol.1., Part 2, CH. II, pp.417-582. Longmans, Green, New York.

DODD, J.M. and DODD, M.H.I. (1980): The ventral lobe and rachendach. Pituitary gland of cartilaginous fishes. Abstract. Ninth International Symposium on Comparative Endocrinology. Hongkong. 7-11 Dec. 1981. Pg. 82.

DOERR-SCHOTT, J. (1976): Immunohistochemical detection by light and electron microscopy of Pituitary hormones in cold-blooded vertebrates. I. Fish and Amphibians. Gen. Comp. Endocrinol. 28, 487-512.

DUMONT, J.N. (1972): Oogenesis in Xenopus laevis (Daudin). I. Stages of oocyte development in laboratory maintained animals. J. Morphol. 136: 153-180.

EKENGREN, B., PEUTE, J. and FRIDBERG, G. (1978a): Gonadotropic cells in the Atlantic salmon, Salmo salar. Cell & Tiss. Res. 191: 187-203.

EKENGREN, B., LINDAHL, K. and FRIDBERG, G. (1978b): Immunocytology and innervation of the gonadotropic cells in the teleost fish Rutilus rutilus. Acta. zool (Stockh). 59: 125-133.

EVENNETT, P.J. and THORNTON, V.F. (1971): The distribution and development of gonadotropic activity in the pituitary gland of Xenopus laevis.

Gen. Comp. Endocrinol. 16: 606-607.

FARMER, S.W., LICHT, P., PAPKOFF, H. and DANIELS, E.L. (1977): Purification of gonadotropins in the leopard frog Rana pipiens. Gen. Comp. Endocrinol. 32:158-162.

FERNHOLM, B. and OLSSON, R. (1969): A cytopharmacological study of the Myxine adenohypophysis. Gen. Comp. Endocrinol. 13: 336-356.

FIRTH, J.A. and VOLLRATH, L. (1973): Determination of the distribution of Luteinizing hormone-like gonadotropic activity within the dogfish pituitary gland by means of the Xenopus oocyte meiosis assay. J. Endocrinol. 58: 347-348.

FONTAINE, M. and OLIVEREAU, M. (1975): Some aspects of the organization and evolution of the vertebrate pituitary. American Zoologist 15 (Suppl. I). 7-11. Trends in comparative endocrinology.

FRANCHI, L.L. (1962): The structure of the ovary. B. Vertebrates. In "The Ovary" , Vol. I (S. Zuckermann, ed.), pp. 121-142. Academic Press, New York.

GOOS, H.J. Th., SELDENRIJIK, R. and PEUTE, J. (1976): The gonadotropic cells in the pituitary of the black molly, Mollienisia latipinna and other teleosts, identified by the immunofluorescence technique in normal and androgen-treated animals. Cell Tiss. Res. 167: 211-219.

GOTTFRIED, H., HUNT, S.V., SIMPSON, T.H. and WRIGHT, R.S. (1962): Sex hormones in fish. The Oestro-

gens of cod (Gadus callarias). J. Endocrinol. 24:425-430.

GREENWOOD, P.H. (1957): Reproduction in the East African lungfish Protopterus aethiopicus Heckel. Proc. Zool. Soc. Lond. Vol. 130. pp. 547-567.

GRESIK, E., QUIRK, J.G. and HAMILTON, J.B. (1973): A fine structural and histochemical study of the Leydig cells in the testis of the teleost Oryzias latipes (Cyprinodontiformes). Gen. Comp. Endocrinol. 20: 86-98.

GRIER, H.J. (1981): Cellular organization of the testis and spermatogenesis in fishes. Amer. Zool., 21: 345-357.

GRIER, H.J. and LINTON, J.R. (1977): Ultrastructural identification of the Sertoli cell in the testis of the Northern pike Esox lucius. Amer. Jnl. Anat. Vol. 149. No. 2 pp. 283-288.

GRIFFIN, R.L. (1972): Ultramicrotomy. Balliere Tindall.

GURAYA, S.S. (1969): Histochemical observation on the corpora atretica of amphibian ovary. Gen. Comp. Endocrinol. 12: 165-167.

GURAYA, S.S. (1978): Maturation of the follicle wall of nonmammalian vertebrates. In "The Vertebrate Ovary", (Ed. by Jones, R.E.), Ch. 8, pp. 261-329. Plenum Press, New York and London.

