SOME ASPECTS OF THE REPRODUCTIVE ENDOCRINOLOGY OF THE CATFISH, *CLARIAS GARIEPINUS* (BURCHELL, 1822).

Dissertation

Submitted in partial fulfilment of the

requirements for the Degree of Master of Science

of Rhodes University

by

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LIST OF TABLES AND FIGURES

ABSTRACT

A description is given of the location, anatomy and morphology of the endocrine glands likely to be involved in controlling the reproductive cycle of C. gariepinus. Methods of identifying the secretory tissues in the light and electron microscopes are outlined. A review of the published results on homologous tissues from other teleosts is included and directions for further research on the endocrinology of C. gariepinus are discussed.

INTRODUCTION AND AIM

The survival of any species in an environment that varies daily and seasonally is dependent on its ability to adapt to, and make optimum use of, these variations.

Photoperiod, temperature, water chemistry and turbidity are only some of the factors which vary in the environment of teleosts. In tropical or semi-tropical regions many species of freshwater fish spawn at the onset of the rainy season, when conditions are most favourable for the development of eggs and fry (de Vlaming, 1974). The neuroendocrine and endocrine systems together form the transducing system by which cues from the changing environment, as perceived by the nervous system, are translated into physiological responses (de Vlaming, 1974; Olivereau, 1977; Scott, 1979).

Neuroendocrine cells are located in the hypothalamus of the brain and also in the pineal gland. Axons arising from the neuroendocrine cells in the hypothalamus penetrate, and form the bulk of, the neurohypophysis of the pituitary. In fish the neurosecretory fibres end in close contact with the secretory cells of the adenohypophysis of the putuitary. The neuroendocrine system secretes what are known as releasing- and inhibiting- 'factors', which influence the secretion of the various adenohypophysial hormones.

The pineal gland has recently been credited with a major role in the control of reproduction (Scott, 1979). The pineal of teleosts is photosensitive and may play a part in the perception of changing light regimes (de Vlaming, 1974; Scott, 1979). There is also a possibility that secretions from the pineal may have a direct effect on gonadotropin secretion from the pituitary (Scott, 1979). A hypothalamus-pituitary complex is well established in teleosts, and there is increasing evidence for a pineal-hypothalamus-pituitary complex (Olivereau, 1977; Scott, 1979).

Several endocrine tissues are involved in the control of reproduction in teleosts (de Vlaming, 1974). Factors influencing the activity of these tissues are complex, and an intricate system of positive and negative feedback reactions is in operation. The complete 'pattern' of hormone interaction is not understood as yet in any teleost species. Although secretions from other tissues may also play a part, information available to date indicates that hormones secreted by the pituitary, gonads, adrenocortical tissue and possibly thyroid are important in the regulation of the reproductive cycle (de Vlaming, 1974; Olivereau, 1977), and it is for this reason that these tissues were selected for study in the present thesis.

A brief description of what is known to date about the nature and action of the hormones secreted by these tissues may help to clarify the situation, although it must be stressed that the conclusions quoted have been drawn from studies on a wide variety of teleost species, and extrapolation of these results to apply to different species is not necessarily reliable.

The endocrine tissues studied in this thesis all secrete several hormones. Hormones secreted by the adenohypophysis of the pituitary are all proteinaceous and include adrenocorticotropin, gonadotropins, thyrotropin, prolactin, somatotropin and melanotropin. Some of these hormones (e.g. gonadotropin) act directly on the gonads, while others (e.g. adrenocorticotropin) affect the gonads indirectly, usually with another endocrine gland as an intermediary (de Vlaming, 1974).

Hormones secreted by the testis are steroids containing 19 carbon atoms, and are commonly known as androgens. Androgens found in fish tissues include testosterone, 17 α-hydroxyprogesterone, androsterone and 11-ketotestosterone (Ozon, 1972a). Hormones secreted by the testis have been shown to be responsible for secondary sex characters and sexual behaviour (Pickford and Atz, 1957; Dodd, 1960; Woodhead, 1975). In addition, it is apparent that the sex steroids play some part in controlling the stages of spermatogenesis, but the relative roles of the androgens and the gonadotropins is still under discussion. (Lofts, Pickford and Atz, 1966; Sundararaj and Nayyar, 1967; Woodhead, 1975).

The oestrogenic activity of the teleost ovary has been well established (Barr, 1968; Hoar, 1969; de Vlaming, 1974; Woodhead, 1975), but the nature of action of oestrogens is not entirely clear. Oestrogens, like androgens, are steroid hormones, but have only 18 carbon atoms. The three most frequently occurring oestrogens in fish are oestradiol 17β, oestrone and oestriol (Ozon, 1972b). There is considerable evidence that oestrogens influence the action of pituitary gonadotropins on the teleost ovary, and that high levels of oestrogens found prior to spawning may result in the maintenance of the ovaries in a gravid state (Barr, 1968; Hoar, 1969; de Vlaming, 1974; Viswanathan and Sundararaj, 1974). There is also a possibility that oestrogens may influence sexual behaviour in female fish (Ball, 1960; Liley, 1969).

Hormones secreted by the adrenocortical tissue are steroids with 21 carbon atoms, and can be separated into two categories, the glucocorticoids and the mineralocorticoids. The mineralocorticoids are deoxycorticosterone and aldosterone, and are believed to be involved in the control of ovulation (Chester Jones *et al.*, 1969; de Vlaming, 1974; Goswami and Sundararaj, 1974; Katz and Eckstein, 1974; Bentley, 1976). The glucocorticoids found in fish include cortisol, cortisone and corticosterone, of which cortisol is the most prominent (Bentley, 1976). Cortisol in fish may be involved in ionic regulation as well as intermediary metabolism (Ball *et al.*, 1971). Corticosteroids appear to be involved in counteracting 'stress' (Hane *et al.*, 1966; Love, 1970; Fuller *et al.*, 1976). In addition there are indications that cortisol may be directly involved in the reproductive cycle (Fuller *et al.*, 1976).

Many roles have been suggested for the hormones triiodothyronine and thyroxine (tetraiodothyronine), which are elaborated by the thyroid gland. These include influence on growth control, morphogenesis, metamorphosis of larvae, deposition of guanine in scales, melanogenesis, haemotopoiesis, metabolism of lipids, cardiac rhythm, behaviour and sexual maturation and spawning (Pickford and Atz, 1957; Gorbman, 1959; Ball, 1960; Olivereau, 1960a, 1977; Matty, 1966; Hoar, 1969; Sage, 1973; Bentley, 1976). The involvement of thyroid hormones in reproduction has not been definitely established, but variations in thyroid activity appear in many cases to be closely linked to variations in gametogenic activity (Barrington and Matty, 1954; Pickford and Atz, 1957; Ball, 1960).

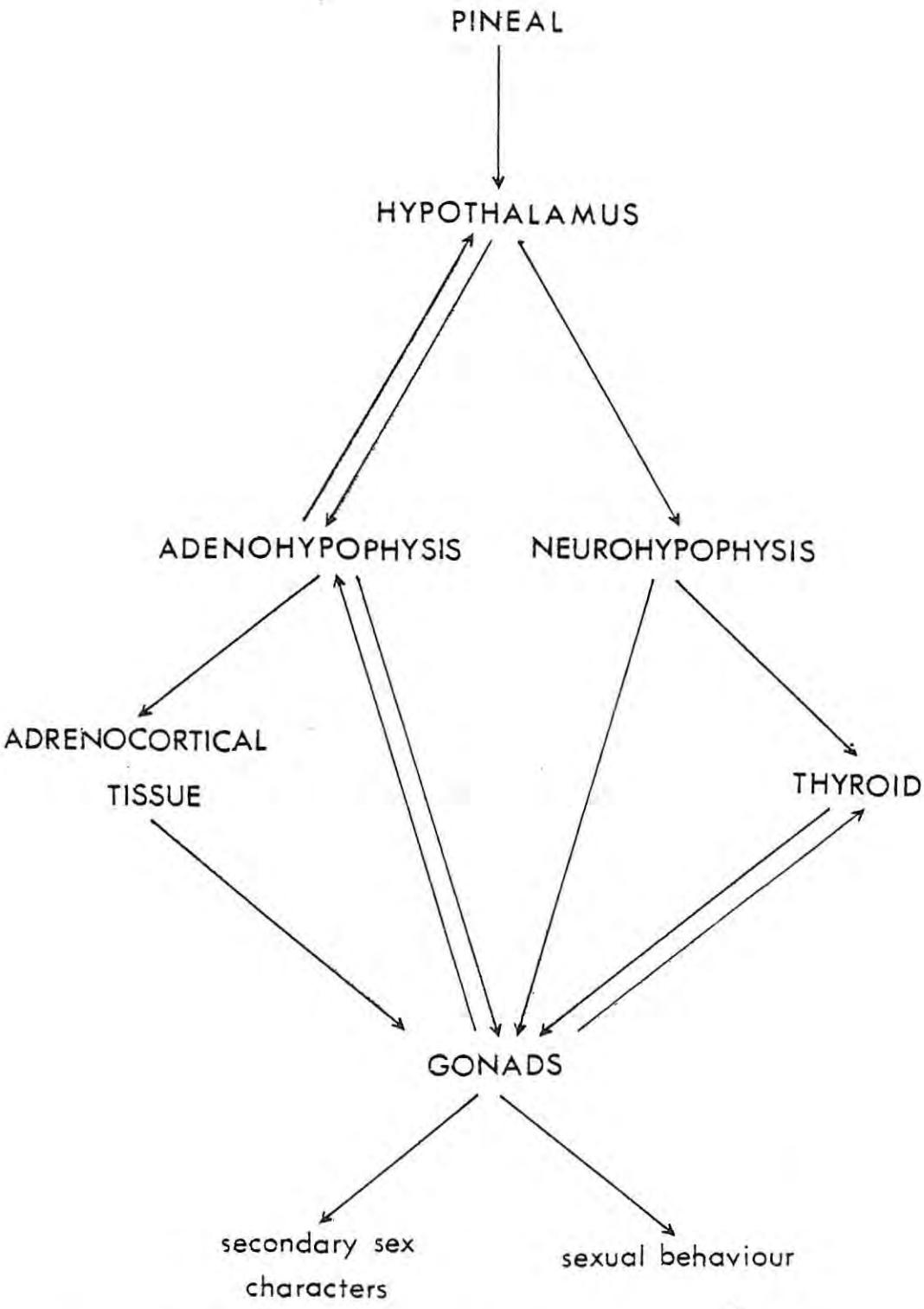

Figure 1 Highly simplified diagrammatic representation of the basic hormone interactions controlling the reproductive cycle of teleost fish, as understood to date.

The many collateral physiological changes associated with spawning, especially in migratory fish, may be the cause of this thyroid activity, rather than gonad development as such (Ball, 1960; Woodhead, 1975; Bentley, 1976). Cases have also been reported of depressed thyroid activity during spawning (Ball, 1960). Sage (1973) suggests that thyroid is involved in the reproductive process of fish 'as it is necessary for gonad development in some species'. Olivereau (1977) postulates that a very small amount of thyroid stimulating hormone may be necessary to activate gonadotropins. She also mentions the fact that anti-thyroid drugs often interfere with reproduction in fish (see also Barrington and Matty, 1952; Pickford and Atz, 1957; Ball, 1960), but suggests that this may be the result of a specific non-toxic action. Several authors have also suggested that hormone output of the gonads influences thyroid activity rather than vice versa (Matty, 1966; Singh, 1969).

Figure 1 represents a highly simplified diagram of the basic hormone interactions controlling reproduction in teleost fish, as understood to date.

The information outlined above has been drawn from studies on a wide variety of teleost species. Scott (1979), in a recent review on the control of teleost reproduction, emphasises the need for a comprehensive study on a single fish. Very little work has been done on the endocrinology of southern African fish, and the need for such studies is great, due especially to the increasing role of these fish in the provision of protein, and the necessity for aquaculture. Clarias gariepinus (Burchell), a freshwater catfish, was chosen for this study, and it is hoped that this will be the first in a series of endocrine investigations on this species. C. gariepinus was chosen for its large size, its importance as a food fish and its potential as a species suitable for aquaculture.

The aims of this project are therefore:

1. To summarise the literature available on the anatomy and morphology of the pituitary, gonads, adrenocortical tissue and thyroid of teleosts in general, for the benefit of South African zoologists, to whom this literature is not always readily available.

2. To build up a basic framework of knowledge on which further studies on C. gariepinus can be founded, by locating and identifying these tissues and providing a description of the anatomy, morphology and fine structure of these tissues in this catfish.
3. To compare the results from C. gariepinus with those found by other authors in closely related species.
4. To discuss directions for further research.

This project has been set out in such a way that a review of the literature on the study of each secretory tissue precedes the results of C. gariepinus material. This was done in order to provide some background for the reader and to point out the most relevant publications for others attempting similar studies.

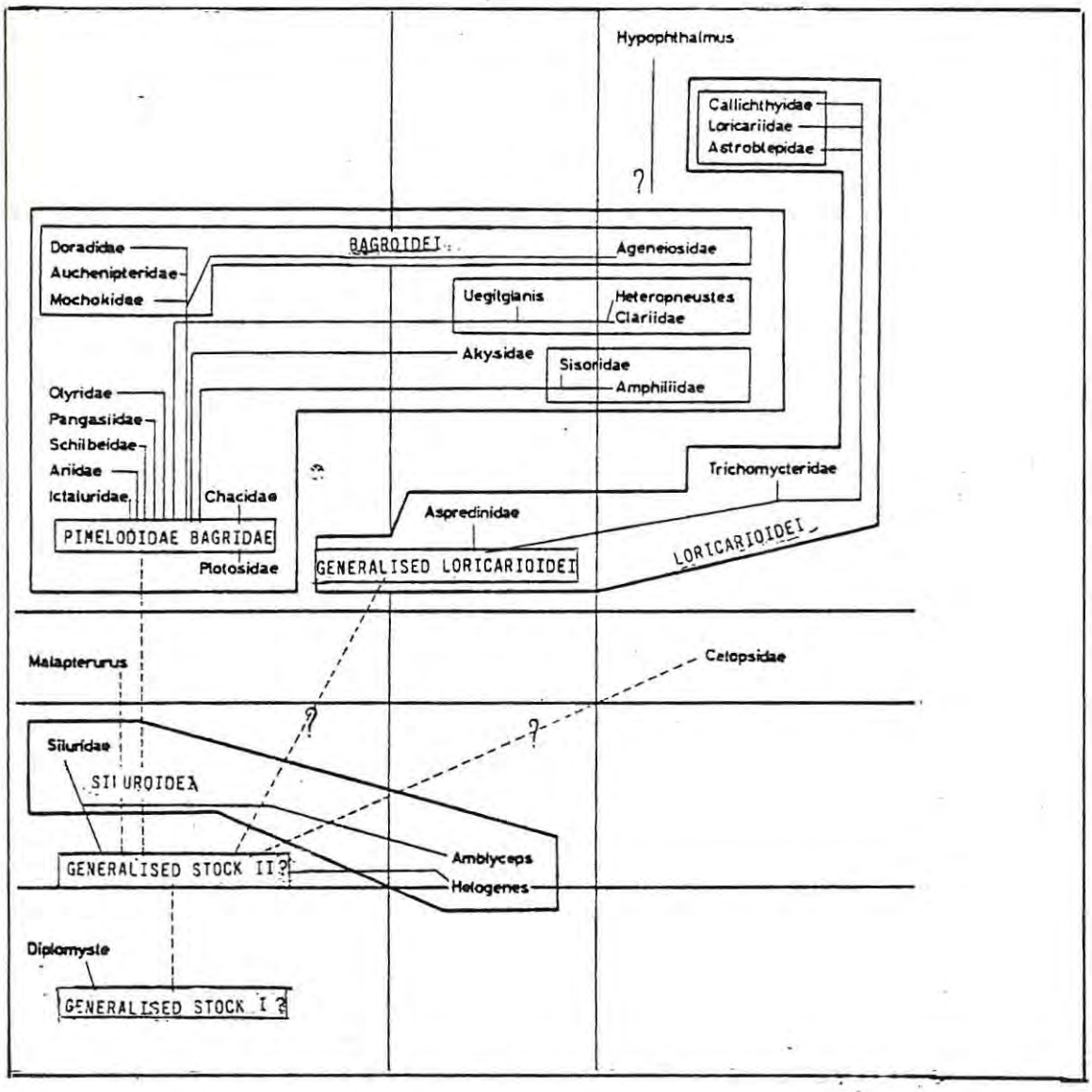

Figure 2 Interrelationships among the catfish from Chardon's (1968) work on the anatomy of the Weberian apparatus and swimbladder.

MATERIALS AND METHODS

1. STUDY MATERIAL

Clarias gariepinus: Taxonomic status

C. gariepinus is a teleost fish, of the order Cypriniformes, or Ostariophysi (Lagler *et al.*, 1977). It belongs to the superfamily Siluroidea, which includes, among others, the families Ictaluridae (North American freshwater catfish), Hetoropneustidae (stinging catfish) and Clariidae. The interrelationships within the Siluroidea are complicated and the subject of much debate. Chardon (1968) has proposed a classification based largely on the comparative anatomy of the Weberian apparatus (Figure 2). Chardon's figure has been included to give some idea of the interrelationships between the Heteropneustidae, Ictaluridae and Clariidae as these are three families on which some endocrine research has been done, and with which the findings on C. gariepinus can be compared. Table 1 lists all the siluroids mentioned in this thesis.

TABLE 1 Catfish mention in this thesis

FAMILY	SPECIES
Clariidae	Clarias gariepinus
	Clarias lazera
	Clarias senegalensis
	Clarias mossambicus
	Clarias anguillaris
	Clarias batrachus
	Clarias macrocephalus
	Clarias buthupogon
	Uegitglanis zammaranoi
Heteropneustidae	Heteropneustes fossilis
Ictaluridae	Ictalurus punctatus
	Ictalurus nebulosus nebulosus

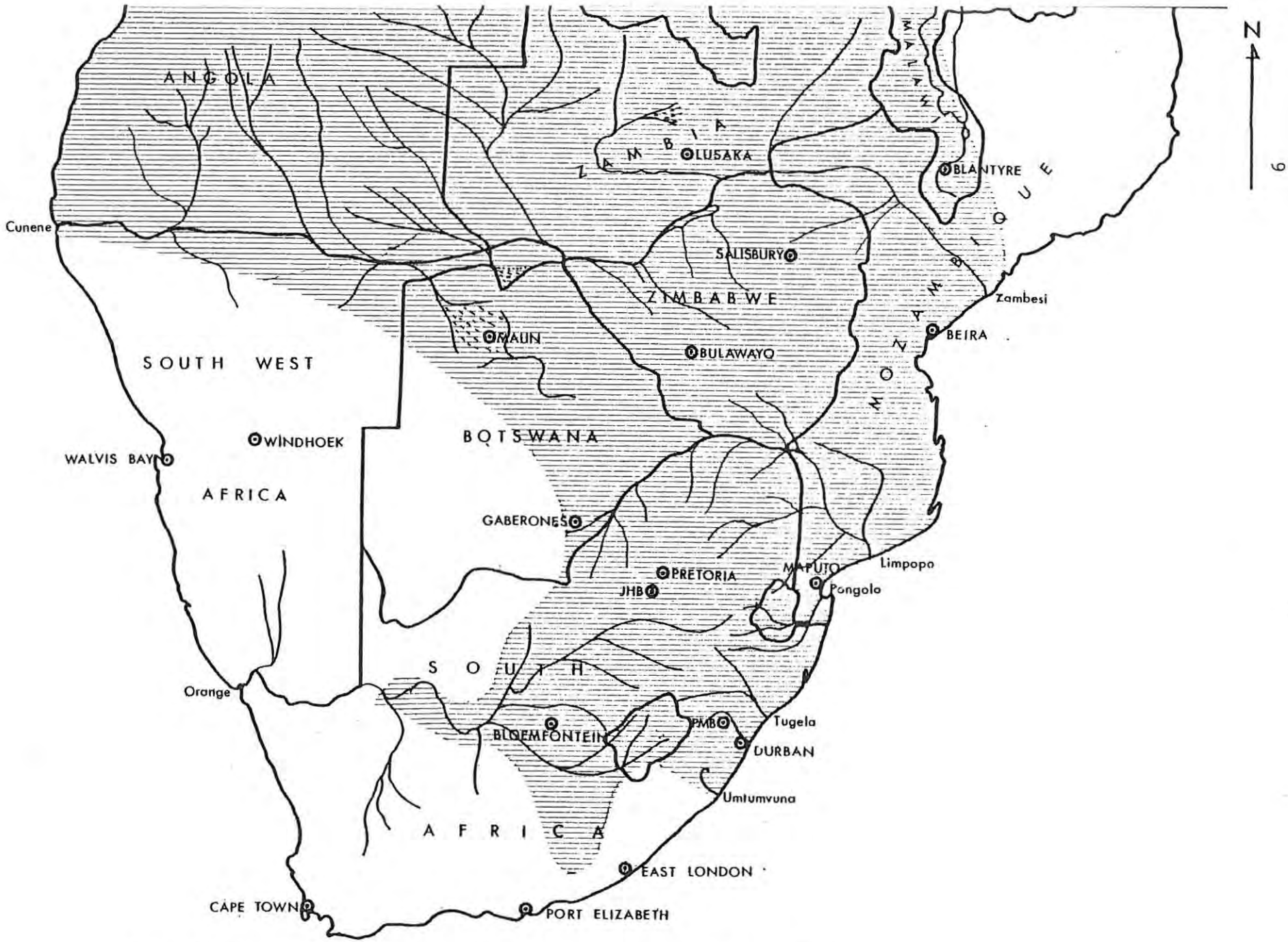

Figure 3 Southern Africa, showing the distribution of C. gariepinus .
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There is considerable disagreement among taxonomists at present as to whether all the species of African catfish at present recognised are valid, or whether some are merely synonyms. In this work all species are referred to by the names given to them by the authors cited, but it should be borne in mind that several of these clariids are either closely related or synonyms. Those suggested as synonymous are C. lazera and C. senegalensis, C. gariepinus and C. mossambicus (Jubb, 1967; Dehuisser, 1975; Bell-Cross, 1976; Clay, 1977; Bruton, 1979a). The Asian catfish, C. batrachus and C. macrocephalus are quite distinct from the African catfish (Bruton, 1979a).

Specimens of C. gariepinus from the collection localities used for this work have been lodged in the J.L.B. Smith Institute of Ichthyology in Grahamstown under catalogue numbers:

RUSI 13354	from the Pongolo River
RUS1 13355	from the Albert Falls Dam
RUS1 13356	from the Van Ryneveld's Dam

The general biology and ecology of C. gariepinus

C. gariepinus, the sharp-tooth catfish, is a common inhabitant of lakes, rivers, swamps and impoundments of southern Africa. Its distribution ranges from the Zambesi in the north to the Umtumwuna and Orange rivers in the south (Jubb, 1967; Cambray and Jubb, 1977). It has also been recorded in the Great Fish river in the Eastern Cape, but this extended distribution is due to the penetration of individuals through the Orange-Fish tunnel, which was constructed in 1974 (Jubb, 1978) (Figure 3).

The size reached by C. gariepinus depends on food quality and availability, varying from 1,5 kg to 30 kg or more (Bruton,1979b). The head is dorso-ventrally and the tail laterally flattened. Four pairs of barbels surround the mouth. The skull is hard and bony with broad plates lying beneath the skin (Crass, 1964). The skin is smooth and tough and covered with a mucous layer. There are no scales.