GURAYA, S.S., KAUR, R. and SAXENA, P.K. (1975): Morphology of ovarian changes during the repro-

ductive cycle of the fish Mystus tengara (Ham.) Acta. anat. 91: 222-260.

HANSEN, G.N., HANSEN, B.L. and HUMMER, L. (1980): The cell types in the adenohypophysis of the South American lungfish Lepidosiren paradoxa with special reference to the immunocytochemical identification of the corticotropin-containing cells. Cell Tiss. Res. 209: 147-160.

HARDISTY, M.W. (1965a): Sex differentiation and gonadogenesis in lampreys. I. The ammocoete gonads of the brook lamprey, Lampetra planeri. J. Zool. 146: 305-345.

HARDISTY, M.W. (1965b): Sex differentiation and gonadogenesis in lampreys. II. The ammocoete gonads of the landlocked sea lamprey, Petromyzon marinus. J. Zool. 146: 346-387.

HARDISTY, M.W. and BARNES, K. (1968): Steroid 3B-oldehydrogenase activity in the cyclostome gonad. Nature (London). 218: 880.

HARDISTY, M.W., ROTHWELL, B. and STEELE, K. (1967): The interstitial tissue of the testis of the river lamprey Lampetra fluviatilis. J. Zool. Lond. 152: 9-18.

HAYAT, M.A. (1970): Principles and techniques of electron microscopy. Biological applications. Vol. I. Van Nostrand Reinhold Company.

HENDERSON, N.E. (1962): The annual cycle in the testis of the Eastern brook trout Salvelinus fontinalis (Mitchill). Canadian Journal of Zoology, Vol. 40. pp. 631-641.

HIROSE, K. (1972): The ultrastructure of the ovarian follicle of medaka Oryzias latipes. Z. Zellforsch. 123: 316-329.

HOAR, W.S. (1969): Reproduction. In "Fish Physiology" (Ed. Hoar, W.S. and Randall, D.J.) Vol. III Ch. I pp. 1-72. Academic Press, London and New York.

HOPE, J., HUMPHRIES Jr., A.A. and BOURNE, G.H. (1963): Ultrastructural studies on developing oocytes of the salamander Triturus viridescens. J. Ultrastructure Research 9: 302-324.

HUMASON, G.L. (1972): Animal tissue techniques. 3rd. ed. W.H. Freeman and Co. San Francisco.

HYDER, M. (1972): Endocrine regulation of reproduction in Tilapia. Gen. Comp. Endocrinol. Suppl. 3, pp. 729-740.

HYDER, M., SHAH, A.V., CAMPBELL, C.M. and DADZIE, S. (1974): Methallibure studies on Tilapia. II. Effect of Tilapia pituitary homogenate (TPH), human chorionic gonadotropin (HCG) and testosterone propionate (TP) on the testes of methallibure-treated Tilapia nigra. Gen. Comp. Endocrinol, 23: 245-255.

JONES, R.E. (1978): Ovarian cycles in nonmammalian vertebrates. In "The Vertebrate Ovary" (Ed. by Jones, R.E.) Ch. 21, pp. 731-762. Plenum Press New York and London.

KATZ, Y., ECKSTEIN, B., IKAN, R. and GOTTLIEB, R. (1971): Estrone and estradiol-17B in the ovaries of Tilapia aurea (Teleostei, Cichlidae). Comp. Biochem. Physiol. Vol. 40B. pp. 1005-1009.

KAUL, S. and VOLLRATH, L. (1974): The goldfish pituitary. I. Cytology. Cell Tiss. Res. 154: 211-230.

KERR, T. (1965): Histology of the distal lobe of the pituitary of Xenopus laevis Daudin. Gen. Comp. Endocrinol. 5: 232-240.

KERR, T. and VAN OORDT, P.G.W.J. (1966): The pituitary of the African lungfish Protopterus sp. Gen. Comp. Endocrinol. 7: 549-558.

KIME, D.E. (1980): Comparative aspects of testicular androgen biosynthesis in nonmammalian vertebrates. Steroids and their mechanism of action in nonmammalian vertebrates. Capri Oct. 1979. Raven Press. pp.17-31.

KING, J.A. and MILLAR, R.P. (1979): Hypothalamic LH-RH

content in relation to the seasonal reproductive cycle of Xenopus laevis. Gen. Comp. Endocrinol. 39: 309-312.

KUHN, O. and THENIUS, E. (1974): Origin of the Tetrapods. In "Grzimek's Animal Life Encyclopaedia" Vol. 5 Fishes II Amphibians. pp. 273-282.

LAGIOS, M.D. (1965): Seasonal changes in the cytology of the adenohypophysis, testes and ovaries of the black surfperch, Embiotoca jacksoni, a viviparous percomorph fish. Gen. Comp. Endocrinol. 5: 207-221.

LAMBERT, J.G.D. (1970): The ovary of the guppy Poecilia reticulata. The granulosa cells as sites of steroid biosynthesis. Gen. Comp. Endocrinol. 15: 464-476.

LANCE, V.A. and CALLARD, I.P. (1969): A histochemical study of ovarian function in the ovoviviparous elasmobranch, Squalus acanthias. Gen. Comp. Endocrinol. 13: 255-267.

LANZING, W.J.R. (1959): Studies on the river lamprey Lampetra fluviatilis during its anadramous migration. Ph.D. Thesis. Univ. Utrecht.

LAZARD, L. (1979): Steroidogenesis in axolotl testis. Histochemistry of two major enzymes related to cell type, spermatogenesis and substrate. Gen. Comp. Endocrinol. 39: 381-387.

LICHT, P. (1979): Reproductive endocrinology of reptiles and amphibians. Gonadotropins. Ann. Rev. Physiol. 41: 337-351.

LICHT, P. and PAPKOFF, H. (1974): Separation of two distinct gonadotropins from the pituitary gland of the bullfrog Rana catesbeina. Endocrinology, 94: 1587-1594.

LINDAHL, K. (1980): The gonadotropic cell in parr, precocious parr male and smolt of the Atlantic salmon Salmo salar. An immunocytological, light and electron microscope study. Acta zool (Stockh). 61: 117-125.

LOFTS, B. (1964): Seasonal changes in the functional activity of the interstitial and spermatogenetic tissues of the green frog, Rana esculenta. Gen. Comp. Endocrinol. 4: 550-562.

LOFTS, B. (1968): Patterns of testicular activity. In "Perspectives in Endocrinology", Hormones in the lives of lower vertebrates. (Ed. Barrington, E.J.W. and Jorgensen, G.B.). Ch. 14. pp.239-304. Academic Press. London and New York.

LOFTS, B. and BOSWELL, C. (1960): Cyclical changes in the distribution of the testis lipids in the common frog, Rana temporaria. Nature. 187: 708.

LOFTS, B. and MARSHALL, A.J. (1957): Cyclical changes in the distribution of the testis lipids of a teleost fish, Esox lucius. Quart. J. Microscop. Sci. 98: 79-88.

LOFTS, B., PICKFORD, G.E. and ATZ, J.W. (1966): Effects of methyl testosterone on the testis of a hypophysectomized cyprinodont fish, Fundulus heteroclitus. Gen. Comp. Endocrinol. 6: 74-88.

LOFTS, B., WELLEN, J.J. and BENRAAD, Th. J. (1972):

Seasonal changes in the endocrine organs of the male common frog, Rana temporaria. Gen. Comp. Endocrinol. 18: 344-363.

LUPO DI PRISCO, C., DELRIO, G., CHIEFFI, G., CORDELLINI, L.B. and MAGNI, A.P. (1971): Identification and biosynthesis of steroid hormones in the ovary and fat bodies of female Triturus cristatus carnifex. Comp. Biochem. Physiol. Vol. 40B. pp. 53-60.

MARSHALL, A.J. and LOFTS, B. (1956): The Leydig cell homologue in certain teleost fishes. Nature, 171: 704-705.

MATHEWS, L.H. and MARSHALL, F.H.A. (1956): Cyclical changes in the reproductive organs of the lower vertebrates. In "Marshall's Physiology of Reproduction" (A.S. Parkes, ed). 3rd ed. Vol. I Pt.I. Ch.3 pp. 156-218. Longmans, Green and Co. London.

MELLINGER, J. (1966): Variation de la structure hypophysaire de Scyliorhinus caniculus (L) et d'autres poissons elasmobranches. Microscopie optique et microscopie electronique. C.R. Acad. Sci. (Paris) 255: 2294-2296.