C. gariepinus is omnivorous, and is an opportunistic feeder. Food items range from plankton, through insects, crustacea, young birds and other fish,

including smaller individuals of their own species (Groenewald, 1964; Jubb, 1967; Murray, 1975; Clay, 1977; Bruton, 1979b). Catfish are readily eaten by many African tribes, who fish throughout the year with rod and line, handlines and nets. In some areas of shallow lakes and swamps extensive use is made of 'isi-fonya' (thrust) baskets (Tinley, 1964). At certain times of year catfish enter shallow water to spawn and are caught by African fishermen using single or multiple 'u-mono' baskets, clubs, spears and nets (Bruton, 1979a).

Reproduction of C. gariepinus

C. gariepinus undergoes an annual cycle of gonadal maturation, spawning and gonad regression. First maturity is reached at modal lengths of between 200 and 750 mm (normally between 200 and 350 mm), depending on environmental conditions (Mulder, 1971; Gaigher, 1977; Bruton, 1979a; Clay, 1979).

The cycle of gonadal maturation and spawning is associated with environmental changes. Maturation begins in the early spring, coinciding with increasing daylength and water temperature. Gonads reach a mature state within 1 - 2 months, and may remain mature for several months before spawning takes place (Bruton, 1979a). Spawning occurs after heavy rainfall and subsequent flooding of rivers and lake margins (Groenewald, 1957; Holl, 1966; 1968; van der Waal, 1972; Bowmaker, 1973; Bruton, 1979a).

The time lapse between heavy rainfall and the initiation of spawning is between 8 and 36 hours (Bruton, 1979a). Spawning takes place at night. Flooded marginal areas of lakes and impoundments and the flooded banks of the rivers are utilized as spawning grounds. Spawning behaviour is a complex routine which is described in detail by Bruton (1979a). Intra-specific aggression is followed by courtship. The male approaches a female and butts her lightly on the body. The pair then swim together among the vegetation, and after a while they stop, and spawning occurs. The male curves his body around the head of the female in a loose mating posture and eggs and sperm are shed into the water (Bruton, 1979a).

Mortality of eggs and larvae is high. Unlike the Asian species, C. batrachus and C. macrocephalus, no nest building or parental care occurs in C. gariepinus. It is thought that the choice of spawning grounds where

flooding is so recent and water so shallow that predator numbers are relatively low offers some protection for the development of eggs and fry (Bruton, 1979a).

Environmental control of the reproductive cycle

C. gariepinus is an inhabitant of semi-tropical regions, which are characterised by a definite winter and summer with heavy rains in the summer months followed by long periods of drought.

Factors inducing the early stages of gonad maturation have not been studied in C. gariepinus, but it is probable that either increase in temperature or photoperiod or both is responsible, as has been found to be the case in a great number of teleosts (see de Vlaming, 1974 for review), including the Indian catfish, Heteropneustes fossilis (Sundararaj and Sehgal, 1970a and b); Sundararaj and Vasal, 1973; Sundararaj *et al.*, 1973).

It is not known what factor in the floodwater is responsible for releasing spawning behaviour in flood-dependant spawners. A number of factors have been suggested, including high oxygen content and pH of the water, changes in water chemistry, conductivity, clarity, flow velocity and sudden drops in temperature. There is also a possibility that biological factors such as chemical change in the water associated with the inundation of marginal vegetation or simply availability of suitable spawning grounds may be involved (Qasim and Qayyum, 1961; de Bont and Maes, 1965; Bruton, 1979a). Lake (1967) working on Australian freshwater fish, postulated that it may be the presence of a substance known as 'petrichor' in the water which is responsible for triggering spawning. Petrichor is an oily substance, first isolated by Bear and Thomas (1966), which is produced when water flows over ground which has been exposed to hot dry conditions and is believed to cause the pleasant smell after a shower of rain. Pott (1969) suggests this that petrichor may be the stimulus for spawning of C. gariepinus and other annual spawning fish in the Pongolo river. Bowmaker (1973) and van der Waal (1974) both mention petrichor in connection with the spawning of Clarias species. Bruton (1979a) is wary of accepting this as an important factor triggering C. gariepinus spawning in Lake Sibaya. It is more likely a combination of factors and not any single one which controls the triggering mechanism.

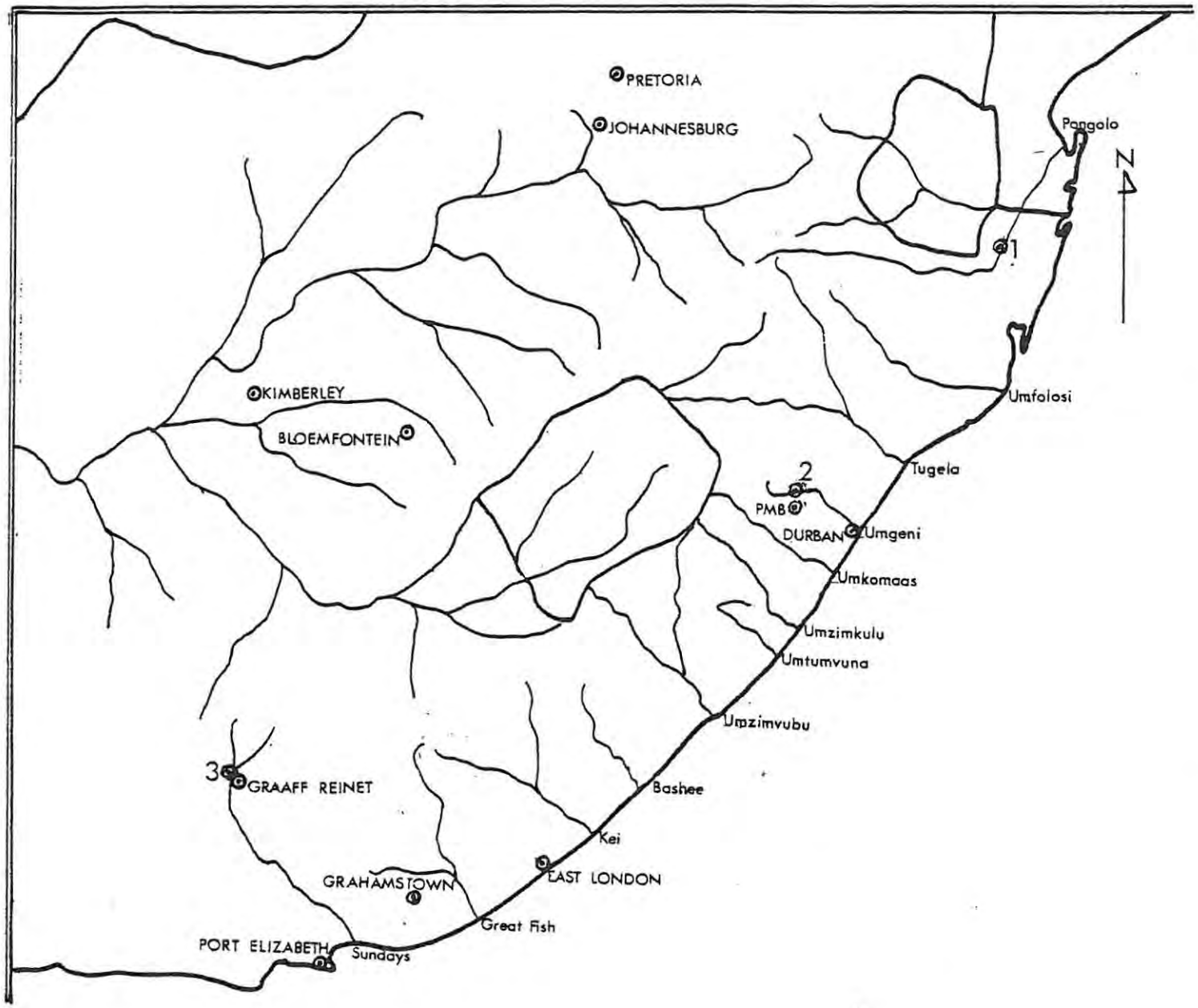

Figure 4 Collection localities for the C. gariepinus specimens used in this study. The fish were kept in outdoor ponds and aquaria in Grahamstown.

1. Mzinyeni Pan (27° 15'S, 32° 15'E)
2. Albert Falls Dam (29° 26'S, 30° 25'E)
3 van Ryneveld's Pass Dam (32° 18'S, 24° 37'E)

Scale 1:4 000 000

2. GENERAL METHODS

This project makes use of general dissection, light microscopy and electron microscopy methods to describe the location and structure of the endocrine tissues likely to be involved in reproduction in C. gariepinus.

Catching and killing techniques

Specimens of C. gariepinus were obtained from three different localities in South Africa (Figure 4).

1. Mzinyeni pan (27°S, 32,5°E) on the floodplain of the Pongolo River in Northern Zululand. This is a shallow turbid lake, with little aquatic vegetation and sandy beaches.
2. Albert Falls Dam (30 S, 30 E) on the Umgeni river, near Pietermaritzburg, Natal. This is a recent impoundment with both steep-sloping rocky shores, and occasional grassy-sloped shores.
3. Van Ryneveld's Pass Dam (32 S, 24 E) on the Sundays river near Graaff Reinet, in the Eastern Cape; a large impoundment with gently sloping shores. C. gariepinus has only penetrated into this region of the Eastern Cape since construction of the Orange-Fish tunnel in 1974 (Jubb, 1978).

Specimens of C. gariepinus were caught using one of several methods:

(a) Seine nets

A beach seine 50 m long, 2,5 m deep and 2,5 cm knot-to-knot mesh, was set by boat about 25 m offshore and pulled in by hand. Seine nets were most successful in the Mzinyeni Pan and the van Ryneveld's Pass Dam.

(b) Gill nets

Gill nets were used when seine-netting proved unsuccessful. C. gariepinus could not be caught in seine nets on the shallow slopes of the Albert Falls Dam during the winter months, and

Table 11 Details of C. gariepinus specimens used for histological work

Specimen No.	Collection Locality	Month of capture	Standard length (mm)	Mass (g)	Sex	Method of capture	Time held in captivity before dissection
1	Mzinyeni Pan	April	430		M	Seine net	0
2	Mzinyeni Pan	April	400		F	"	0
3	Mzinyeni Pan	April	860		F	"	0
4	Mzinyeni Pan	April	490		M	"	0
5	Albert Falls Dam	October	635	325	F	Gill net	0
6	Albert Falls Dam	October	600	270	F	"	0
7	Albert Falls Dam	October	630	300	M	"	0
8	Albert Falls Dam	October	625	320	F	"	0
9	Albert Falls Dam	October	610	290	M	"	0
10	Albert Falls Dam	November	575	240	F	"	0
11	Albert Falls Dam	December	740	520	M	"	0
12	Albert Falls Dam	December	660	320	M	"	0
13	Albert Falls Dam	December	680	350	F	"	0
14	Albert Falls Dam	December	665	330	M	"	0
15	Albert Falls Dam	January	720	370	M	Longline	0
16	Albert Falls Dam	January	620	250	M	"	0
17	Van Ryneveld's Dam	February	605	283	F	Seine net	0
18	Van Ryneveld's Dam	February	705		F	"	3 days
19	Van Ryneveld's Dam	February	530		M	"	3 days
20	Van Ryneveld's Dam	February	140		F		3 days
21	Van Ryneveld's Dam	October	420		F	"	6 days
22	Van Ryneveld's Dam	October	360	310	M	"	6 days
23	Van Ryneveld's Dam	May	399	400	M		1 month

gill nets were therefore used. 120 mm stretch mesh monofilament gills nets were used. Nets were checked every 2 - 3 hours throughout the night and any captive fish were removed, as C. gariepinus drowns if it is confined underwater.

(c) Longlines

Longlines of the design described by Bruton (1978) were set at depths of 5 - 25 m in the Albert Falls Dam. Meat, liver or fresh fish fillets were used as bait. The lines were pulled in as soon as movement on the buoys indicated that a fish had been hooked. The catfish were landed using a handnet.

Most C. gariepinus specimens were killed within 5 mins in a lethal concentration(1 mℓ/ℓ)of MS 222 anaesthetic. Others were transported live to the laboratory where they were kept in glass aquarium tanks (1,5 m ×0,75 m ×0,5 m) or concrete ponds (2,5 m × 2,5 m × 1 m). The catfish were fed on grated beef heart or fillets of frozen mullet. Care had to be taken not to overstock small tanks as the fish became aggressive towards each other and caused severe injuries by tearing the skin and muscle with their pectoral spines. No heating or aeration of the tanks was necessary. Fish held in tanks were caught with a handnet and killed by immersion in MS 222 anaesthetic.

Table II gives the particulars of place and time of catching, and killing techniques for the fish used in histological work.

The standard length and weight of each fish was recorded and the gonads were removed and weighed before the fish were dissected.

All fish were dissected as quickly as possible after death. Those which had not been completed within 1 hour were discarded.

Dissection methods

Of the tissues described in this project only the gonads and pituitary (van der Waal, 1974; 1978) had previously been located in C. gariepinus. Initial dissections were therefore largely exploratory. Once the tissues had been identified the following dissection methods were used:

I. Pituitary

The head was severed from the body immediately posterior to the bony shield. The upper jaw was then removed from the lower jaw (Figure 5.1) and the branchial trees, skin and connective tissue cut away to expose the cranium (Figure 5.2).

Incisions through the cranium were made anteriorly and posteriorly to its widest point, through the parasphenoid bone. Lateral incisions through the parasphenoid bone freed a complete section of this bone, which could then be lifted clear, exposing the pituitary gland attached to the brain (Figure 5.3).

Bouin's Hollande sublimate (fixative), was poured over the brain and pituitary *in situ*. The optic nerves and spinal nerve were severed. The whole brain, with pituitary attached, was removed and placed in Bouin's Hollande sublimate. (See Appendix 1)

II. Thyroid

Thyroid follicles lie in the connective tissue surrounding the ventral aorta and the afferent branchial arteries.

The lower jaw was removed prior to the dissection of the pituitary (Figure 6.1). The mucosa of the buccal cavity and pharynx, together with the pharyngeal teeth, was carefully removed to expose the heart and ventral aorta lying ventrally (Figure 6.2)

An incision was then made through the ventral aorta near the point of emergence from the bulbus arteriosus. Incisions were also made across each of the afferent branchial arteries, as far from their emergence from the ventral aorta as possible. The piece of tissue thus freed, consisting largely of blood vessels and connective tissue, was lifted out and placed in Bouin's fixative.

In fish which were dissected to show the overall distribution of thyroid follicles the whole lower jaw was fixed and sectioned.

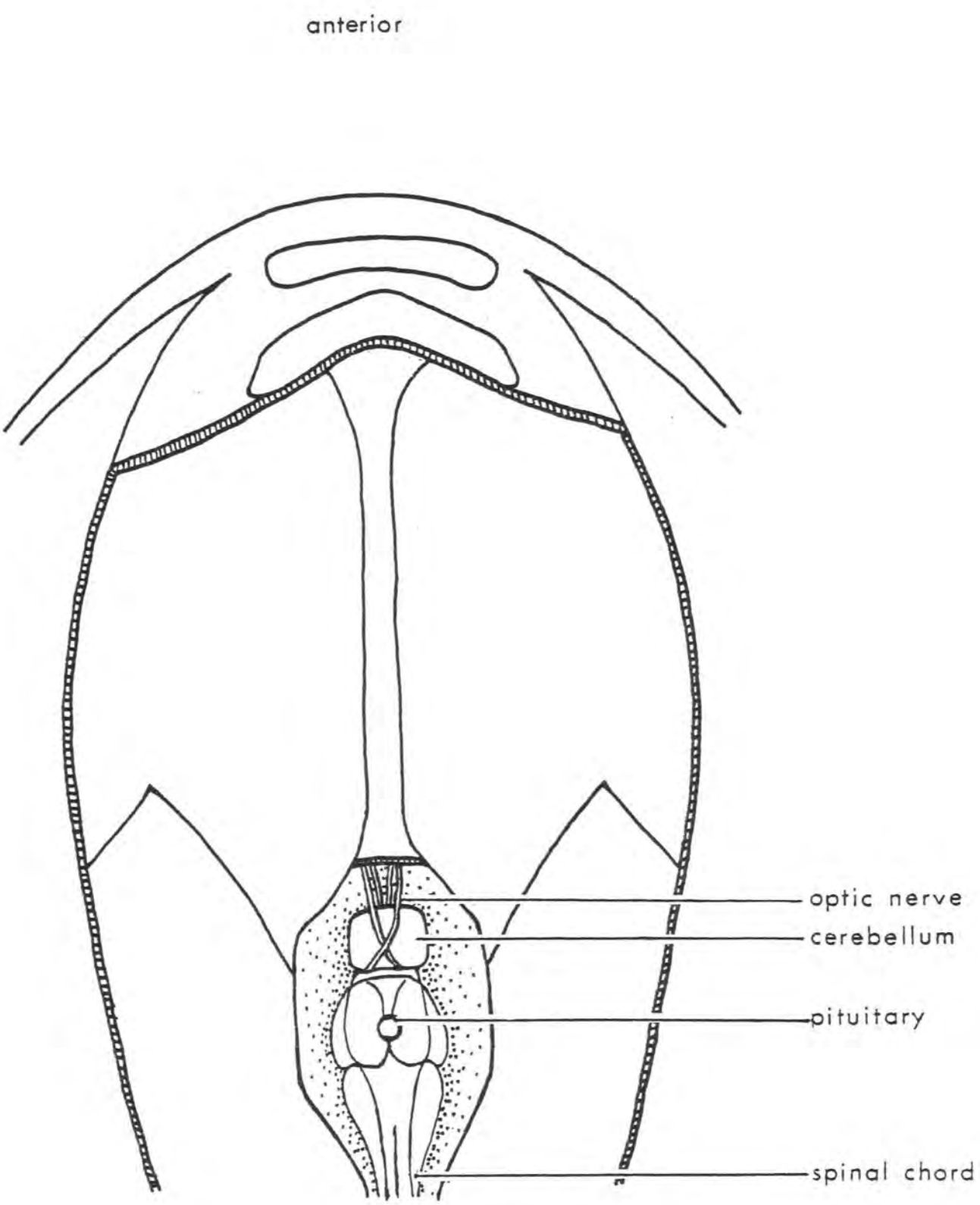

Figure 5.3 Dissection of pituitary. Part 3.

anterior

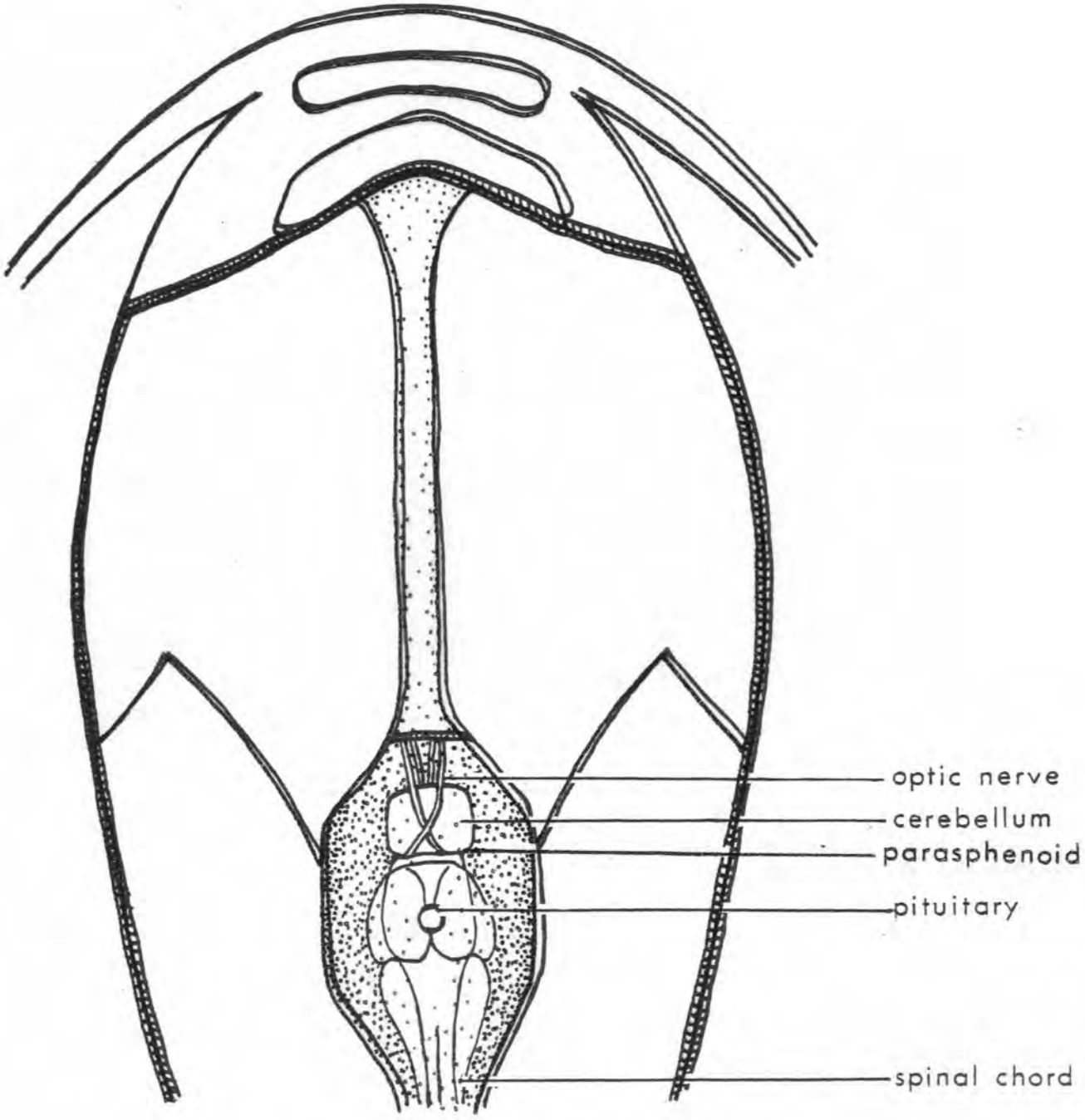

Figure 5.3 Dissection of pituitary. Part 3.
Figure 5.2 Dissection of pituitary. Part 2.