NAGAHAMA, Y., CLARKE, W.C. and HOAR, W.S. (1978): Ultrastructure of putative steroid-producing cells in the gonads of coho (Onchorynchus kisutch) and pink salmon (Onchorynchus gorbuscha). Can. J. Zool. 56: 12, 2508-2519.

NICHOLLS', T.J. and GRAHAM, G.P. (1972): The ultrastructure of the lobule boundary cells and Leydig cell homologs in the testis of a cichlid

fish, Cichlasoma nigrofasciatum. Gen. Comp. Endocrinol. 19: 133-146.

NICHOLLS', T.J. and MAPLE, G. (1972): Ultrastructural observations on possible sites of steroid biosynthesis in the ovarian follicular epithelium of two species of cichlid fish, Cichlasoma nigrofasciatum and Haplochromis multicolor. Z. Zellforsch. 128: 317-335.

O'HALLORAN, M.J. and IDLER, D.R. (1970): Identification and distribution of the Leydig cell homolog in the testis of sexually mature Atlantic salmon Salmo salar. Gen. Comp. Endocrinol. 15: 361-364.

PATZNER, R. (1977): Cyclical changes in the testis of the hagfish Eptatretus burgeri (Cyclostomata). Acta zool. (Stockh). 58: 223-226.

PETER, R.E. and CRIM, L.W. (1979): Reproductive endocrinology of fishes: Gonadal cycles and gonadotropin in teleosts. Ann. Rev. Physiol. 41: 323-335.

PEUTE, J., TERLOW, M., BRETON, B., GOOS, H.J.Th. and VAN OORDT, P.G.J.W. (1980): Effect of ovariectomy of gonadotrophs and NLT of the rainbow trout. Gen. Comp. Endocrinol. 40: 340.

PEYROT, A. and VELLANO, C. (1980): Recent acquisition on the thyroid impact on reproduction in the vertebrates. Abstract. Gen. Comp. Endocrinol. 40: 318.

RAI, B.P. (1965): Cyclical changes in the testis of the mahseer Barbus tor (Tor Tor). Acta anat. 62: 461-475.

RAJALAKSHMI, M. (1966): Atresia of oocytes and ruptured follicles in Gobius giuris (Hamilton-Buchanan). Gen. Comp. Endocrinol. 6: 378-385.

RASTOGI, R.K. and CHIEFFI, G.(1970): A cytological study of the pars distalis of Pituitary gland of normal, gonadectomized, and gonadectomized steroid hormone-treated green frog, Rana esculenta L. Gen. Comp. Endocrinol. 15: 247-263.

REDSHAW, M.R. and NICHOLLS', T.J. (1971): Oestrogen biosynthesis by ovarian tissue of the South African clawed toad Xenopus laevis Daudin. Gen. Comp. Endocrinol. 16: 85-96.

ROMER, A.W. (1954): I. Vertebrate beginnings. In "Man and the Vertebrates" Vol. I. A Pelican book.

ROWLANDS, W. and WEIR, B.J. (1977): The ovarian cycle in vertebrates. In "The Ovary" (ed. by Prof. Lord Zuckermann and Barbara J. Weir). 2nd. ed. Vol. II. Ch.6.

RUBY, S.M. and McMILLAN, D.B. (1970): Cyclic changes in the testis of the brook stickleback Eucalia inconstans (Kirkland). J. Morphol., 131:447-466.

RUBY, S.M., and McMILLAN, D.B. (1972): The interstitial origin of germinal cells in the testis of the stickleback. J. Morphol., 145: 295-318.

SAIDAPUR, S.K. and NADKARNI, V.B. (1973): Histochemical localization of -3B-Hydroxysteroid dehydrogenase and glucose 6-phosphate dehydrogenase in the testis of Indian skipper frog Rana cyanophlyctis. Gen. bomp. Endocrinol. 21: 225-230.

SAIDAPUR, S.K. and NADKARNI, V.B. (1974): Steroid synthesizing cellular sites in amphibian ovary. A histochemical study. Gen. Comp. Endocrinol. 22: 459-462.

SCHARRER, B. and WURZELMANN, S. (1969): Ultrastructural study on nuclear-cytoplasmic relationships in oocytes of the African lungfish, Protopterus aethiopicus. I. Nucleolo-cytoplasmic pathways. Z. Zellforsch. 96: 325-343.