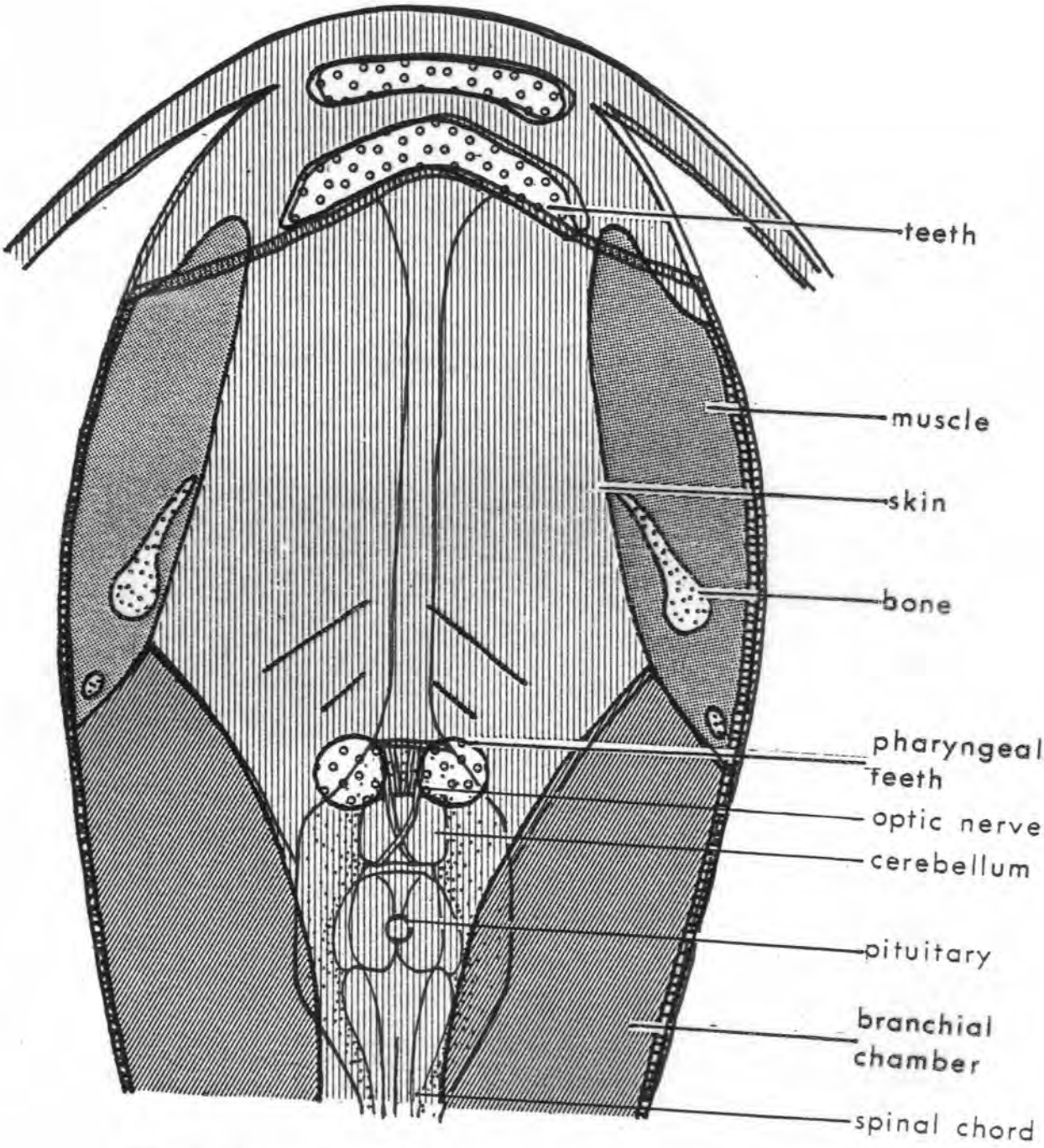

Figure 5 13 Dissection of pituitary. Part 13.

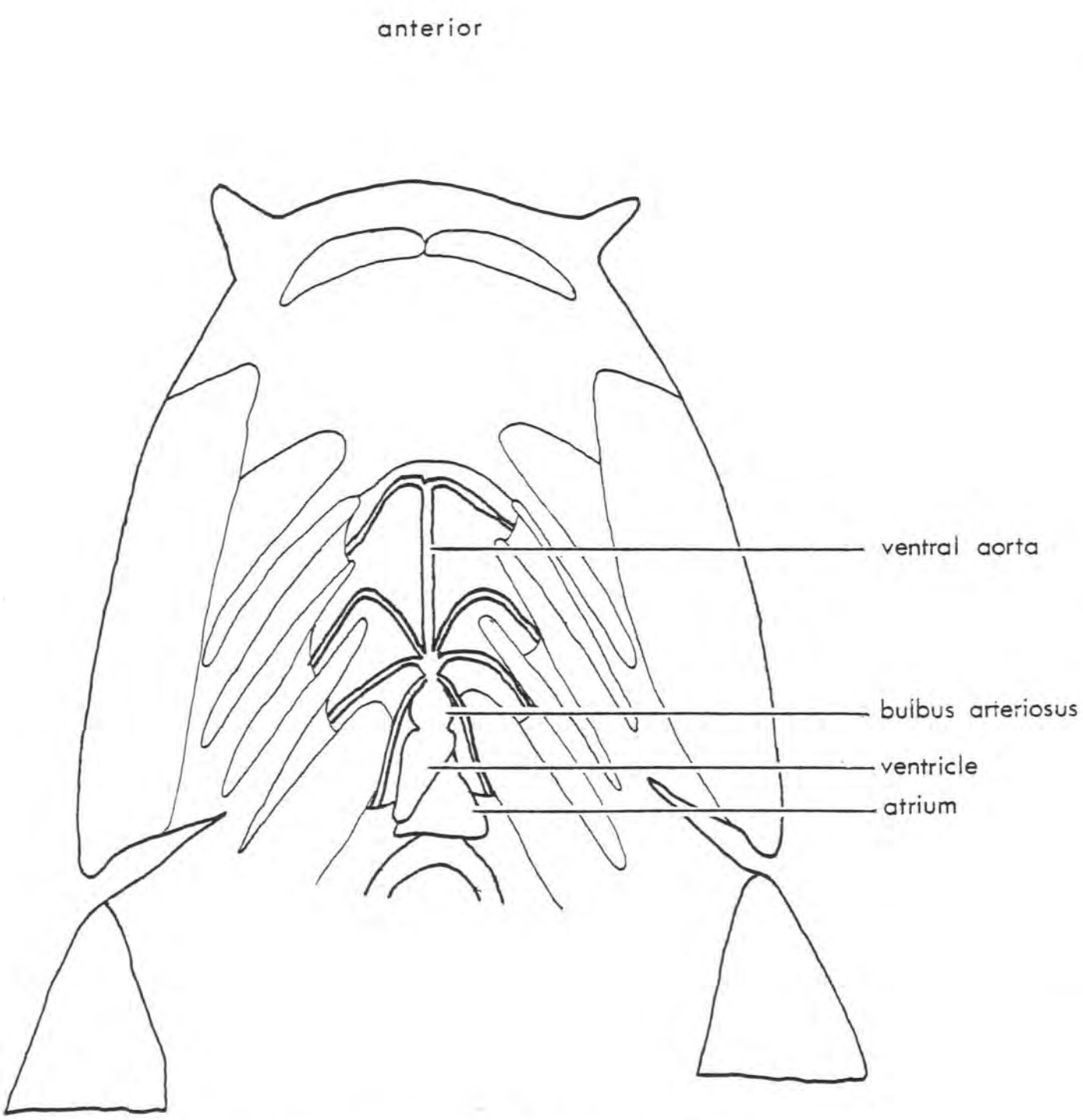

Figure 6.2 Dissection of thyroid, Part 2.

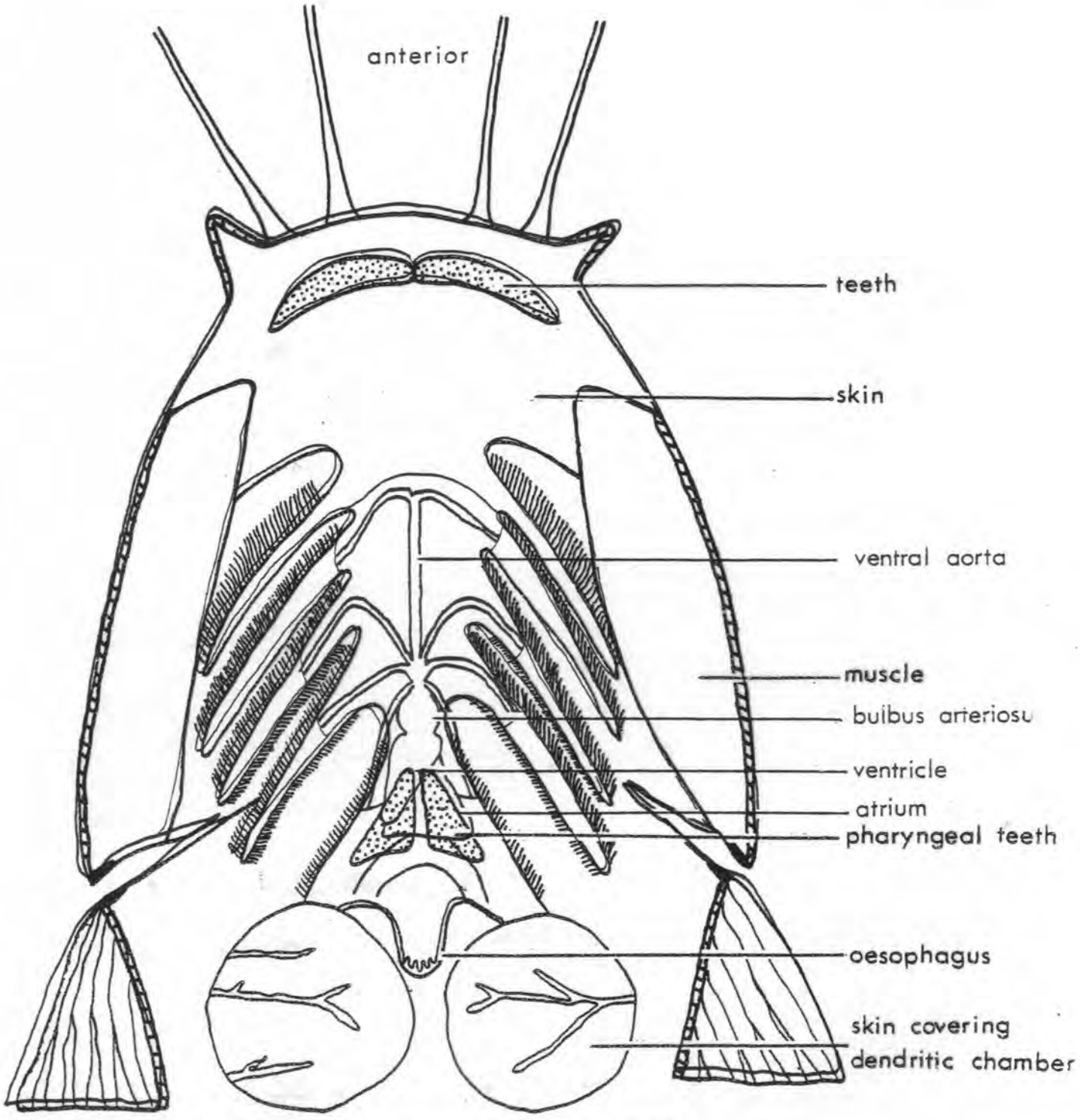

Figure 6.2 Dissection of thyroid, Part 2.

Figure 6.1 Dissection of thyroid. Part 1.

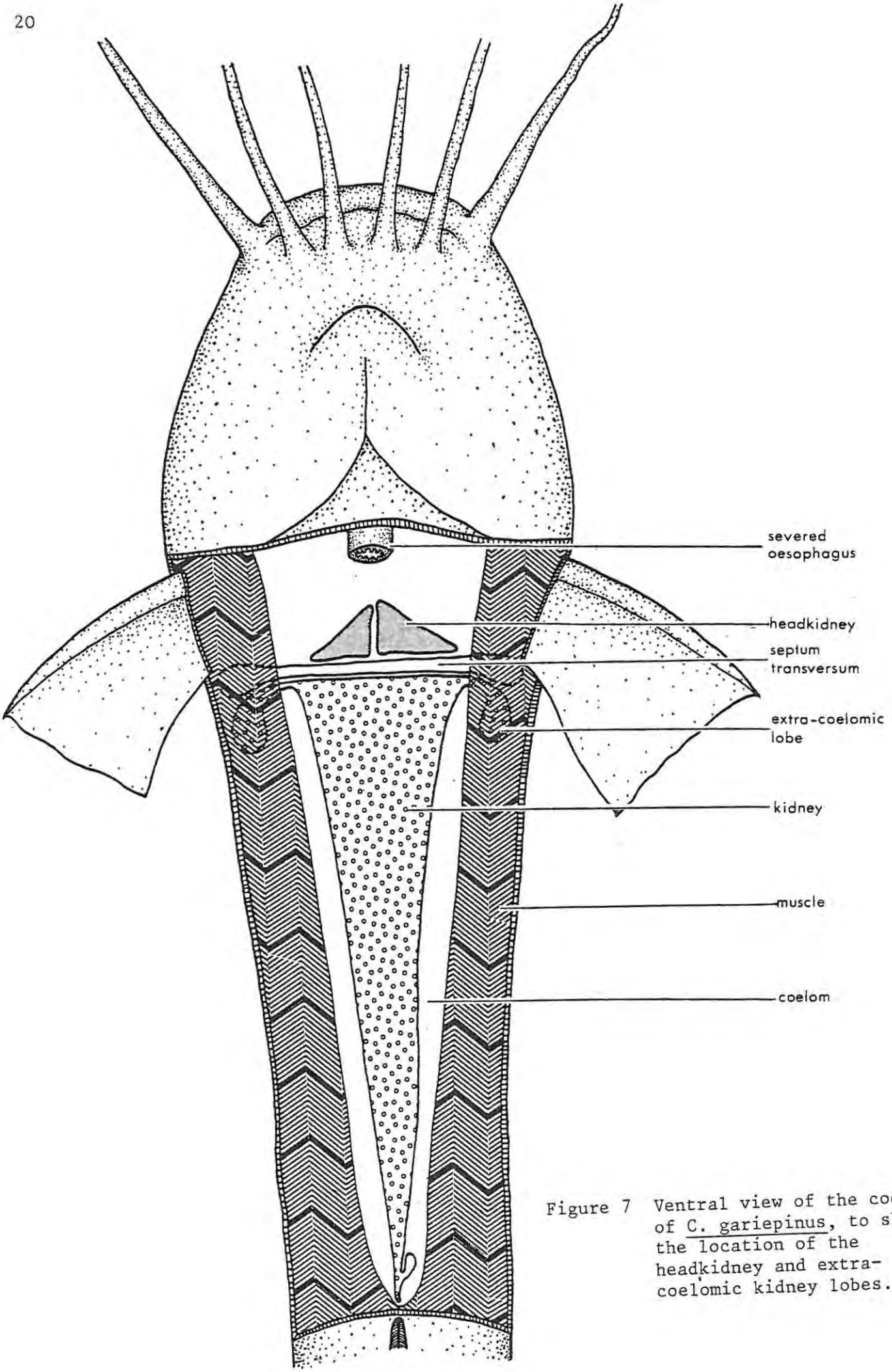

Figure 7 Ventral view of the coelo
of C. gariepinus, to show
the location of the
headkidney and extra-
coelomic kidney lobes.

III. Gonads

A slit was made in the abdominal body wall from the urogenital papilla anteriorly to the pectoral girdle, thus exposing the gonads, attached by mesenteries to the dorsal coelomic wall. This connective tissue was cut, and the gonads removed and weighed to the nearest 0,5 g with a Pesola spring balance. A section of gonad, about ½ - 1 cm long, was fixed in Bouin's fixative.

In some male fish a section of gonad from the region nearest to the urogenital papilla was also removed and fixed so that the structure of the seminal vesicles could be examined.

IV. Headkidneys

The slit made in the abdominal body wall for the removal of the gonads was extended a few centimetres by cutting through the bone of the pectoral girdle. The oesophagus was severed and the alimentary canal removed to reveal the kidney. The septum transversum could now be seen lying anterior to the kidney. Between the septum transversum and the severed oesophagus is a large mass of connective tissue, embedded in which are two small brownish-red triangular structures, the left and right headkidney (Figure 7.). The headkidneys were dissected out and fixed in Bouin's fixative.

The extra-coelomic lobes of the kidney (see Page 94) were also fixed in some cases. The thick, muscular layers lateral to the anteriormost part of the kidney were cut away until two dark red structures were revealed lying dorsally near the vertebral column. Each of these is attached to the kidney by a narrow isthmus which was severed, and the extracoelomic lobes were removed and fixed.

3. LIGHT MICROSCOPY METHODS

Methods used for gonads, thyroid and headkidney tissue

Gonad, thyroid and headkidney tissue were fixed in Bouin's fixative for 12 - 18 hours.

Tissues were then transferred to 70% ethanol, in which they were kept for at least two weeks, the ethanol being changed for a fresh solution every 24 hours. Tissues which were kept for several months before embedding were stored in 70% ethanol.

Dehydration and embedding procedure:

Pieces of tissue measuring up to 3 mm × 3 mm × 1 mm were dehydrated and embedded according to the following schedule:

Solution	No. of times changed	Time in each change	Total time
70% ethanol	14	24 hours	336 hours
96% ethanol	3	1 hour	3 hours
100% ethanol	3	30 - 45 mins	1 - 2 hours
Toluene	3	30 - 45 mins	1 - 2 hours
Paraffin wax or 'paraplast' embedding medium at 4-6° above m.p.	3	30 - 45 mins	1 - 2 hours

Tissues were then embedded in fresh catfish wax at 10 - 15°C above melting point (m.p.).

Methods used for pituitary material

The pituitary and brain were fixed *in situ* by flooding the exposed brain cavity with Bouin's Hollande sublimate (see Appendix 1). The whole brain, with pituitary attached was then lifted out and placed in this same fixative for 12 - 18 hours. The tissue was trimmed until only the pituitary, stalk, and a small amount of brain tissue remained. This material was then immersed in a 0,5% solution of iodine in 70% ethanol for 1 - 2 hours. This treatment removes the black deposit left in the tissue by the mercuric chloride in the fixative. After iodine treatment the tissue was placed in pure 70% ethanol, and processed in the same way as described for the gonad, thyroid and headkidney tissue in the Table given above.

Sections of all embedded material were cut using a rotary microtome. Sections were cut at 5 µm or 7 µm.

Sections were mounted onto glass slides which had been cleaned in 96% ethanol, dried and then smeared with a thin film of Mayer's albumen (see Appendix 11). Sections were then floated on warm water (approx. 4°C below the melting point of the wax) until they expanded, then an albumenised slide was slipped beneath the sections, which were then lifted onto the slide. Excess water was drained off and the slides left to dry overnight on a hotplate at approximately 25°C. The slides were dried just clear of the surface of the hotplate.

Slides were stained using one of the following methods:

1. Stains used for general material (ovaries, testes, thyroid, headkidney).
   (a) Masson's trichrome method (Humason, 1972).
   (b) Ehrlich's haematoxylin and eosin method. (Humason, 1972).
2. Stains used for pituitary material.
   (a) Heidenhain's Azan method (Heidenhain, 1915).
   (b) Herlant's tetrachrome method (Herlant, 1960; Racadot, 1963).
   (c) Periodic acid - Schiff - orange G method (Pearse, 1953).
   (d) Gabe's modification of Gomori's aldehyde fuchsin method (Gabe, 1953).
   (e) Acid permanganate - alcian blue - periodic acid - Schiff - orange G method (Herlant, 1960).
   (f) Luxol fast blue - periodic acid - Schiff - orange G method (Herlant, 1960).
   (g) MacConnaill's lead haematoxylin method (MacConnaill, 1947; Olivereau, 1970).

Stained sections were mounted in DPX mountant or Euparal, then covered with coverslips.

4. ELECTRON MICROSCOPY METHODS

Small blocks of tissue (< 1 mm^2) were excised and pre-fixed in cold (4°C) 5% glutaraldehyde (Polaron Ltd., UK) in 0.1M phosphate buffer (pH 7.2) for a minimum of 12 hours. The tissue blocks were washed in two exchanges of phosphate buffer (10 mins each) and transferred to the secondary fixative, 1% osmium tetroxide in phosphate buffer prepared according to Millonig (1961).

Secondary fixation was carried out for 90 mins at 4°C and was followed by a further two washes in phosphate buffer prior to dehydration. Dehydration was carried out through an ethanol series (30, 50, 70, 80, 90, 100%) with 10 mins at each concentration and two treatments of absolute ethanol. The temperature was maintained at 4°C until the 80% stage. Dehydration was followed by two changes of the transitional solvent, propylene oxide,for 20 mins each. The tissue was then subjected to increasing concentrations of the embedding medium, Emix (Emscope Laboratories, UK), in propylene oxide (25:75, 50:50; 75:25) to ensure adequate infiltration of resin. The tissue blocks were then transferred to pure resin and allowed to infiltrate for one hour at 40°C after which they were transferred to fresh resin in BEEM capsules and allowed to polymerise for 16 hours at 60°C.

Ultrathin (40 - 50 nm) sections were cut on an LKB UM III ultramicrotome with glass knives. The sections were collected on uncoated 200 or 400 mesh copper grids and stained with 5% aqueous uranyl acetate followed by lead citrate prepared according to Reynolds (1963). Staining times were variable depending upon the tissue under examination but were generally in the region of 20 - 30 mins for the uranyl acetate and 3 - 6 mins for the lead citrate. The examination of the sections took place using a Hitachi HU 11B transmission electron microscope operating at an accelerating voltage of 75 kV. Images were recorded on Kodak 4463 electron image film.

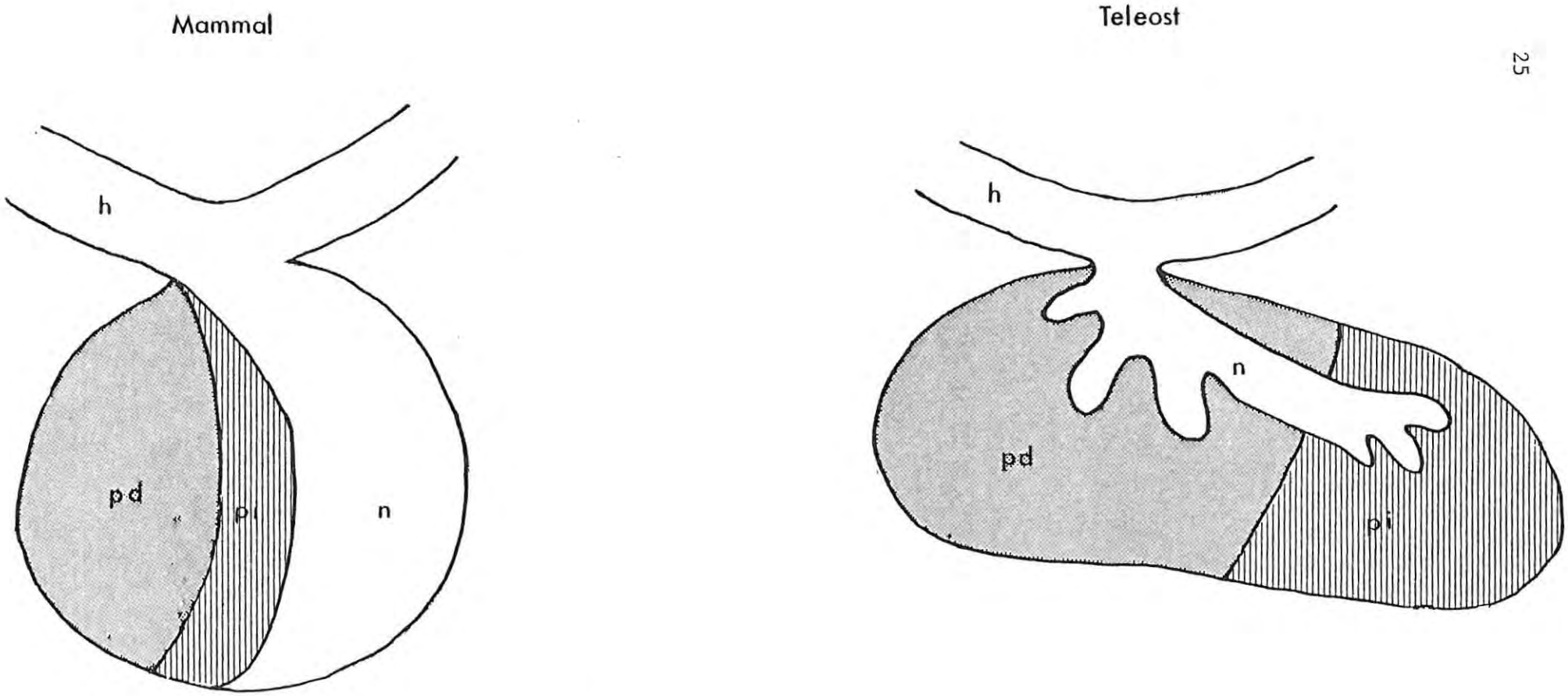

Figure 8 Diagrammatic representation of a comparison between the basic arrangement of components of mammalian and teleost pituitary glands. h = hypothalamus, n = neurohypophysis, pd = pars distalis, pi = pars intermedia.

REVIEW AND RESULTS

THE PITUITARY OF TELEOSTS

Comprehensive reviews of publications on the pituitary gland of teleosts are published fairly regularly, and include those of Kerr (1942), Pickford and Atz (1957), Hoar (1966), Purves (1966), van Oordt (1968), Ball and Baker (1969), Sage and Bern (1971) and Schreibman *et al.*, (1973).

The pituitary gland of all vertebrates is made up of two distinct components, the neurohypophysis and the adenohypophysis. Embryologically the neurohypophysis is formed from a downgrowth from the infundibular floor of the diencephalon (third verticle) of the brain, and the adenohypophysis is formed from an ectodermal upgrowth known as Rathke's pouch from the floor of the mouth or stomadeum (Romeis, 1940; Bentley, 1976). The form and interrelationships of these two components vary in the different classes of vertebrates (Bentley, 1976). The best documented organisation is that found in man, and for this reason a brief comparison between the pituitary of teleosts and that of man is given. The adenohypophysis and neurohypophysis of the human pituitary have come to be known as the anterior and posterior lobes respectively, due to their relative positions (Figure 8). The neurohypophysis of the teleost gland, in contrast, does not form a discrete lobe but interdigitates with the adenohypophysis. In some fish, as is the case in man, the neurohypophysis is connected to the brain by a definite infundibular stalk which consists of axonal fibres of neurosecretory cells, whose cell bodies are located in the hypothalamus of the brain. In others the pituitary is closely applied to the brain and the broad cavity of the third ventricle extends downwards in a shallow hypophysial recess lined by ependymal cells of various shapes, in the centre of an oval ring of fibres (Kerr, 1942; Bentley, 1976).

The neurohypophysial core consists largely of the endings of these fibres interspersed with cells known as pituicytes. The neurohypophysis is basically a storage-release centre for materials which are synthesised in the hypothalamus. These are transported to the neurohypophysial core along neurosecretory axons. The adenohypophysis is the site of synthesis, storage and release into the bloodstream of seven or eight different

hormones, and consists of the corresponding number of secretory tissue types and blood capillaries.

The blood supply to the pituitary of teleosts is different from that of all other vertebrates, including other actinopterygians (Bentley, 1976). In all other vertebrates there are portal blood vessels connecting the hypothalamus and the adenohypophysis. Considerable variation has been observed among the teleosts but there is always little or no connection through the brain connection or stalk (Kerr, 1942; Pickford and Atz, 1957; Ball and Baker, 1969; Bentley, 1976) although a hypophysial portal system has been reported for Channa punctatus (Belsare, 1965).

The blood supply to the adenohypophysis of teleosts usually passes initially through the neurohypophysis. Hypophysial arteries which branch off from the internal carotid artery bring blood to the neurohypophysis. These break up into a collection of capillaries forming a vascular plexus known as the primary longitudinal plexus, in the neurohypophysial core. This plexus is usually found close to, or actually at the adeno-hypophysial boundary. From this plexus a series of capillaries passes into the adenohypophysis and ramifies through this glandular region. This plexus is known as the secondary centrifugal plexus. Blood is then collected into a capillary network in the connective tissue capsule enclosing the gland and this superficial network of vessels drains posteriorly through a vein which enters the systematic venous system via the hypophysial or hypocranial vein (Kerr, 1942; Bretschneider and de Wit, 1947; Green, 1951; Pickford and Atz, 1957; Wingstrand, 1966; Ball and Baker, 1969).

A saccus vasculosus is present in many teleosts. This is a thin-walled sac growing out from the third ventricle (infundibular recess) posterior to the pituitary. The function of this structure is unknown.

The adenohypophysis or glandular lobe of the teleost pituitary can be subdivided into two regions, the pars intermedia and the pars distalis. In the pars distalis two distinct areas can again be distinguished, and these are known as the rostral and proximal divisions of the pars distalis.

A great variety of different names have been used for these areas which makes the literature somewhat confusing (Table III).

Stendell (1914), one of the earliest workers on teleost pituitaries used the names pars anterior, middle and posterior glandular regions. Olivereau (1954) used pars follicularis, middle glandular region and pars intermedia. Pickford and Atz (1957) suggested a totally new terminology based solely on morphological considerations. They felt that the names previously used suggested homology with the regions of the same name in the pituitary of higher vertebrates,which had not be proven, and thus could lead to confusion. They therefore suggested that the names pro-, meso- and meta-adenohypophysis should replace the earlier names. These names were adopted by several authors in subsequent publications, but gradually it has become evident that the regions of the teleost pituitary do seem to be responsible for the secretion of the same or similar hormones to those of higher vertebrates, and it now seems justified in view of the body of experimental evidence available, the prevalence of use, and in the interest of uniformity, that the terms of Pickford and Atz be superceded by those which have functional homologies with the tetrapod gland (Ball and Baker, 1969; Schreibman *et al.*, 1973). This is also preferable as some cells of the teleost hypophysis may be found in either the rostral or the proximal regions of the pars distalis, or both, and these regions do not merit the same level of distinction from each other as they do from the pars intermedia.

TABLE III/...

TABLE III Summary of names given to different regions of the adenohypophysis by various authors

	Author			
1.	Stendell (1914)	pars anterior	ubergangsteil	pars intermedia
2.	Kerr (1942)	anterior glandular region	middle glandular region	posterior glandular region
3.	Olivereau (1954)	pars follicularis	middle glandular region	pars intermedia
4.	Pickford and Atz (1957)	pro-adenohypophysis	meso-adenohypophysis	meta-adenohypophysis
5.	Various	anterior lobe	dorsal or transitional lobe	ventral or intermediate lobe
6.	Most modern authors	rostral pars distalis	proximal pars distalis	pars intermedia

The arrangement, shape and proportions of these regions vary within the teleosts. In some species, e.g. the eel Anguilla anguilla, the rostral and proximal pars distalis and pars intermedia are arranged sequentially along the antero-posterior axis (Knowles and Vollrath, 1966c; Olivereau, 1967; Ball and Baker, 1969), while in others, e.g. the cichlid Sarotherodon mossambicus, and the molly Poecilia latipinna, the pituitary is shorter antero-posteriorly but much deeper dorso-ventrally. The pars intermedia is then located ventral to the pars distalis (van Oordt, 1968; Ball and Baker, 1969; Sage and Bern, 1971).

The hormones known to be secreted by the adenohypophysis are:

1. Thyrotropic hormone (also known as thyrotropin, thyroid-stimulating hormone, or TSH).
2. and
3. Gonadotropic hormones (also known as gonadotropins, or GTH). In mammals gonadotropins are follicle stimulating hormone (FSH) and interstitial cell stimulating hormone (ICSH), which is also sometimes called luetinising hormone (LH).
4. Lactotropic hormone (also known as prolactin, lactotropin, luteotropic hormone, mammotropic hormone, lactogen, or LTH).
5. Somatotropic hormone (also known as somatotropin, growth hormone, or STH).

6. Adrenocorticotropic hormone (also known as adrenocorticotropin, or ACTH).
7. Melanotropic hormone (also known as melanophore-stimulating hormone, intermedin or MSH).

This terminology is based mainly on the prominent physiological activity of the hormone in mammals. This may not be the same in fish, although the hormones appear to be biochemically similar (though not identical) to their mammalian counterparts (Ball and Baker, 1969; Sage and Bern, 1971).

Each of the three divisions of the adenohypophysis is made up of two or more different secretory cell types. The problem facing the endocrinologist is to distinguish between these cell types and allocate the correct function to each.

The distinction of the different cells can be achieved by various types of staining methods. The cells can be divided into chromophils (cells with stainable cytoplasmic granules) and chromophobes (cells with no stainable cytoplasmic granules). Chromophils may be acidophils, with an affinity for acid dyes, or basophils, with an affinity for basic dyes.

Separation of different cells of one staining type in the same region of the gland can be difficult, and new staining methods are always being sought to aid in this type of distinction. The development of electron microscopy has been a great aid to this end and cells can then be identified according to the size and shape of their secretory granules, size and position of nucleus, relationship to other parts of the pituitary, etc.

There are several methods which can be used in the identification of the source of the adenohypophysial hormones. These rely on looking for coincidental or related changes in the pituitary and its target organs; isolation of one cell type and assay of its hormone content; extirpation of one cell type and study of resultant deficiencies and use of histochemical or immunochemical means for the identification of the hormones within the cells (van Oordt, 1968).

I. ROSTRAL PARS DISTALIS (RPD)

This region of the gland usually contains two secretory cell types that are discernible in the light microscope, one acidophil and one chromophobe (in some cases this is slightly basophilic). Sometimes another basophil is also present (e.g. in the eel, Anguilla anguilla, Ball and Baker, 1969).

(a) Prolactin cells:

The acidophil cell type of the rostral pars distalis is now generally believed to be the site of synthesis of prolactin. This was proved experimentally by various authors (reviewed by van Oordt, 1968; Ball and Baker, 1969 and Sage and Bern, 1971), who discovered that the killifish, Fundulus heteroclitus, could not survive in fresh water without prolactin, and then discovered that an acidophil cell type in the rostral pars distalis appeared active in fish held in seawater or dilute seawater.

Further evidence for these acidophils being the source of prolactin comes from experiments involving monitoring the effects of hypophysectomy, followed by grafts of the rostral pars distalis acidophils, and also from immunocytochemical staining (Ball and Baker, 1969; Sage and Bern, 1971 and Schreibman *et al.*, 1973).

These presumed prolactin-secreting cells are the predominant cell type in the rostral pars distalis, and are generally round or oval in shape, with an eccentric nucleus. The cytoplasm of these cells is packed with large coarse granules, which are fairly evenly distributed. These granules stain intensely with azocarmine, acid fuchsin, erythrosin and orange G. The nuclei are usually oval or round, although in many cyprinodontids a kidney-shaped nucleus has been found (Ball and Baker, 1969).

In certain species of fish, e.g. the eel, Anguilla anguilla, the prolactin cells are arranged in the form of follicles. Each cell is columnar in shape and bears cilia projecting into the follicular lumen. In other species this follicular arrangement is not apparent (Ball and Baker, 1969; Sage and Bern, 1973).

(b) Adrenocorticotropic cells

Adrenocorticotropin-secreting cells are also generally located in the rostral pars distalis. The presence of adrenocorticotropin in the teleost pituitary was demonstrated directly by assay methods. Experiments involving the treatment of pituitaries of Poecilia latipinna and Anguilla anguilla with the adrenocortical inhibitor, metapirone, showed a subsequent hypertrophy and hyperplasia of these pituitary cells. Similarly, surgical removal of the adrenal gland in A. anguilla resulted in the specific activation of these cells (Olivereau and Olivereau, 1968; Ball and Baker, 1969; Schreibman *et al.*, 1973).

The adrenocorticotropic cells of the teleost pituitary are located in the rostral pars distalis, and form a border between the neurohypophysis and the prolactin cells. Adrenocorticotropic cells are polymorphic, cuboidal or columnar in shape. A difference in staining responses between species has been recorded (Schreibman *et al.*, 1973). Very fine granules which may be erythrosinophilic, fuchsinophilic and positive also to acid alizarin blue, may be present and these are the only cells of the rostral pars distalis which stain with lead haematoxylin (van Oordt, 1968). Adrenocorticotropic cells have sometimes been described as chromophobes, particularly in cyprinodontid pituitaries (Ball and Baker, 1969; Schreibman *et al.*, 1973), though this may be due to hypoactivity of the cells at the time of death (Ball and Baker, 1969).

Nuclei of these cells are elongate, particularly in actively secreting cells, and may have a prominent nucleolus, or may show a scattering of chromatin and no nucleolus, as is the case in Poecilia sp. (Ball and Baker, 1969).

(c) Other cells of the rostral pars distalis

The location of the cells responsible for the secretion of thyroid stimulating hormone (TSH cells) is very variable. These cells are always found in the pars distalis, but are not confined to either rostral or proximal regions. They have been described in the rostral

pars distalis of some fish,(e.g. clupeoids, cyprinids and some others), and in the proximal pars distalis of others (e.g. cyprinodontids) and intermediate between these two regions in others (e.g. cichlids, salmonids, Ball and Baker, 1969). They will be described here with the proximal pars distalis cells.

The gonadotropic cells are also fairly variable in position. They are usually found in the proximal pars distalis but some gonadotrops are occasionally found in the rostral region, particularly in certain fish (e.g. Anguilla and Salmo spp., Ball and Baker, 1969). These cells will be considered under the heading of proximal pars distalis.

Agranular chromophobic cells have been found in the rostral pars distalis of many species (e.g. Anguilla anguilla, Mugil cephalus, Gasterosteus aculeatus, Carassius auratus, Oncorhynchus nerka and several Tilapia and Sarotherodon spp., but their function is unknown.

II. PROXIMAL PARS DISTALIS (PPD)

This region of the pituitary usually contains at least two basophil and one acidophil cell types.

(a) Somatotropic cells

The acidophil cell type of the proximal pars distalis is generally accepted to be the source of somatotropin, the STH cells, although evidence for this is fairly scanty. Somatotropic cells of teleosts have been identified by their similarities in staining properties to mammalian somatotropic cells, and the fact that they are usually large cells with a well-developed Golgi apparatus in rapidly growing fish, and show an accumulation of secretory material when growth is artificially retarded (Sage and Bern, 1971), and are present at birth in Xiphophorus sp. (Schreibman *et al.*, 1973).

These are usually the most prominent cells of the pars distalis and occupy a large proportion of the proximal pars distalis. The cells are usually large and rounded, oval or pyramidal in shape. They are typically crowded with extremely fine orangeophilic granules

which may be packed so densely that they give the impression of a homogeneous mass. Somatotropic cells are the only serous (i.e. not glycoproteinaceous) cells in the proximal pars distalis, and are negative to periodic acid-Schiff staining technique, aldehyde fuchsin and alcian blue. The nucleus is ovoid or round in section, and is frequently eccentrically placed. It may appear distorted due to the dense packing of the cytoplasmic granules. The nucleolus is usually prominent. Ball and Baker (1969) report that great variations occur within a single fish in the size of the nucleolus of these cells.

Matty (1957) describes two types of acidophil in the proximal pars distalis of Pseudoscarus guacamaia, but their functional identity is unknown, and this does not appear to be a common phenomenon in teleosts.

Sexual dimorphism in somatotropic cells is sometimes apparent, in which case the somatotropic cells of the female are usually larger and more heavily granulated than those of the male. This phenomenon can usually be attributed to species where the adult female reaches a larger size than the adult male (e.g. Anguilla and Poecilia spp. Ball and Baker, 1969).

(b) Gonadotropic cells

The cells which are now accepted as being the source of gonadotropins in the telost pituitary are basophils of the proximal pars distalis. They have been identified mainly as a result of the changes observed in these cells during reproduction. They are also quiescent or absent before sexual maturity and show pronounced changes in their secretory activity which correlate with the gonadal cycle. These basophilic cells also exhibit distinct changes after castration. Similar changes are observed after treatment with 'methallibure', a chemical substance which inhibits gonadotropin production (Sokol, 1955; Robertson and Wexler, 1962a and b; Ball and Baker, 1969; Sage and Bern, 1971; Schreibman *et al.*, 1973).

The type of gonadotropins elaborated in the teleost pituitary, and the

number of cell types involved remains unresolved. In mammals two gonadotropins, follicle stimulating hormone and luetinising hormone are produced, and the cells producing them are of two types known as β-and γ-cells respectively. Two types of gonadotrop have been identified in several species of teleosts, e.g. Carassius auratus (the goldfish), Anguilla anguilla (the European eel) and Salmo salar (the Atlantic salmon, Ball and Baker, 1969; Leatherland, 1972; Olivereau, 1976). Electron microscope studies sometimes reveal two cell types when only one was apparent in the light microscope (Leatherland, 1972). Teleost gonadotropins are chemically more similar to mammalian luetinising hormone than follicle stimulating hormone, though activities similar to those brought about by both these hormones have been demonstrated in teleost pituitary extracts (Sage and Bern, 1971; de Vlaming, 1974). Recent biochemical and biological evidence suggests that there may be only one type of gonadotropin which serves the double role of follicle stimulating and luetinising hormones (Schreibman *et al.*, 1973; Dodd, 1975; Bentley, 1976). It would therefore appear inadvisable to apply the names β-and γ-cells to the gonadotropic cells of teleosts unless homology of these cells with their mammalian counterparts is incontrovertibly proved.

Gonadotropic cells of teleosts lack uniformity in shape and nuclear structure. In poeciliids, which have been well described, they have irregular cell boundaries with considerable intracellular spaces and intercellular vacuoles. The nuclei are large and round with a prominent nucleolus (Ball and Baker, 1969; Sage and Bern, 1971). In all teleosts the gonadotropic cells are typically mucoid cells, containing granules which stain strongly with periodic acid-Schiff, indicating a glycoproteinaceous nature. They also stain positively with alcian blue, and sometimes with aldehyde fuchsin. The granules are larger than those of the thyrotrops but have similar staining reactions (see following section). Gonadotropic cells also often contain a variable number of unique spherical hyaline droplets which are positive to periodic acid-Schiff,but negative to aldehyde fuchsin (Sage and Bern, 1971; Schreibman *et al.*, 1973). Vacuoles are often present in these cells. Active cells have reduced cytoplasmic staining, enlarged nuclei and nucleoli and more extensive vacuolisation.

(c) Thyrotropic cells

The thyrotropic cells are probably the most diverse in structure of all pituitary cell types. The methods used to identify these cells are typified by work on Phoxinus phoxinus, which demonstrated that these cells were degranulated in correlation with hypertrophy of the thyroid following treatment with goitrogens (Barrington and Matty, 1955), and on Carassius auratus which showed that destruction of the thyroid by I^{131} resulted in thyrotropic cell degranulation (Chavin *et al.*, 1962).

Thyrotropic cells are angular or polyhedral in outline. The nuclei have chromatin granules and a round nucleolus that varies in size with the activity of the cell.

The separation of thyrotropic from gonadotropic cells can be difficult as they have very similar staining features. Thyrotrops are periodic acid-Schiff positive, aldehyde fuchsin positive, and also stain with chrome-alum haematoxylin. In some fish thryotrops are aldehyde fuchsin positive and gonodotrops aldehyde fuchsin negative, but in others gonadotrops are also positive to this stain (Ball and Baker, 1969; Sage and Bern, 1971). In Poecilia latipinna the gonadotrops stain deep blue with alizarin blue, while the thyrotrops stain a slatey grey-blue (Ball and Baker, 1969).

Topographical separation of the two cell types is also sometimes possible as the thyrotropic cells may be in the rostral pars distalis, and the gonadotrops are nearly always in the proximal pars distalis. Thyrotrops in the proximal pars distalis are usually dorsally (cyprinodontids) or anterdorsally (cichlids and some mullet) situated, but this distinction is not always clear. The thyrotrops have smaller secretory granules than the gonadotrops, and in fact these are often the smallest of those of all the basophilic cells of the pituitary. The cytoplasm is sometimes vacuolated (e.g. Sarotherodon mossambicus) and in this species has also been reported to sometimes contain crystalline-like inclusions (Sage and Bern, 1971).

Examination of the adenohypophysial cells under the electron microscope reveal greater details of the structure of these cells. Results

published to date are not extensive, and too variable to allow useful generalisations to be made (Schreibman *et al.*, 1973).

III. PARS INTERMEDIA

There are two cell types in the teleost pars intermedia. One of these is believed to be the source of melanophore-stimulating hormone, and the function of the other is not yet clear. The shape and staining reactions of these cells vary with the species of teleost.

Neither of the cell types of the pars intermedia are thought to be in any way involved with reproduction, nor can they easily be confused with others which are (Sage and Bern, 1971), and for this reason these cells will not be considered in any detail here.

IV. NEUROHYPOPHYSIS

The neurohypophysis of teleosts consists largely of axonal nerve fibres the cell bodies of which are located in the hypothalamus of the brain. The fibres are mostly unmyelinated, and extend down the pituitary stalk or hypothalamus-hypophysial connection to form the bulk of the neurohypophysial core (Ball and Baker, 1969).

The means by which the pituitary is attached to the brain varies in different species of teleosts. In some the gland is closely applied to the brain, while in others it is suspended from the brain by the presence of a distinct stalk. Kerr (1942) categorised teleost pituitaries into two types, type A and type B, based on the absence or presence (respectively) of a stalk. Bretschneider and de Wit (1947) renamed these platybasic and leptobasic types. In the leptobasic type variations occur in the place of attachment to the brain, and on the basis of this Bretschneider and de Wit (1947) designated the name caudobasic, dorsobasic and craniobasic.

The neurohypophysial core forms the bulk of the neurohypophysis. It sends out many fingerlike processes through the pars distalis of the adrenohypophysis, and penetrates the pars intermedia extensively. Many of the fibres terminate in the core of the neurohypophysis in close connection to the blood vessels, while others extend beyond the core in protrusions which invade the

neurohypophysis. Scattered cells known as neuroglial cells or pituicytes occur throughout the neurohypophysis, but are most common in close association with the pars intermedia (Perks, 1969).

The neurohypophysial fibres are of several different types. Two of these may be distinguished in the light microscope as one type is stainable with typical neurosecretory stains (i.e. aldehyde fuchsin, chrome-alum haematoxylin, aldehyde thionin and alcian blue), and the other is not. Ultrastructural study of the eel pituitary led to the synonymisation of these stainable and non-stainable fibres with the type A and type B fibres of Knowles and Vollrath (1966a and b). These ultrastructural studies have also led to the further separation of type A fibres into types A1 and A2 on the basis of the size of the secretory granules, and the relationship between the endings of these fibres with the adenohypophysial cells, blood vessels, etc. (Knowles and Vollrath, 1966a and b; Ball and Baker, 1969; Perks, 1969). The endings of the neurohypophysial fibres make contact with the adenohypophysial cells in various ways: - they may make immediate contact (synaptic in some cases), may end on a single or double basement membrane separating the nerve fibre from the adenohypophysial cell (through which the neurosecretory material is believed to diffuse), or may end in an extravascular space which separates the fibre terminal from the endocrine cell. Neurosecretory fibres have also been reported to terminate on capillaries of the neurohypophysial core, and their secretions therefore probably pass into the blood stream, and thence into the adenohypophysis (Ball and Baker, 1969; Peter, 1973; Dodd, 1975).

The hypothalamus and neurohypophysis therefore form a link between the nervous and endocrine tissues and the external and internal environment of teleosts as is the case in higher vertebrates. This has been emphasised by hypophysectomy experiments and experiments involving lesions of the pituitary stalk, which have resulted in drastic changes in the histology of the pituitary (Peter, 1973). In addition the neurohypophysis of teleosts acts as a storage and release centre for the octapeptide hormones arginine vasotocin and isotocin. There is suggestive evidence that the latter may have a role in bringing about the act of spawning in some teleosts, but as yet little is known about these hormones (Ball and Baker, 1969; Sage and Bern, 1971; de Vlaming, 1974; Dodd, 1975).