SHACKLEY, S.E. and KING, P.E. (1977): Oogenesis in a marine teleost, Blennius pholis L. Cell Tiss. Res. 181: 105-128.

SIMPSON, T.H., WRIGHT, R.S. and HUNT, S.V. (1963): Sex hormones in fish. Pt. II. The oestrogens of Scyliorhinus caniculus. J. Endocrinol. 26: 499-507.

SPECKER, J.L. and MOORE, F.L. (1980): Annual cycle of plasma androgens and testicular composition in the rough-skinned newt, Taricha granulosa. Gen. Comp. Endocrinol. 42: 297-303.

SUMPTER, J.P., FOLLETT, B.K., JENKINS, N. and DODD, J.M. (1978): Studies on the purification and properties of gonadotrophin from ventral lobes of the pituitary gland of the dogfish (Scyliorhinus canicula L.). Gen. Comp. Endocrinol. 36: 264-274.

SUNDARARAJ, B.I. (1960): Correlation between the structure of the pituitary and the changes in the testis of the Indian catfish Heteropneustes. Acta anat. 40: 305-322.

THENIUS, E. and TEROFAL, F. (1974): Coelacanths and lungfishes. In "Grzimek's Animal Life Encyclopaedia", Vol. 5. Fishes II Amphibians. pp.261-267.

THORNTON, V.F. and EVENNETT, P.J. (1973): Changes in the fine structure of the ovarian follicle of the toad (Bufo bufo) prior to induced ovulation. Gen. Comp. Endocrinol. 20: 413-423.

TOKARZ, R.R. (1978): Oogonial proliferation, oogenesis and folliculogenesis in nonmammalian vertebrates. In "The Vertebrate Ovary", (Ed. by Jones, R.E.), Ch. 5, pp. 145-173. Plenum Press, New York and London.

TSO, E.C.-F. and LOFTS, B. (1977a): Seasonal changes in the newt Trituroides hongkongenesis testis. I. A histological and histochemical study. Acta zool. (stockh). 58: 1-8.

TSO, E.C.-F. and LOFTS, B. (1977b): Seasonal changes in the newt Trituroides hongkongenesis testis. II. An ultrastructural study on the lobule boundary cells. Acta zool. (Stockh). 58: 9-15.

TSUNEKI, K. and GORBMAN, A. (1977a): Ultrastructure of the testicular interstitial tissue of the hagfish Eptatretus stouti. Acta. zool. (Stockh). 58: 17-25.

TSUNEKI, K. and GORBMAN, A. (1977b): Ultrastructure of the ovary of the hagfish Eptatretus stouti. Acta zool. (Stockh). 58: 27-40.

UPADHYAY, S.N. and GURAYA, S.S. (1971): Histochemical observations on the interstitial tissue of

of fish testis. Gen. Comp. Endocrinol. 16: 504-510.

UNSICKER, K. (1975): Fine structure of the male genital tract and kidney in the anura Xenopus laevis Daudin, Rana temporaria L. and Bufo bufo L. under normal and experimental conditions. I. Testicular interstitial tissue and seminal efferent ducts. Cell Tiss. Res. 158: 215-240.

VAN OORDT, P.G.W.J. and BRANDS, F. (1970): The Sertoli cells in the testis of the common frog Rana temporaria. J. Endocrinol. 48:1.

VAN OORDT, P.G.W.J. (1968): The analysis and identification of the hormone-producing cells of the adenohypophysis. In "Perspectives in Endocrinology", Hormones in the lives of lower vertebrates. I. Fish and Amphibians. Gen. Comp. Endocrinol. 28: 487-512.

de VLAMING, V.L. (1972): Reproductive cycling in the estuarine gobiid fish, Gillichthys mirabilis. Copeia. No.2: 278-291.

WALVIG, F. (1963): The gonads and the formation of the sexual cells. In "The Biology of the Myxine" (Ed. by Brodal, A. and Fange, R.) Ch. VIII. The urinary and reproductive systems. pp.516-581. Universitetsforlaget, Oslo-Norway.

YARON, Z. (1971): Observations on the granulosa cells of Acanthobrama terrae-sanctae and Tilapia nilotica (Teleostei). Gen. Comp. Endocrinol. 17: 247-252.