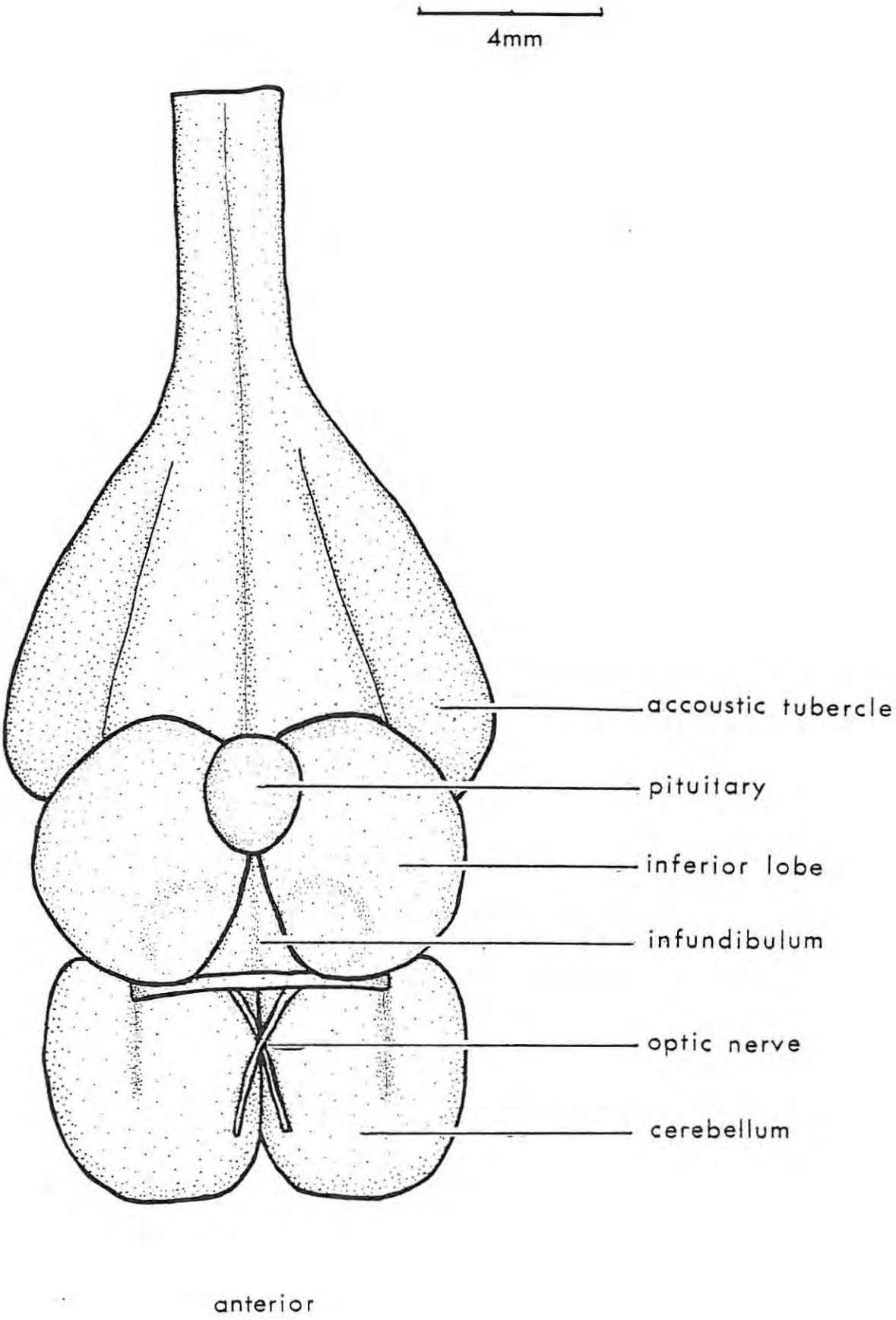

Figure 9 Ventral view of the pituitary of C. gariepinus.

RESULTS ON THE PITUITARY OF C. GARIEPINUS, AND COMPARISON WITH THAT OF OTHER SILUROIDS

The pituitary of C. gariepinus is an oval, off-white coloured body lying ventral to the brain, posterior to the optic chiasma, in a notch between the two inferior lobes (Figure 9). The pituitary is attached to the brain by a definite but short stalk, thus corresponding to Bretschneider and de Wit's classification as leptobasic (Bretschneider and de Wit, 1947). The whole gland is covered by a network of small blood vessels. The brain and pituitary are protected in an extremely strong bony case or cranium, and the pituitary itself rests on the parasphenoid bone.

In general structure and location the pituitary of C. gariepinus was found to be similar to that described for C. lazera (Rizkalla, 1963) and C. batrachus (Lehri, 1966), but both these authors claimed to be able to make our different regions of the pituitary before histological sections were made. Lehri reported several distinctive depressions in the external appearance of the pituitary of C. batrachus, while Rizkalla actually assigned the names anterior, posterior and middle glandular regions to the different areas formed by similar depressions in C. lazera.

The pituitaries of the 30 specimens of C. gariepinus showed little consistency in outward appearance, and although similar depressions were clearly discernible in some of the examples, in others the pituitary was a completely smooth oval, with no depressions whatever. This difference appeared to be correlated with the closeness of application of the gland to the brain. In the majority of cases the pituitary showed no depressions and was not so closely applied to the brain as to be influenced in shape by the shape of the brain. In the few cases where definite depressions in the external appearance of the pituitary were apparent it was found that the gland was unusually closely applied to the brain, and the depressions in the pituitary seemed to have been imposed on this tissue by corresponding protruberances of the brain. Histological sections revealed that the depressions, when present, did not indicate different regions of the pituitary.

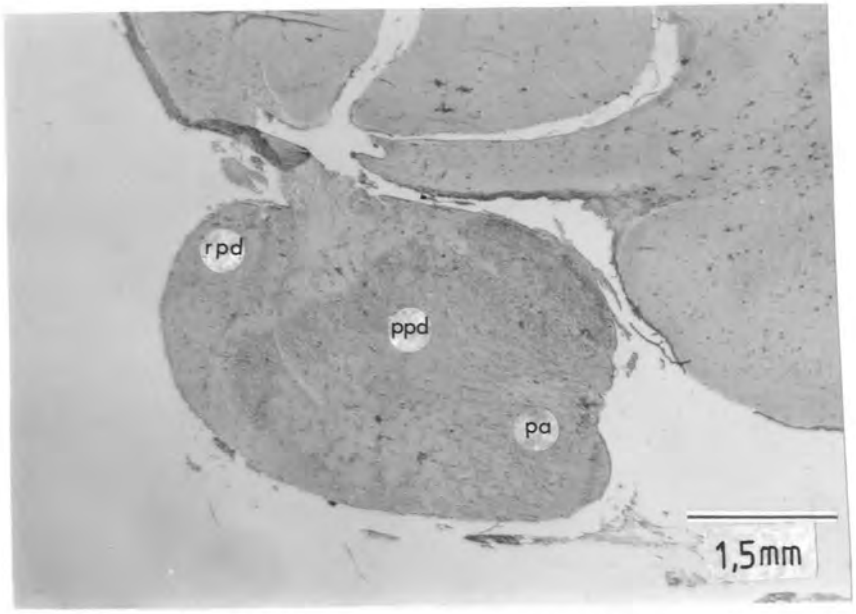

Figure 10 Mid-sagittal section through the pituitary of C. gariepinus to illustrate relative position of the pars intermedia (pa), rostral pars distalis (rpd) and proximal pars distalis (ppd). Periodic acid-Schiff-orange G. 5 µm.

Lehri (1966) describes the pituitary of C. batrachus as having the anterior end broader than the posterior. The pituitary of C. gariepinus showed variability in this respect too. Pituitaries which were smooth and without distortions were completely oval in shape, while those which had external distortions were also slightly broader anteriorly and more pointed posteriorly. The pituitary of C. gariepinus is uniform in colour. The area occupied by the rostral pars distalis alone is more transparent than the posterior regions, which are completely opaque.

Histology and Cytology

A median sagittal section through the pituitary of C. gariepinus reveals that this gland is made up of two distinct regions, the neurohypophysis and the adenohypophysis. The adenohypophysis is further subdivided into three parts, the rostral and proximal pars distalis and the pars intermedia.

In teleosts the adenohypophysis components vary in relative position, from being arranged linearly along the antero-posterior axis to being arranged dorso-ventrally, with the pars intermedia located ventral to the pars distalis (see Ball and Baker, 1969 for review). The pituitary of C. gariepinus was found to exhibit an organization intermediate between these two extremes. The arrangement is basically linear, but with a definite tendency for migration of the pars intermedia to a more ventral position. The linear arrangement is not rigid as in many cases the pars intermedia assumes a postero-ventral position, with the proximal pars distalis extending in a narrow band around this region, and the rostral pars distalis assuming an antero-dorsal position. (Figure 10). Such a situation was not reported in the pituitary of either C. lazera (Rizkalla, 1963), C. batrachus (Lehri, 1966) or Ictalurus punctatus (Grizzle and Rogers, 1976). In the three abovementioned species the three regions of the adenohypophysis were linearly arranged.

Blood supply to the pituitary

The blood supply to the pituitary of C. gariepinus was found to be derived largely from a ring vessel or hypophysial artery. No highly vascularised region of the hypothalamus exists, and there is no evidence of a hypothalamo-hypophysial portal system, comparable to that of higher vertebrates. A small contribution to the blood entering the pituitary of

C. gariepinus does, however, come via the hypothalamus.

Lehri (1966) found no highly vascularised region of the hypothalamus of C. batrachus, nor does he mention any contribution to the blood supply from the hypothalamus. It is possible, however, that a small vessel may connect the hypothalamus and pituitary as in C. gariepinus which may have been overlooked by Lehri. Blood flowing from the hypothalamus to the pituitary, however, is an unusual phenomenon in teleosts (see Ball and Baker, 1969).

Immediately anterior to the pituitary of C. gariepinus the hypophysial artery divides into several smaller blood vessels. The largest and most dorsally situated blood vessel formed by this division passes above the upper surface of the pituitary, between the connective tissue capsule covering the rostral pars distalis and the hypothalamus. This blood vessel enters the gland between the neurohypophysis and the rostral pars distalis, on the ventral side of the neurohypophysial stalk, and forms the primary longitudinal plexus of the pituitary. The primary longitudinal plexus divides up to form a large number of capillaries which radiate through the three regions of the adenohypophysis, thus forming the secondary centrifugal plexus (see page 27). In addition smaller blood vessels formed from the division of the hypophysial artery pass along the ventral surface of the rostral pars distalis, in the connective tissue capsule surrounding the gland.

The secondary centrifugal plexus forms a network of capillaries and sinuses between the secretory cells of the adenohypophysis. All regions of the pituitary of C. gariepinus are richly supplied with blood capillaries. This is in contrast to the findings of Rizkalla (1963) on C. lazera, who found only a few small capillaries in the proximal pars distalis, and of Lehri (1966) on C. batrachus who claims that the pars distalis is poorly vascularized in this species. Both authors illustrate these statements with convincing photographs.

The largest blood vessels of all the pituitary regions of C. gariepinus were found in the pars intermedia, but both the pars distalis regions were also well supplied, although with smaller vessels.

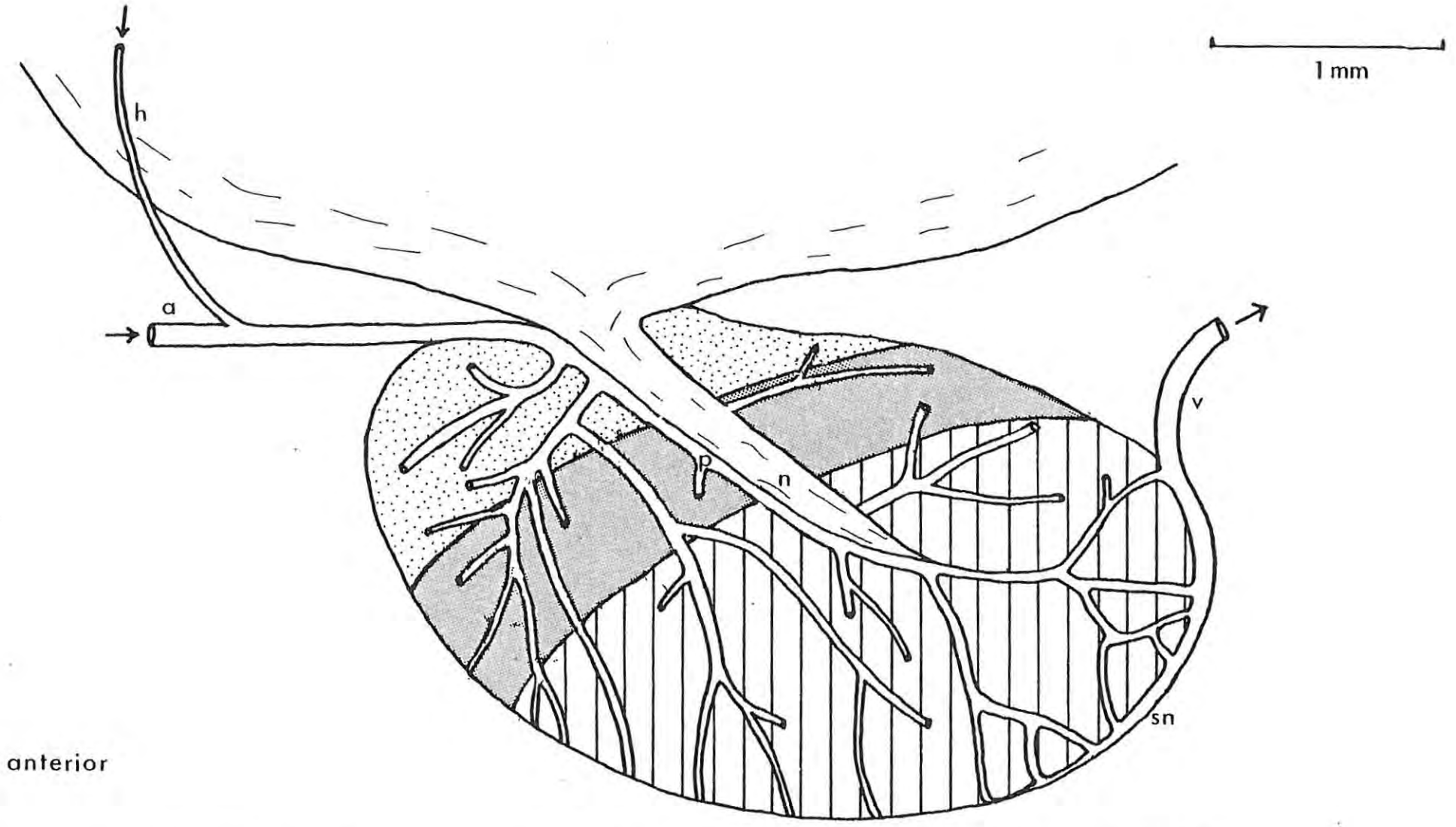

Figure 11 Diagrammatic representation of the blood supply to the pituitary of C. gariepinus.
a = hypophysial artery, h = contribution of blood supply from the hypothalamus,
p = primary longitudinal plexus, sn = superficial network of veins, v = hypophysial vein.

Blood drains from the pituitary of C. gariepinus through a series of small capillaries from the secondary centrifugal plexus, which pass out of the gland into the connective tissue capsule where a superficial venous network is formed. The blood supply to the pituitary of C. gariepinus is illustrated diagrammatically in Figure 11.

Although no saccus vasculosus is evident on macroscopic examination of the brain and pituitary regions, a well-developed vascularised region similar in structure to a saccus vasculosus is revealed in histological sections. This region is immediately posterior to the pituitary. A similar situation was described by Lehri (1966) in C. batrachus.

I. ROSTRAL PARS DISTALIS (RPD)

The rostral pars distalis occupies an antero-dorsal position in the pituitary of C. gariepinus. Three types of cells are distinguishable in the rostral pars distalis. The predominant cell type is a lightly staining, slightly acidophilic cell. Scattered singly or in small groups or clusters between the latter are cells of another type, which are very slightly basophilic, but do not stain strongly with any of the commonly used stains for basophils. These two cells are similar in shape and size. A third type of cell, less numerous and occurring singly, is found in the rostral pars distalis. These cells are identicial in size, shape and staining properties to one of the basophil types found in the proximal pars distalis.

An extensive blood supply in the form of numerous narrow capillaries ramifies through the rostral pars distalis. Neurohypophysial processes split off from the main neurohypophysial core are also found throughout the rostral pars distalis.

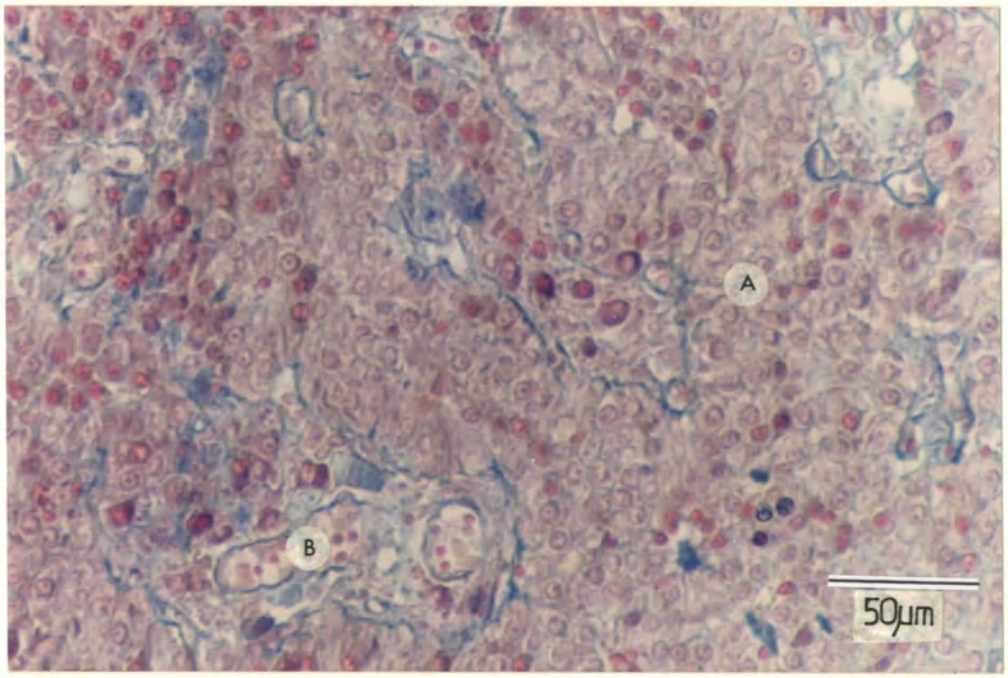

Figure 12 The rostral pars distalis of the C. gariepinus pituitary. Note the cord-like arrangement of the acidophil cells (A), and the large number of blood capillaries (B). Luxol fast blue-periodic acid-Schiff-orange G. 5 µm.

Cell types of the rostral pars distalis :-

(a) Type 1 (Acidophil)

The cytoplasm of the acidophil cells is granular, although the granules are very small. The nucleus is relatively large (average 4,8 µm, n = 40) and is usually rounded and central with a prominent nucleolus. The cells exhibit a wide range of shapes and sizes. Most are polyhedral, but some are elongate. These cells are positive to Orange G, negative to periodic acid-Schiff, and stain a pinkish red with Heidenhain's azan technique.

In the C. gariepinus pituitary a few acidophilic cells in the rostral pars distalis form a follicular-like arrangement around capillaries, but these follicles are not as pronounced as those described in some teleosts (e.g. the eel, Anguilla anguilla, Ball and Baker, 1969), and a cord-like arrangement of these cells was found to be predominant. Cords of cells are separated from each other by very thin connective tissue layers, by branches of the neurohypophysis and by blood capillaries (Figure 12).

These findings contrast markedly with both of those of Rizkalla (1963) on C. lazera and of Lehri (1966) on C. batrachus. According to Rizkalla the anterior glandular region 'appears to be formed of follicles', but his photographs do not indicate a rigid follicular arrangement like that found in the eel (see Ball and Baker, 1969). Lehri, on the other hand, states that the acidophil cells of the rostral pars distalis in C. batrachus 'do not exhibit any regular arrangement in the form of cords as described for other fishes'. The cord-like arrangement of the homologous cells in the C. gariepinus pituitary is indisputable. Most of the cords are two to three cells thick, but some consist only of a single row of cells. Cells lining the walls of the blood vessels are columnar in shape, while others are more polyhedral. Due to their location, cord-like arrangement and staining reaction, these cells are believed to be responsible for the secretion of prolactin in the C. gariepinus pituitary.

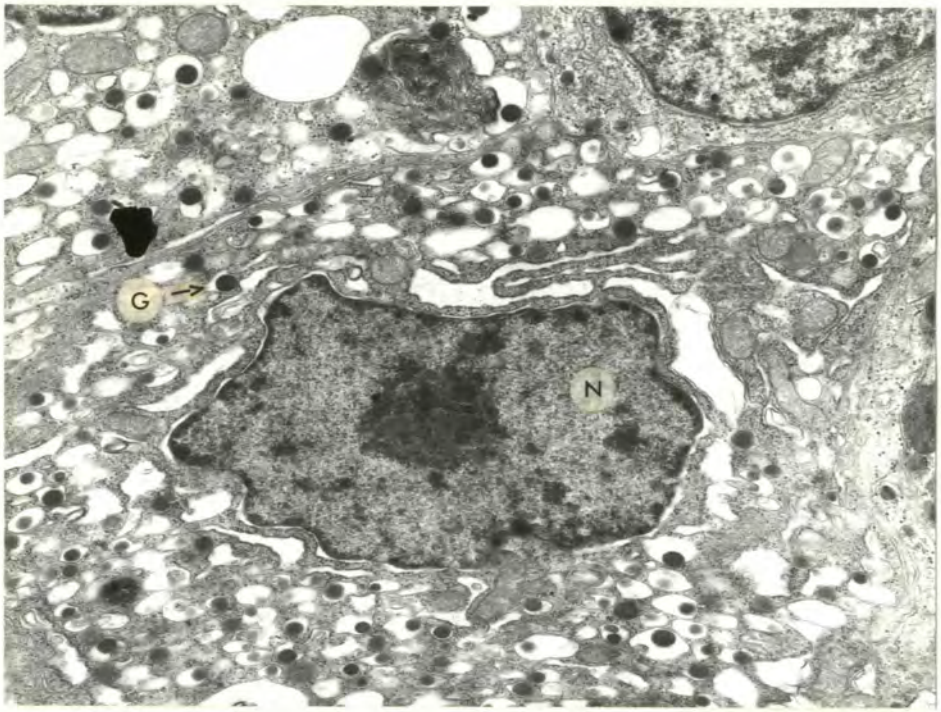

Figure 13 Electron micrograph of a prolactin cell of C. gariepinus. Note the small granules (G), irregular shaped nucleus (N), and vacuolated nature of the cytoplasm. 1200X.

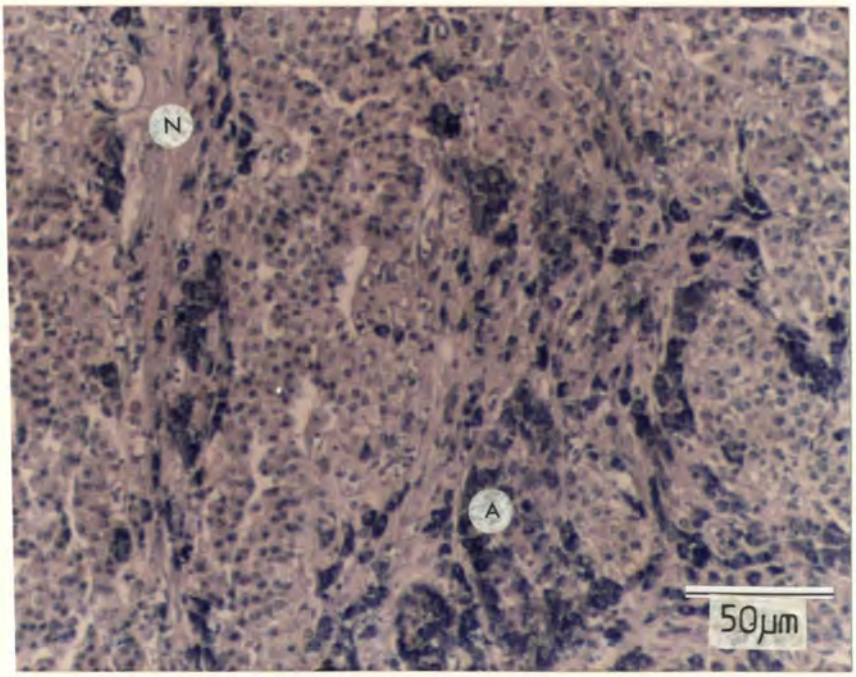

Figure 14 Lead haematoxylin positive cells (A) in the rostral pars distalis. Note the extensive neurohypophysial penetration (N). MacConnaill's lead haematoxylin.

The fine structure of the rostral pars distalis acidophils is unusual (Figure 13). The nucleus is irregular in outline, and the nucleolus is prominent and centrally placed. The cytoplasm has a highly vacuolated appearance, due to a network of membrane-bound structures. Granules are small (average 0,25 µm, n = 40) and are found within these membrane-bound structures. Large round mitochondria are common.

The vacuolated appearance of the cytoplasm may be due to artefacts caused by the preparation of the tissue, or may indicate extreme activity of the secretory cells at the time of death. As this phenomenon was only observed in one type of cell the latter explanation seems more likely.

(b) Type 2 (Slightly basophilic)

The second type of cell in the rostral pars distalis of the C. gariepinus pituitary has a more heavily granulated cytoplasm than that of the acidophils of this region. These type 2 cells stain a faint reddish-orange with the periodic acid-Schiff-orange G staining method, brownish-green with luxol fast blue-periodic acid-Schiff-orange G, and purplish with Heidenhain's azan. The reaction with the abovementioned stains is weak. These cells stain strongly, however with MacConnaill's lead haematoxylin (Figure 14).

Lead haematoxylin positive cells have nuclei of approximately the same size as those of the acidophils of the rostral pars distalis (average 4,7 µm, n = 40). Extensive chromatin material and a definite nucleolus can be distinguished in the nucleus. The cells, which are usually elongate in shape, are found in small groups scattered through the rostral pars distalis, particularly close to neurohypophysial fibres. On the basis of their position in the rostral pars distalis, and their intense affinity for MacConnaill's lead haematoxylin, it can be concluded that these cells are the adenocorticotropin-secreting cells.

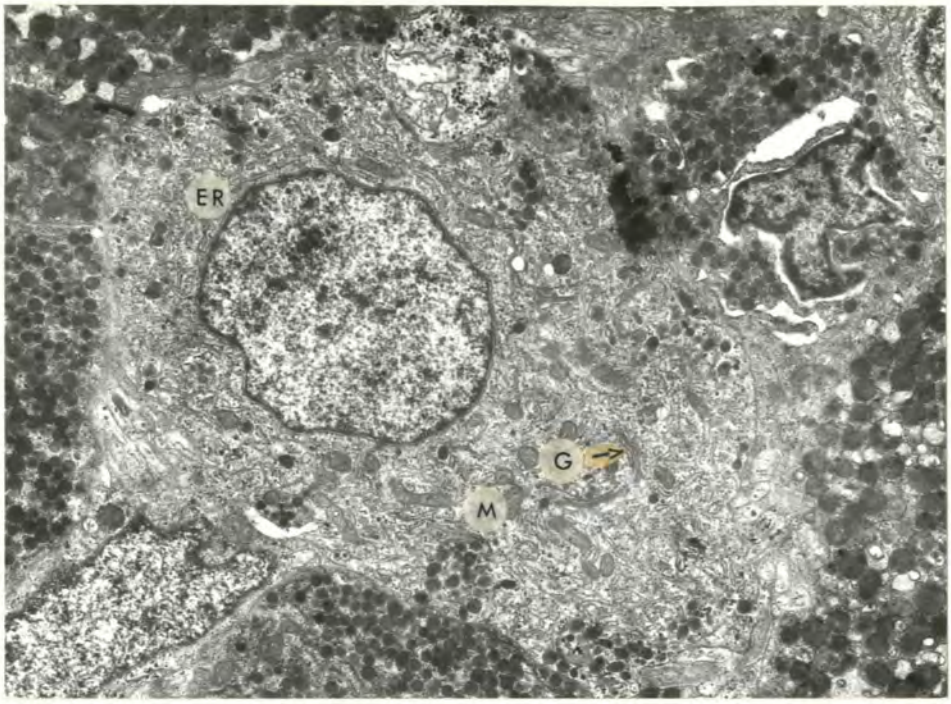

Figure 15 Electron micrograph of an adrenocorticotropic cell. Note the extensive endoplasmic reticulum (ER), Golgi apparatus (G), and mitochondria (M). 7000X.

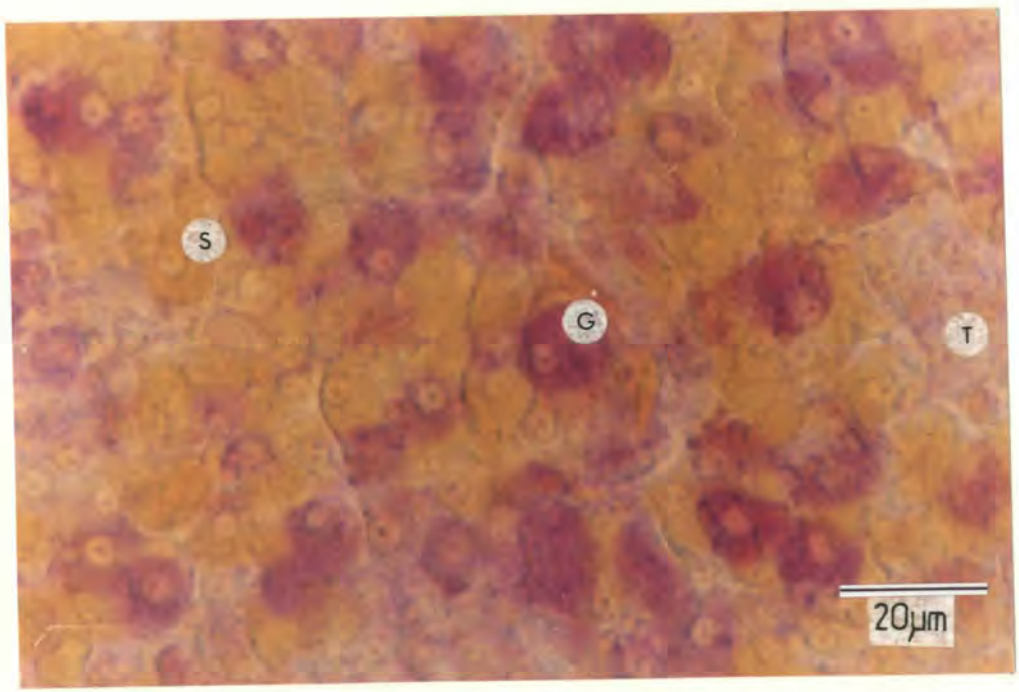

Figure 16 The proximal pars distalis of the C. gariepinus pituitary. Note the large, heavily granulated gonadotrops (G), smaller, orange stained somatotrops (S), and small lightly stained putative thyrotrops (T). Periodic acid-Schiff-orange G. 5 µm.

Electron micrographs show that these cells, which are usually elongate with an oval nucleus, are only sparsely granulated, and the granules are small (average 0,2 µm, n = 40). Rough endoplasmic reticulum and oval mitochondria predominate in these cells, and this fact together with the low percentage of granules indicates that the cells were probably active at the time of death (Figure 15).

(c) Type 3 (Basophil)

The third type of cell occasionally found in the rostral pars distalis of the pituitary of C. gariepinus will be described in the section on the proximal pars distalis, where the majority of cells of this type are found.

II. PROXIMAL PARS DISTALIS (PPD)

The proximal pars distalis lies intermediate between the rostral pars distalis and the pars intermedia. There are three dominant cell types in this division of the pituitary of C. gariepinus, two basophil types and one acidophil.

(a) Type 1 (Basophil)

The largest cell types of the proximal pars distalis are a basophil type, which are also the largest cells of the entire pituitary. These cells attain a diameter of up to 16,8 µm, and are round to polyhedral in outline. The nucleus is large and round with a large, usually eccentric, nucleolus. These cells are heavily granulated, and the granules are the largest found in any cell type of the pituitary of C. gariepinus. The granules fuse to form large secretory globules which are often prominent in the cytoplasm of cells. Both granules and globules stain intensely red with periodic acid-Schiff, dark blue with Heidenhain's azan, and violet with aldehyde fuchsin. The large basophil cells are scattered randomly throughout the proximal pars distalis, and are not found in large groups of one cell type (Figure 16).

The number of basophilic cells vary considerably throughout the year, particularly in male fish. Very few are visible in the pituitary of

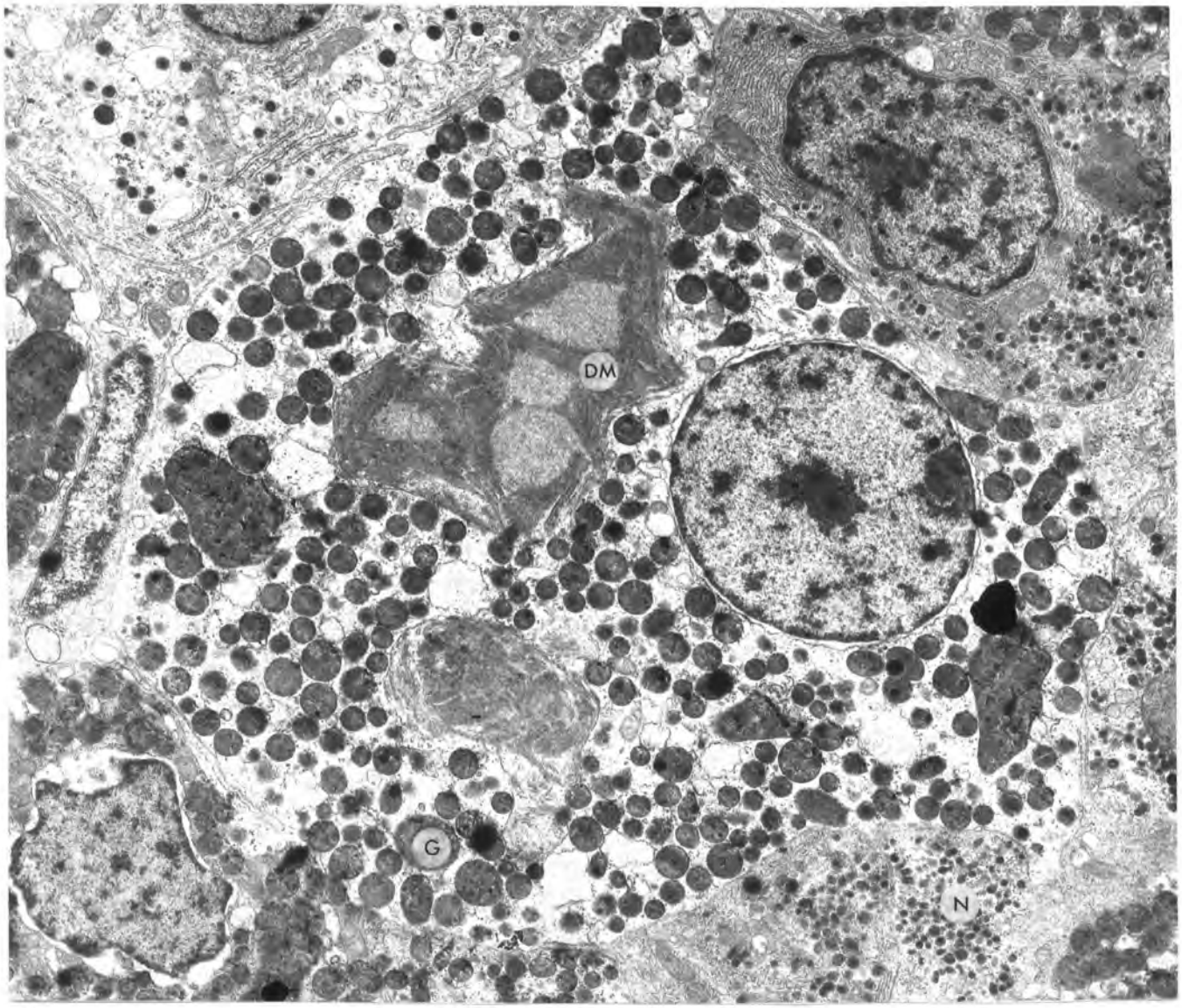

Figure 17 Electron micrograph of a gonadotropic cell. Note the large, irregular granules (G), and electron dense masses (DM). Nerve endings (N) can be seen in the top left-hand corner of the cell. 10 000X.

a newly-spawned male, while in a male whose testes are full of spermatids and ripe sperm, the pituitary is packed with heavily-granulated, type 1 basophil cells. In immature fish large basophilic cells are absent or very sparse.

Due to their staining reactions, heavy granulation, correlation of activity with the reproductive cycle and absence in immature fish, it is concluded that the large, intensely staining basophils of the proximal pars distalis are the gonadotrops of the C. gariepinus pituitary. The arrangement of these cells is not a usual one as in most teleost pituitaries gonadotrops are found in large groups (Ball and Baker, 1969; Sage and Bern, 1971).

The gonadotropic cells correspond closely to the basophils described by Rizkalla (1963) in the proximal pars distalis of the C. lazera pituitary although he describes the globules (large, fused granules) in this species as exhibiting an acidophilic reaction in various intensities. Globules of C. gariepinus gonadotrops were strongly basophilic at all times.

The electron microscope revealed only one type of gonadotrop. These cells were easily discernible by the large size and irregular shape of the granules, and the presence of densely staining masses with a characteristic fine structure (Figure 17). The granules of these cells are round to oval in section, and variable in size (average 0,54 µm, n = 40).

The dark-stained masses are very irregular in shape, and are less electron dense than the granules. The perimeters of these large inclusions have a striated appearance, and the centre may be amorphous or may be similarly striated (Figure 17). Similar inclusions have not been described in the pituitaries of many teleosts but gonadotropic cells of most teleosts have large droplets or globules in the cytoplasm as well as granules (Schreibman *et al.*, 1973), and Kaul and Vollrath (1974) discuss the fusion of these globules in the goldfish (Carassius auratus) pituitary where they form darkly staining masses very similar to those found in the C. gariepinus pituitary. This fusion of globules occurred in the goldfish only after

administration of oestradiol, while the dark stained masses were evident in pituitaries of untreated C. gariepinus. High oestrogen levels are believed to inhibit secretion of gonadotropins (see page 3). The C. gariepinus specimens whose pituitary fine structure was examined in this study had been held in captivity for some time, which inhibits normal reproductive development, possibly causing a build-up of gonadotropins. This could account for the formation of these masses, which are suggested to be storage vessels for secretory products.

The nucleus of the gonadotropic cells is smooth in outline, and round to oval in shape in electron micrographs, with a distinct nucleolus. A large number of membrane bound vesicles are present in the cytoplasm, interspersed with the secretory granules. Free ribosomes are common and elongated oval mitochondria are sometimes present in these cells.

Direct contact between gonadotropic cells and nerve endings was often observed (Figure 17), indicating the neurohypophysial control over gonadotropin release.

(b) Type 2 (Acidophil)

The acidophils of the PPD are also intensely staining cells, and are usually approximately half the size of the type 1 basophils. The outline of the acidophil cells is irregular and angular. The nucleus is relatively large and is comparable in size to that of the basophils of the PPD (average 4 µm diameter, n = 40). The nucleus is generally round to oval in shape, but irregular-shaped nuclei are also sometimes observed. The nucleus may be either centrally or eccentrically placed. Clumps of chromatin are prominent in the nucleus. A distinct nucleolus is occasionally discernible (Figure 16).

Acidophil cells stain intensely with Orange G and assume a greenish hue when the Luxol fast blue-periodic acid-Schiff-Orange G technique is used. They stain deep red with erythrosin when Heidenhain's azan technique is applied. Acidophilic cells are found throughout the proximal pars distalis interspersed with gonadotropic cells and

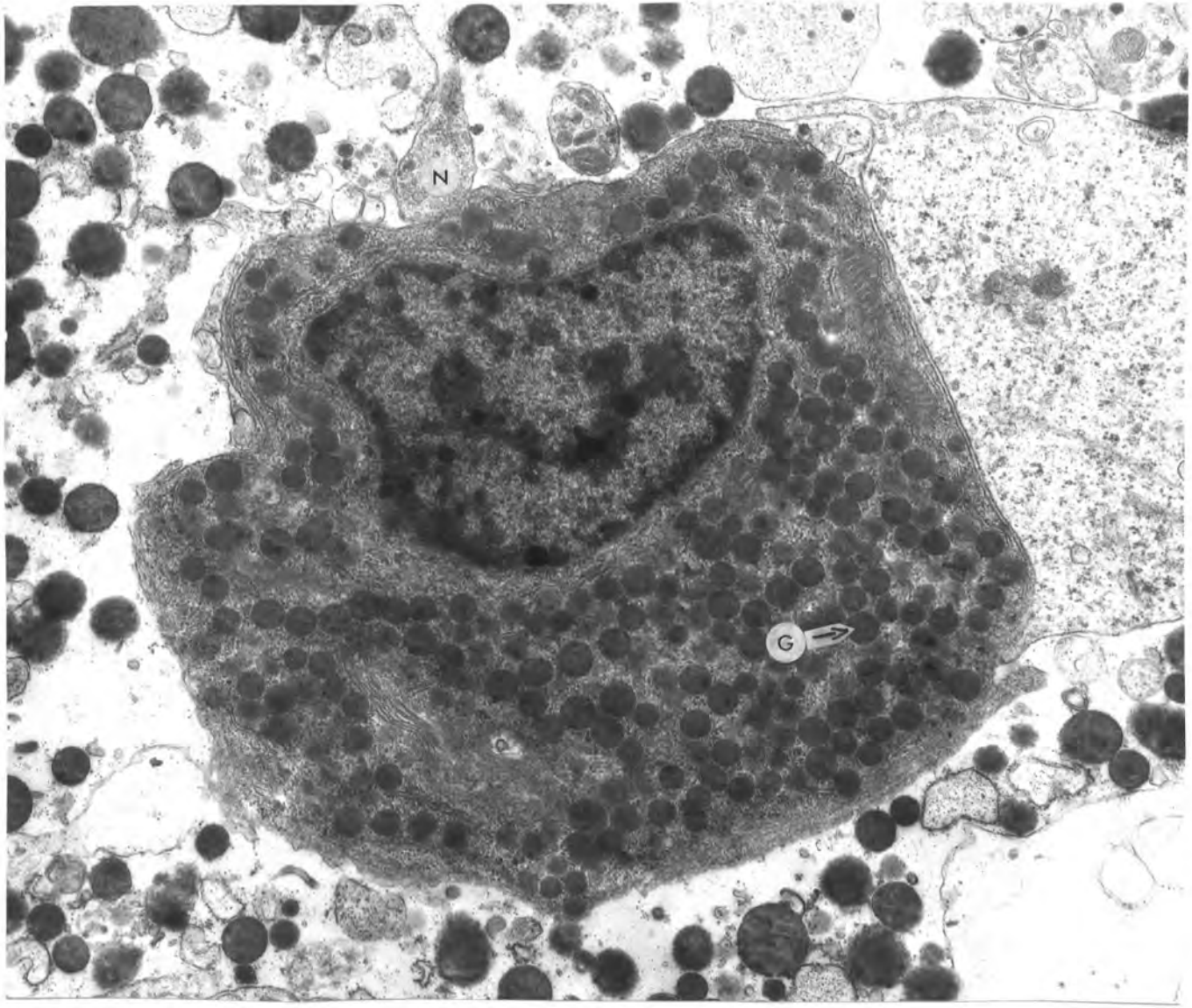

Figure 18 Electron micrograph of somatotropic cell. Note closely packed, regular shaped granules (G), and synapse with nerve ending (N). 22 000X.

are usually the most abundant cell types of the region.

Acidophil cells are assumed to be the somatotrops on the basis of their typical somatotropic staining reactions, their localisation in the proximal pars distalis and their abundance in immature fish.

Examination of the fine structure of these cells shows them to be tightly packed with granules which are considerably smaller (up to 0,20 µm in diameter) than those of the gonadotropic cells. The granules are also more regular in shape and size. Ribosomes and rough endoplasmic reticulum are abundant in the cytoplasm, and elongate mitochondria are found, particularly near the periphery. Contact between nerve endings and somatotropic cells is commonly observed (Figure 18).

(c) Type 3 (Basophil)

The third cell type of the PPD of C. gariepinus is another basophil. Unlike the other proximal pars distalis cells these basophils are found in small clusters of the same cell type and are smaller and less heavily granulated than the other two proximal pars distalis cell types. Type 3 cells also stain less intensely than the other basophils of this area, the gonadotrops (Figure 16).

The shape of the second type of basophil cell shows considerable variation in the pituitaries from different specimens of C. gariepinus. In the majority these cells are small and roughly oval, but in others they are markedly elongate. The nucleus is round and shows a distinct nucleolus.

Type 3 basophil cells stain very slightly purplish-pink with periodic acid-Schiff-Orange G staining method, and a clear light blue with Heidenhain's azan. The luxol fast blue-periodic acid-Schiff-orange G technique distinguishes two different coloured cell types in this area, both very similar in every other way, and indistinguishable with the other staining methods which were applied. With luxol fast-blue periodic acid-Schiff-orange G both types of cell take the stain only lightly, but some appear purplish, and some yellow. One

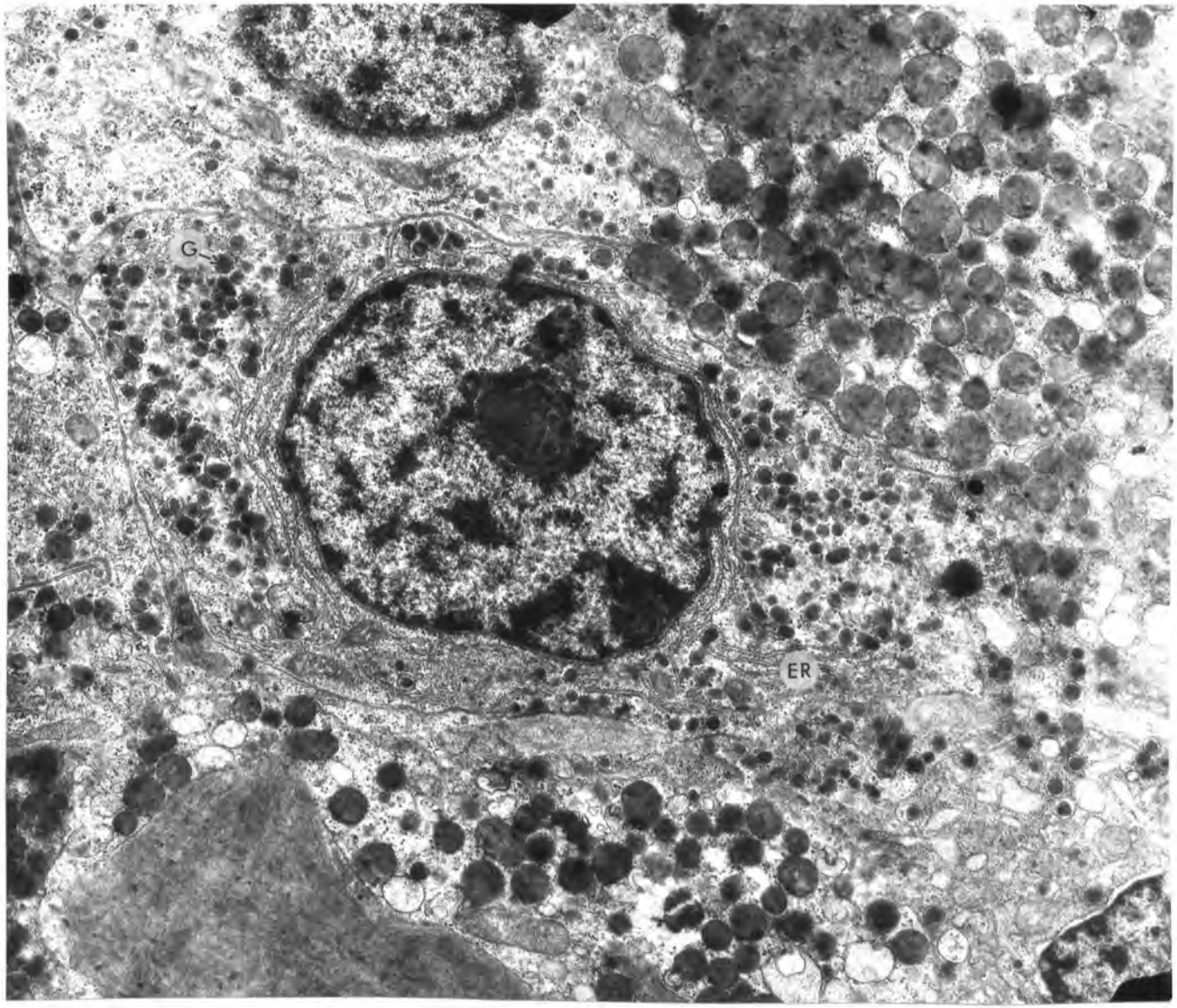

Figure 19 Electron micrograph of putative thyrotrop. Note small granules (G) surrounded by a membranous 'halo', and large amount of rough endoplasmic reticulum. 14 300X.

of these cell types is suggested as the source of thyrotropin, but the function of the other is unknown. There is a possibility that there may be two types of gonadotropic cells in the pituitary of C. gariepinus as is the case in some teleosts (e.g. Salmo salar, Olivereau, 1975), in which case one of these lightly staining basophils may be gonadotropic in function. This is unlikely as only one gonadotropic cell type can be distinguished in electron micrographs.

Electron micrographs of the putative thyrotrops show them to be relatively sparsely granulated cells. The granules are small (0,18 - 0,24 µm, n = 40) and round to oval in section. A distinct membranous 'halo' is apparent around each granule. Rough endoplasmic reticulum is abundant in these cells, particularly around the nucleus. Mitochondria which are round to oval in section are also present (Figure 19).

The small basophils were not described by Rizkalla (1963) for C. lazera or by Lehri (1966) for C. batrachus. Both authors report the presence of chromophobic cells in the proximal pars distalis which were not found in C. gariepinus. The lack of intensity with which these cells stain indicates that the cells described by Lehri and by Rizkalla as chromophobes are homologous with those described by the present author as lightly staining basophils.

III. PARS INTERMEDIA

The pars intermedia is very richly supplied with blood capillaries and nervous material.

Two adenohypophysial cell types are present in the pars intermedia of C. gariepinus, one which is lightly acidophilic, and one very slightly basophilic. These cell types were not studied in detail.

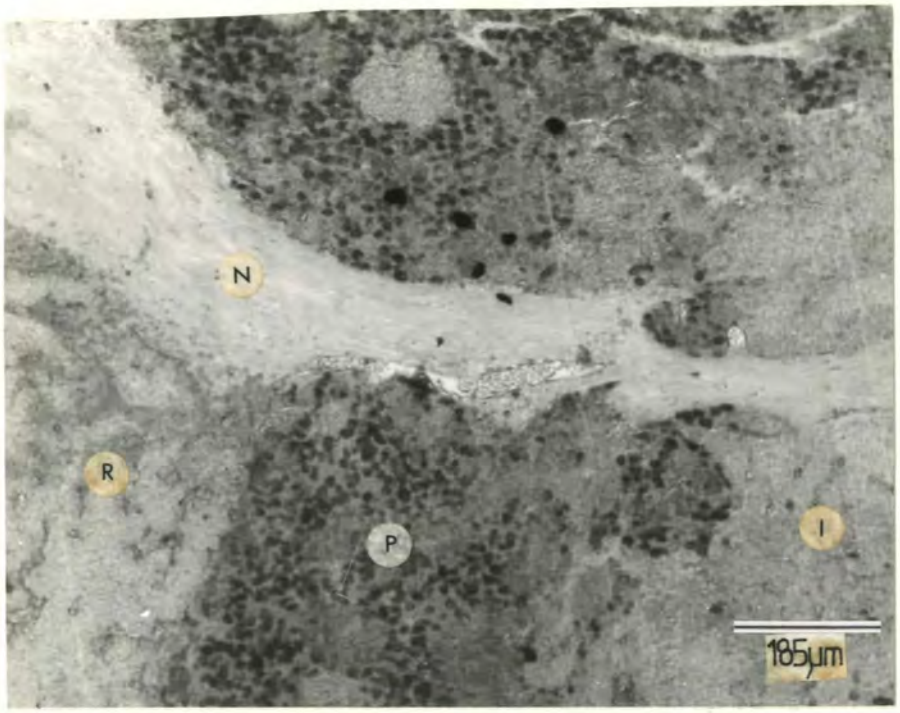

Figure 21 Penetration of the neurohypophysial core (N) through all three regions of the adenohypophysis. R = rostral pars distalis, P = proximal pars distalis, I = pars intermedia. Periodic acid-Schiff-orange G. 7 µm.

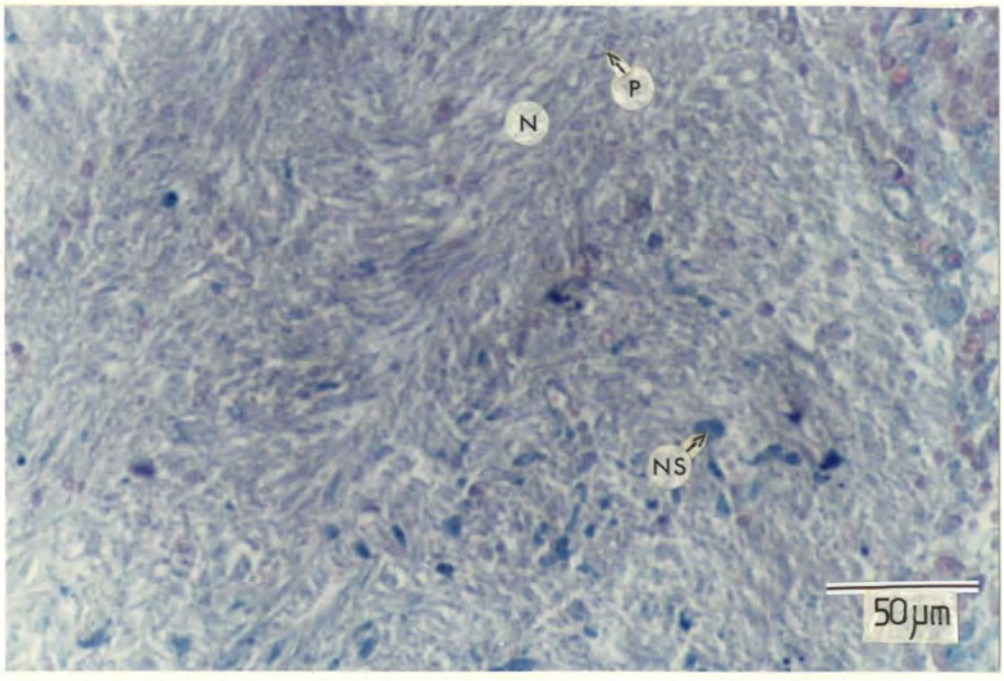

Figure 20 Neurohypophysis of C. gariepinus showing the interlacing nervous fibres (N), pituicytes (P) and neurosecretory material (NS). Aldehyde fuchsin. 7 µm.

IV. NEUROHYPOPHYSIS

The neurohypophysis of all teleosts is made up of two zones - the neurohypophysial or pituitary stalk and the neurohypophysial core. The nervous tissue of both these regions is derived from the third ventricle of the brain and remains attached to the brain throughout life, this providing an essential and direct connection between the nervous and endocrine systems (Liley, 1969).

The pituitary stalk of C. gariepinus is attached to the anterior region of the pituitary and is therefore classified as cranio-leptobasic (Bretschneider and de Wit, 1947). The stalk is made up of a large number of interlacing nervous fibres, connective tissue, and a few neuroglial cells or pituicytes (Figure 20). The neurohypophysial core is similar in composition but is in addition supplied with blood capillaries containing large numbers of red blood corpuscles. The cell bodies from which the nervous fibres of this region arise are located in the hypothalamus. The neurohypophysial core of this species runs from the stalk through to the pars intermedia in an almost solid mass (Figure 21). Branches from this mass penetrate the two regions of the pars distalis, often branching again several times in the process. In the pars intermedia the whole neurohypophysis breaks up into a complex network of small fingerlike processes which ramify throughout this region. The pars intermedia is therefore the region of the adenohypophysis with the greatest neurohypophysial penetration. This is in accordance with the arrangement described in most teleosts (Perks, 1969), including C. batrachus (Lehri, 1966).

Nuclei of pituicytes are visible throughout the neurohypophysis of C. gariepinus. These nuclei assume a variety of different shapes, but are usually roundish, with a pronounced nucleolus. Elongate, oval nuclei are also present, but these are thought to belong to connective tissue cells.

Neurosecretory colloidal material is present in the form of small droplets, and also as larger aggregations known as Herring bodies. The latter stain brightly with acidophilic dyes (Figure 20). The aggregations are usually located in the central part of the nervous tract, and are found

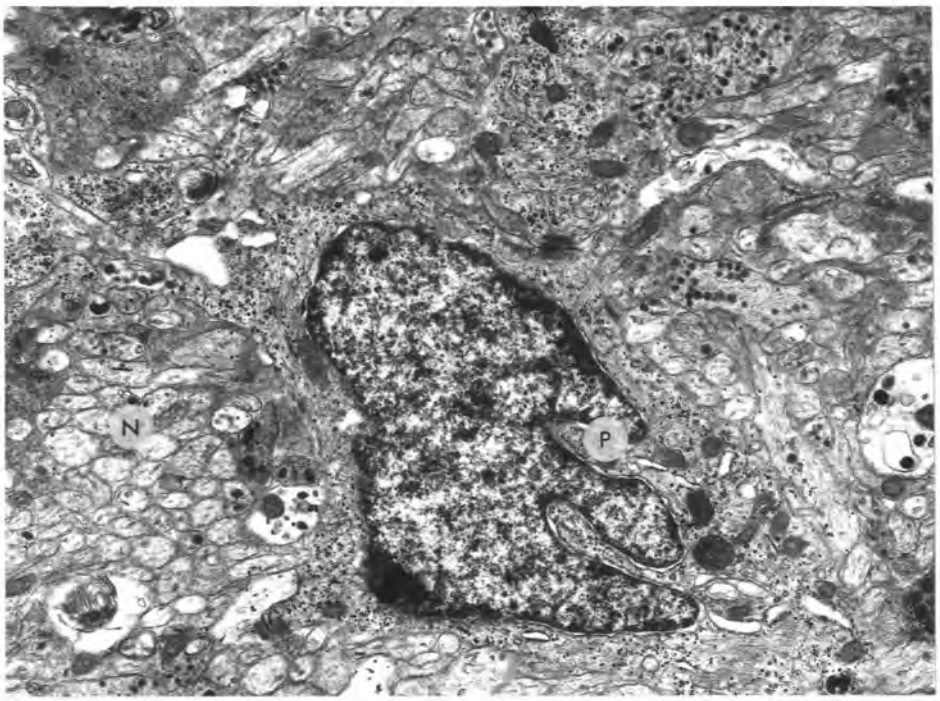

Figure 22 Pituicyte (P) in the neurohypophysial core (N). 10 000X.

at all levels of the neurohypophysial core, with the densest aggregations near the regions of contact between the neurohypophysis and the pars intermedia. Rizkalla (1963) and Lehri (1966) report similar distributions of neurosecretory material in C. lazera and C. batrachus respectively.

Acidophil cells with coarse cytoplasmic granules (described in the neurohypophysis of C. lazera by Rizkalla, 1963) were not seen in C. gariepinus.

In the electron microscope three different neurosecretory fibre types, corresponding to types A_1, A_2 and B of Knowles and Vollrath (1966a and b) can be distinguished on the basis of the size of their secretory granules. Nerve fibres run lengthwise through the neurohypophysial core and then split up into smaller groups of fibres, which ramify between the secretory cells of the adenohypophysis. Endings of these nerve fibres are commonly found adjacent to the cell membranes of the secretory cells (see Figures 17 and 18). Nuclei of pituicytes are occasionally visible in the neurohypophysial core (Figure 22).

ENDOCRINE TISSUES OF TELEOST TESTES

The testes of vertebrates are primarily responsible for the production of male gametes, but this is not their only function. They are also the site of synthesis of the steroid hormones known as androgens.

In most vertebrates the steroidogenic tissue of the testis is in the form of easily recognisable interstitial cells, located between the seminiferous tubules. Recent developments in histological and biochemical techniques have established that the interstitial cells (also known as Leydig cells), are the main site of steroid synthesis in the testes of laboratory mammals (Christensen, 1965). Studies on the ultrastructure of these tissues have reinforced this evidence by showing that the interstitial cells have a fine structure typical of steroid secretory tissue with a well developed smooth endoplasmic reticulum (Christensen, 1965). Bentley (1976) states that a second type of cell, known as Sertoli cells, which are associated with the basement membrane of the seminiferous tubules are also endocrine in function, being responsible for the elaboration of sex hormones 'which are probably involved in the growth and maturation of sperm'. This is contradictory to the opinions of earlier authors who

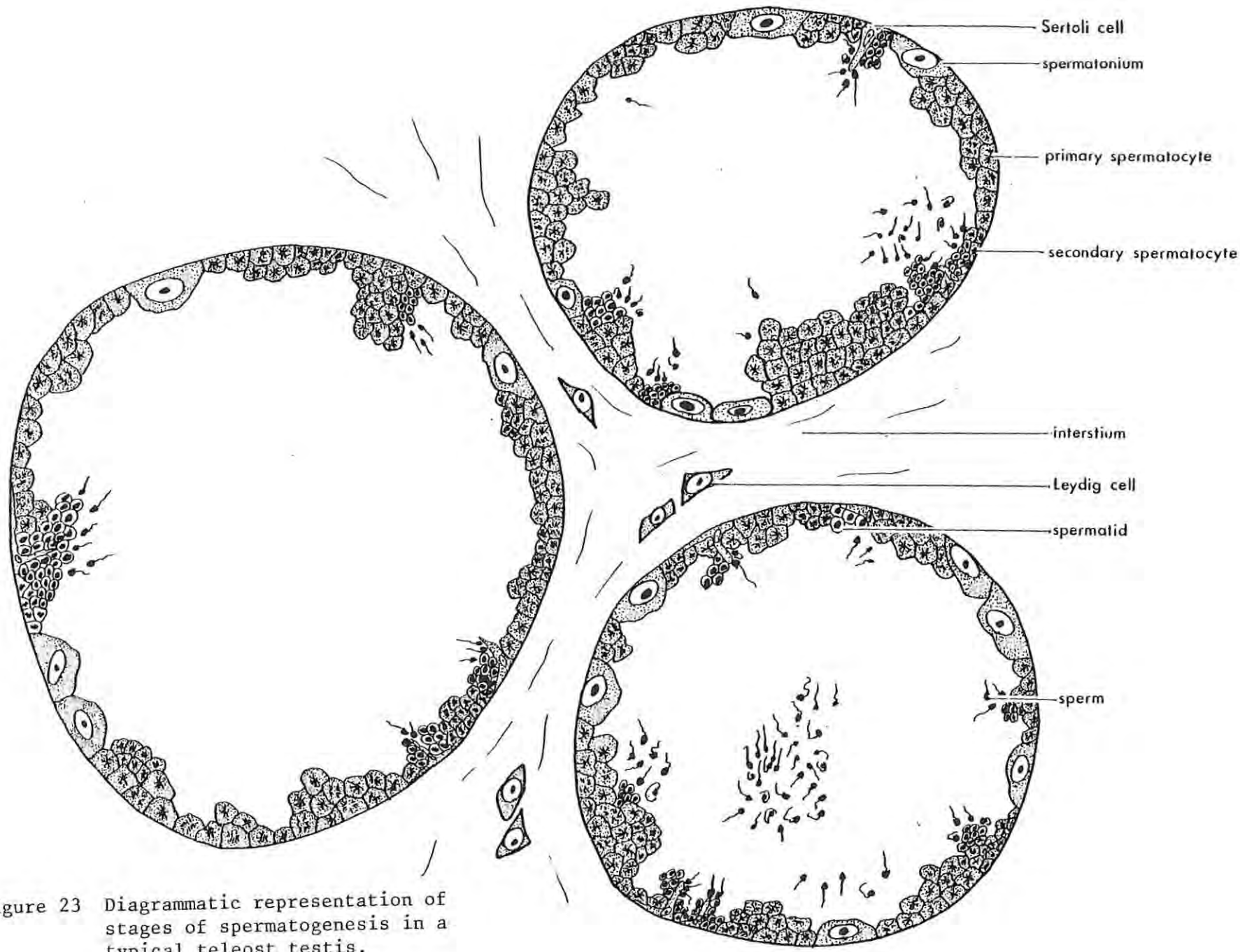

Figure 23 Diagrammatic representation of stages of spermatogenesis in a typical teleost testis.

generally believed that the Sertoli cells were responsible for the nourishment of sperm (and therefore sometimes called sustentacular cells, Lofts and Bern, 1972). Other roles, e.g. contractile, supportive and phagocytic have also been suggested, and although increasing evidence seems to suport the theory of an endocrine function (Hoar, 1969; Lofts, 1972; Lofts and Bern, 1972), in the light of other literature on the subject Bentley's definite statement that these cells are endocrine seems rather premature. He quotes no references or results of his own to justify this statement, and perhaps a safer conclusion would be that the possibility of Sertoli cells having an endocrine function should not be overlooked.

In most teleosts the testes are paired structures lying against the dorsal body wall. Spermatogenesis occurs within testicular units, which may take the form of small sacs, ampullae, lobules or tubules. These lobules, tubules, etc. are separated from one another by a connective tissue layer of varying thickness, and open into the sperm duct, which leads into the urogenital papilla. Teleost testes generally have no permanent germinal epithelium as is found in higher vertebrates. Germ cells proliferate by mitotic division to form nests of spermatogonia or sperm mother cells which migrate from the margin of the testes to lie singly or in small groups scattered along the tubule or lobule walls (Hoar, 1969).

Within the lobules of teleost testes spermatozoa are formed from spermatogonia through a series of cytological changes collectively known as spermatogenesis. Spermatogonia proliferate by mitotic divisions giving rise to primary spermatocytes, which then undergo reduction division to form secondary spermatocytes. The second meiotic division gives rise to spermatids. The spermatids do not divide but undergo metamorphosis to form sperm (also known as spermia or spermatozoa) (Hoar, 1969). The sperm have a distinct head and tail and are the motile functional male gametes. Stages of spermatogenesis in a typical teleost are illustrated in Figure 23.

Early studies on numerous species of teleosts revealed definite interstitial tissue which was well developed when secondary sexual characters were well developed (Courrier, 1921; Kolmer and Scheminsky, 1922). Marshall and Lofts (1956), working on the pike, Esox lucius, and the char, Salvelinus willoughbii, showed that in these fish the usual interstitial or Leydig type of cell is absent but cells found in the walls of the basement

membrane of the seminiferous tubules, which they named lobule boundary cells, appear to perform the same function. Hoar (1969) states that the difference between lobule boundary cells and interstitial cells is one of distribution only as both tissues arise from the same embryological source and are similar histochemically. Hoar and Nagahama (1978) examined the ultrastructure of lobule boundary cells and found them to be homologous with Sertoli cells, rather than Leydig cells. They do not explain which cells are responsible for androgen secretion in fish such as the pike (Esox lucius) which have been reported to have no interstitial Leydig cells (Marshall and Lofts, 1956).

RESULTS ON TESTIS OF C. GARIEPINUS AND COMPARISON WITH OTHER SILUROIDS

The testes of C. gariepinus are elongate, paired, slightly flattened structures. They lie on either side in the posterior region of the coelomic cavity, ventral to the kidneys and attached to the dorsal body wall by mesenteries. Posteriorly the two testes are united to form a common duct which opens into the urogenital sinus. The urogenital sinus is situated within a urogenital papilla which in the male fish is pointed at its free end.

The testes show variations in size, shape and colour at different times of the year. During the late autumn and winter they are thin, elongate and almost translucent. In the spring, when the fish are beginning to mature the testes gradually become thicker and wider with distinct indentations in their distal margins, and lose their translucency. When the gonads are ripe (in the early summer), the testes are relatively wide and the indentations in their distal margins are pronounced. After spawning the testes assume the appearance of deflated sacs. The colour of the testes of C. gariepinus ranges from transparent (immature fish and all fish in non-breeding condition) through pale rose (developing), white and opaque (maturing to mature) to grey-white (spent) (Bruton, 1979a and pers. obs.).

The posterior ends of the testes are modified to form seminal vesicles. These are visible when the testis is mature as turgid fingerlike outgrowths. In the non-breeding season these fingerlike extensions are very much reduced and the seminal vesicles are inconspicuous. The function of the seminal vesicles is not clear. They have been described by Nair (1965) and Lehri (1976) in

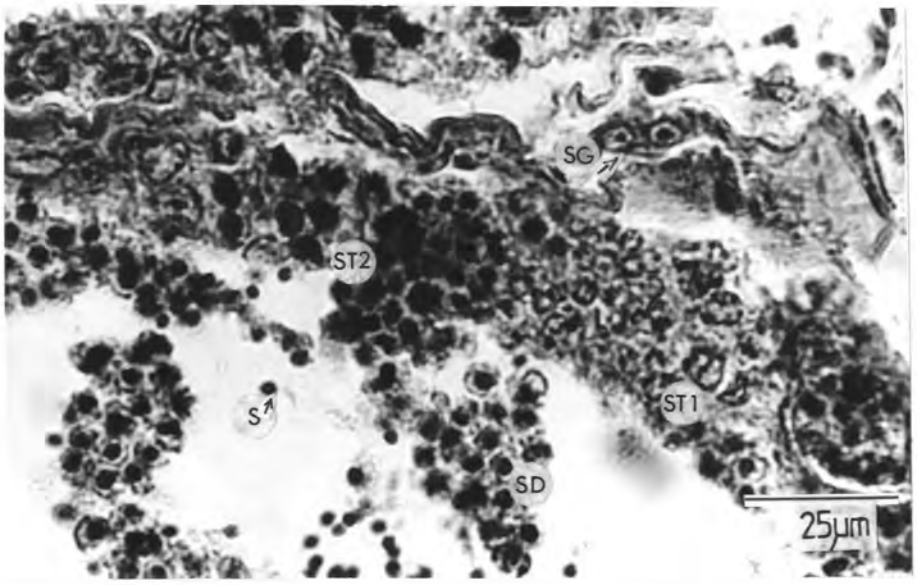

Figure 24 Histological structure of a maturing testis of C. gariepinus. SG = spermatogonium, ST 1 = primary spermatocyte, ST 2 = secondary spermatocyte, SD = spermatid, S = sperm. 5 µm.

C. batrachus and by Nawar (1959) in C. lazera. Their structure is glandular and suggests a secretory function (Nair, 1965; Lehri, 1967). It was thought for some time that these vesicles might be used for the storage of sperm (Nair, 1965; Nawar, 1969) but Lehri (1967) examined sections of these vesicles throughout the year and found no evidence of sperm within them. Other functions which have been suggested are the secretion of a fluid in which sperm are released (Sundararaj, 1958, for Heteropneustes fossilis) or the secretion of an adhesive substance found on Clarias eggs (Nair, 1965, for C. batrachus). Sundararaj also postulated that the secretion may play a part in prolonging the viability of sperm or aiding in fertilisation (See Bruton, 1979a, for review).

Although rather beyond the scope of this project a few sections of seminal vesicles were made and examined under the light microscope. The vesicles were found to be similar in structure to those described by Lehri for C. batrachus and are made up of anastomosing lobules similar to the seminiferous tubules in gross structure. The walls of each lobule consist of simple epithelium. The lumen of each tubule is full of an unidentified fluid substance. No sperm were seen inside the seminal vesicles although sections were made of vesicles from a fish whose testes were found to contain ripe sperm.

Histological structure of the testis

The testis is composed of an intricate structure of numerous convoluted and anastomosing seminiferous lobules. These lobules are separated by stroma tissue made up of loose connective tissue, blood capillaries and interstitial cells. Lobules show a variety of shapes and sizes. The lobules communicate with the lumen of the vas deferens. Each lobule contains several cysts of germ cells which may be in various stages of development. The whole structure is bounded by a covering of connective tissue.

Spermatogonia can easily be recognised in the testis of C. gariepinus. They are large cells (approximately 12 µm, n = 40) with lightly staining cytoplasm and nucleus and a relatively heavily stained eccentric nucleolus. The nucleus occupies a large part of the cell (Figure 24). Primary spermatocytes are smaller than spermatogonia although their size shows considerable variation (6 µm - 7 µm, n = 40). They can be seen in different

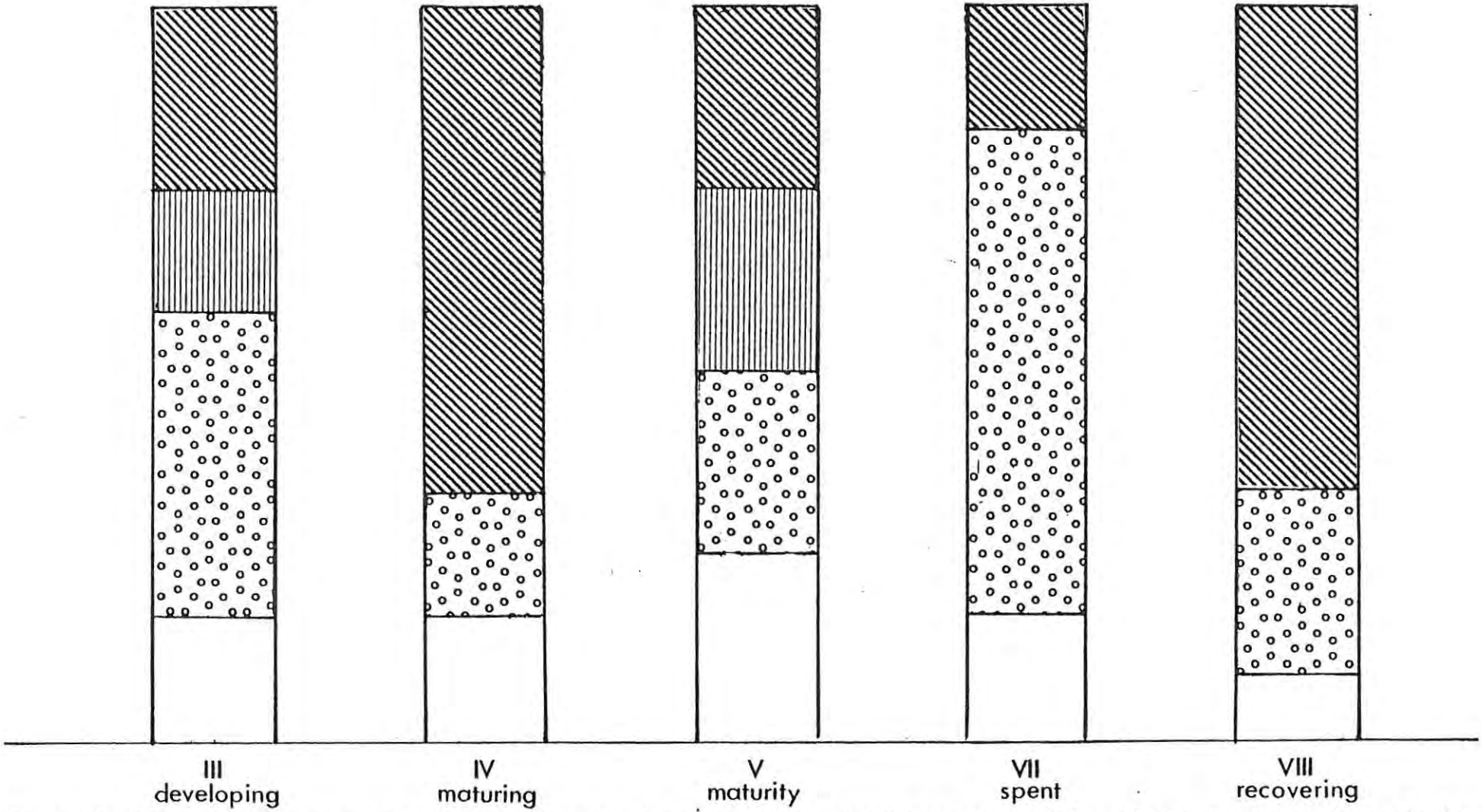

Figure 25 Diagrammatic representation to indicate the approximate percentage composition of the testes of C. gariepinus at different stages of maturity. Numbers 111 to V111 represent maturity states as assessed macroscopically, by classification described by Bruton (1979a). Key to shadings: Blank = spermatogonia, spots = spermatocytes, vertical lines = spermatids, diagonal stripes = sperm.

stages of division. The nuclear membrane is usually indistinct and chromosomal structure is variable depending on the stage of meiotic division (Figure 24). The secondary spermatocytes are of short duration and therefore are rarely seen. They are smaller than primary spermatocytes (5,5 µm, n = 40) and their nucleus gives the impression of containing a thick clump of chromatin (Figure 24). Spermatids are characterised by an even smaller size (4,5 µm, n = 40) and a very deeply staining clumped chromatin mass. Spermatids have a definite spherical shape (Figure 24). The sperm are easily identified by the presence of a distinct head and tail region, and an even smaller size than that of the spermatids. The head is oval shaped with an indentation at the spot where the tail arises (Figure 24).

The testes of C. gariepinus, appear on macroscopical examination to show an annual cycle of gonadal maturation, spawning, and gonad regression (Bruton, 1979a). Microscopic examination revealed some unexpected results. It was not part of this study to assess statistically the stages of gonadal maturation and correlate them with environmental changes, but some qualitative observations were made. The testes of the fish examined indicated that spermatogenesis begins very shortly after the last spawning has occurred and almost ripe sperm are stored within the lobules over the winter months. The lobules of fish caught in the winter have a very narrow diameter and the overall size of the testes is small. As summer approaches the lobules expand in diameter, and ripe sperm are found in the central lumina of the lobules. Small cysts of spermatogonia are also visible at this stage. Spawning results in an almost complete discharge of sperm from the lobules, although a few residual sperm may be left. The sperm mother cells then multiply profusely and the testis which has a shrunken appearance macroscopically is seen in histological sections to be made up of many narrow lobules which are composed almost entirely of spermatogonia. These results suggest that the method of classifying gonad maturity types into type I - VIII used extensively in field studies may not be as accurate a reflection of gonad developmental state as it has been thought to be. Figure 25 shows the comparison between the maturity state that was assigned to the gonads after macroscopic examination and the true state of maturity as elucidated by microscopical examination of sections.

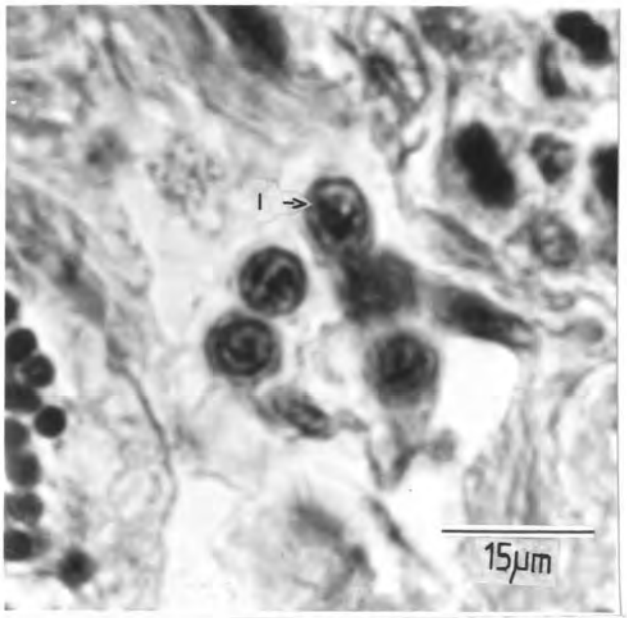

Figure 27 Interstitial cells (I) in an inactive testis. Cytoplasm is much reduced. Masson's trichrome. 7 µm.

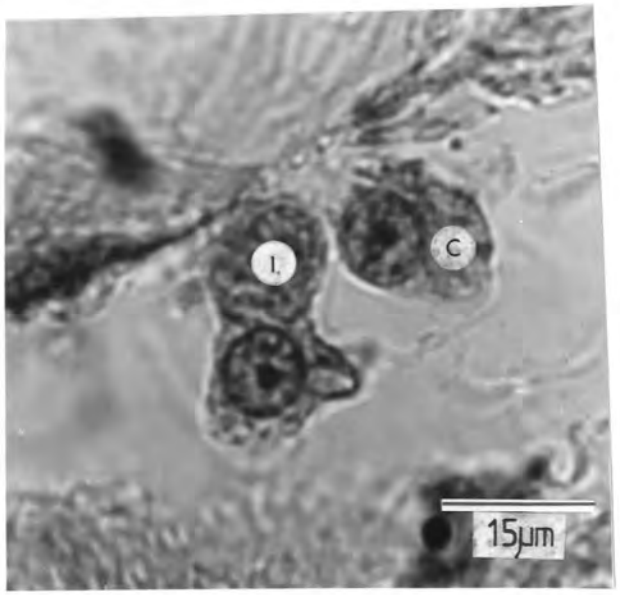

Figure 26 Interstitial cells (I) in an actively maturing testis. Note extensive cytoplasm (C). Masson's trichrome. 5 µm.

Interstitial cells are found in the stroma between the seminiferous lobules. These cells have large nuclei with prominent nucleoli and a varying amount of granulated cytoplasm. They are found singly or in small groups. The stroma consists largely of fibrous connective tissue which Masson's trichrome stain shows to be collagenic. The interstitial cells are closely associated with this connective tissue and it is sometimes difficult to determine whether nuclei belong to connective or interstitial tissue.

Cells corresponding to the lobule boundary cells described in the testis of the pike, Esox lucius, by Marshall and Lofts (1956) and in the rainbow trout, Salmo gairdnerii by Robertson (1958) and subsequently in various other species, were absent in C. gariepinus and it would appear that the role of androgen secretion is fulfilled solely by the Leydig-like interstitial cells.

Interstitial cells show a marked variation in abundance, size, and degree of granulation of the cytoplasm. These variations appear to be closely linked to the stages of spermatogenesis, as would be expected from androgen secretory cells. C. gariepinus shows no marked secondary sexual characters with which the apparent secretory activity of the interstitial cells can be compared as was done by early authors trying to establish an endocrine function for these cells (e.g. Craig-Bennett, 1931, on the stickleback, Gasterosteus aculeatus). Examination of histological preparations reveal that these interstitial cells are most abundant when active spermatogenesis is occurring in the later stages, with spermatids and sperm predominating, and at these times the cytoplasm of the interstitial cells is very much more extensive than at the other stages, and is heavily granulated (Figure 26). It is interesting to note that in the winter months when the testes were full of sperm (although the lobules were narrow, and the sperm obviously not ready to be discharged as milt) the interstitial cells, although abundant, did not appear to be active. The cytoplasm is much reduced, simply forming a 'halo' around the nucleus, which gives the cells a shrunken appearance. Little or no granulation of the cytoplasm is visible in the light microscope (Figure 27).

Electron micrographs of interstitial Leydig cells of C. gariepinus show them to be characterised by extensive smooth endoplasmic reticulum and to include large numbers of mitochondria with distinctive tubular cristae

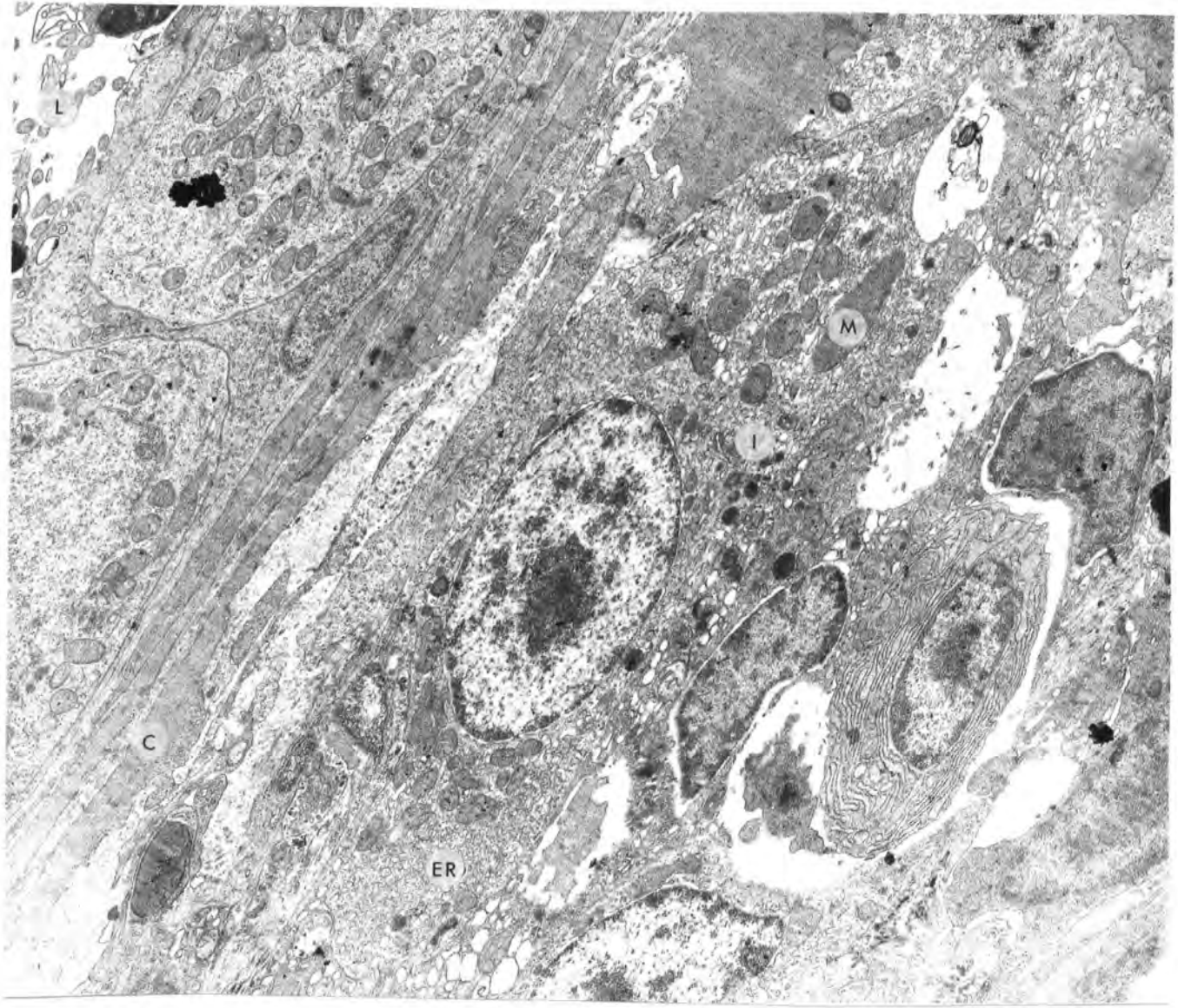

Figure 28 Interstitial Leydig cell (I) in the C. gariepinus testis. Note the large amount of smooth endoplasmic reticulum (ER), and mitochondria (M) with tubular cristae. C = connective tissue of interstitium, L = lumen of seminiferous tubule. 10 000X.

(Figures 28 and 29). These are typical inclusions of steroid secretory cells (Hoar and Nagahama, 1978).

Sertoli cells are sometimes visible in light microscope sections of the testis of ripe C. gariepinus. These cells are irregular in shape and have oval or irregular nuclei, which are found near the basement membrane of the seminiferous lobule. Cytoplasmic extensions penetrate through to the lumen. Electron micrographs illustrate the irregular shapes of the Sertoli cells in more detail. These cytoplasmic extensions can be seen almost enveloping neighbouring cells. The cytoplasm is richly supplied with mitochondria, Golgi apparatus and both rough and smooth endoplasmic reticulum. The mitochondria have lamellar cristae, and smooth endoplasmic reticulum is not present in large enough quantities to indicate a strongly secretory role for the Sertoli cells. The predominant inclusions indicate that a nutritive role is more likely (Figure 30), which is further supported by close contact between developing sperm and Sertoli cells, which can be clearly recognised from electron micrographs (Figure 31).

The testis of C. gariepinus was found to be similar in structure to that described in other siluroids (e.g. Ghosh and Kar, 1952; and Sundararaj, 1960 for Heteropneustes fossilis and Lehri, 1967 for C. batrachus). None of the abovementioned authors, however, described the storage of sperm in the lobules over the winter months.

ENDOCRINE TISSUES OF TELEOST OVARIES

The female gonad, like that of the male, has both gametogenic and endocrine functions. The ovary of teleosts has a variety of morphological forms, but generally varies from that of other vertebrates in that the oviducts are continuous with the connective tissue layers which surround the ovary itself, and thus the ova are not shed into the coelom. The ovary is usually a hollow structure consisting of numerous ovarian follicles embedded in a connective tissue stroma. A germinal epithelium lines this stroma. The connective tissue immediately under the germinal epithelium is rich in blood vessels and is known as the tunica albuginea (see Ball, 1960; Barr, 1968; Hoar, 1969).

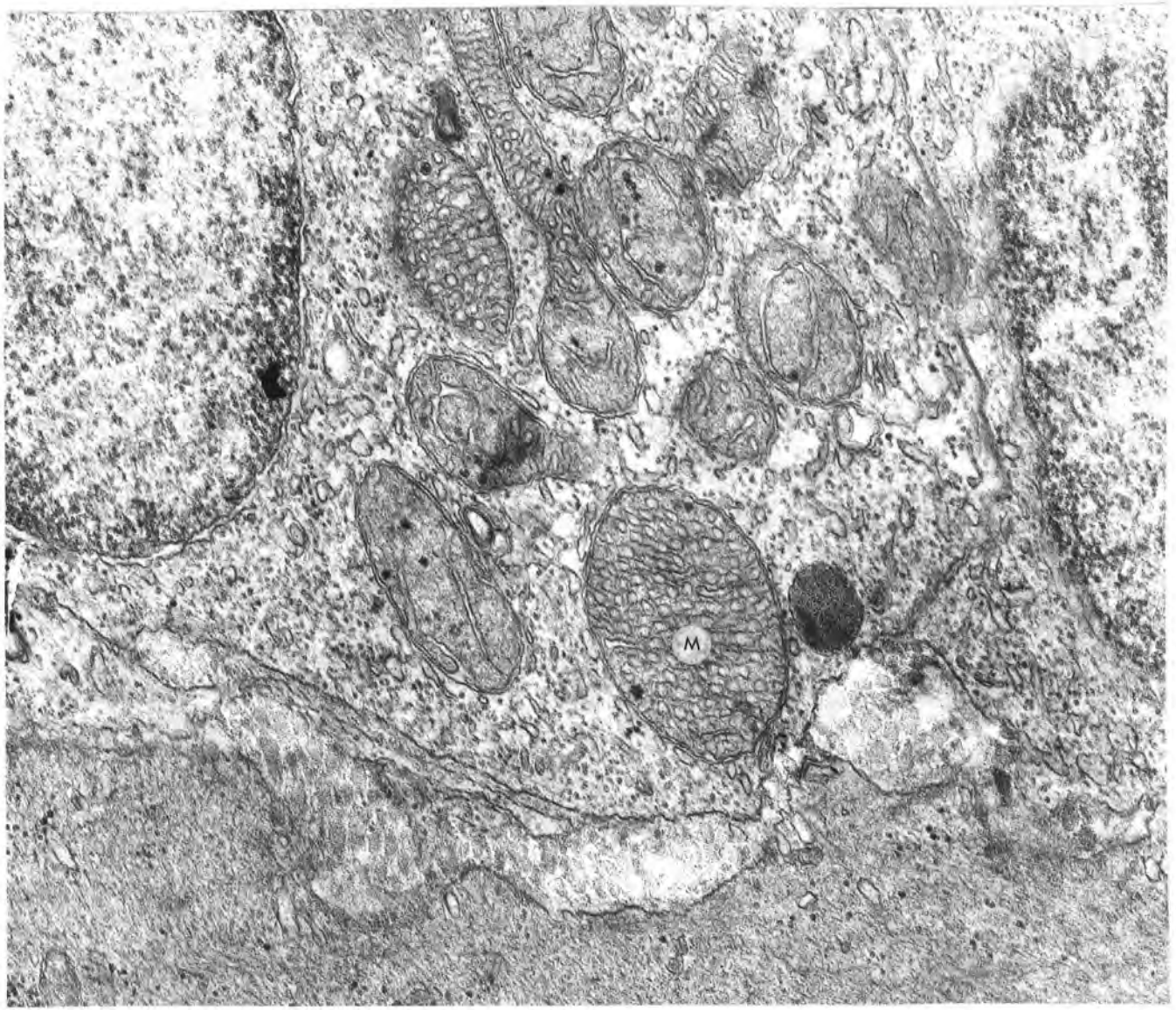

Figure 29 Electron micrograph showing detail of mitochondria (M) of steroidsecretory Leydig cell. Note tubular cristae. 50 000X.

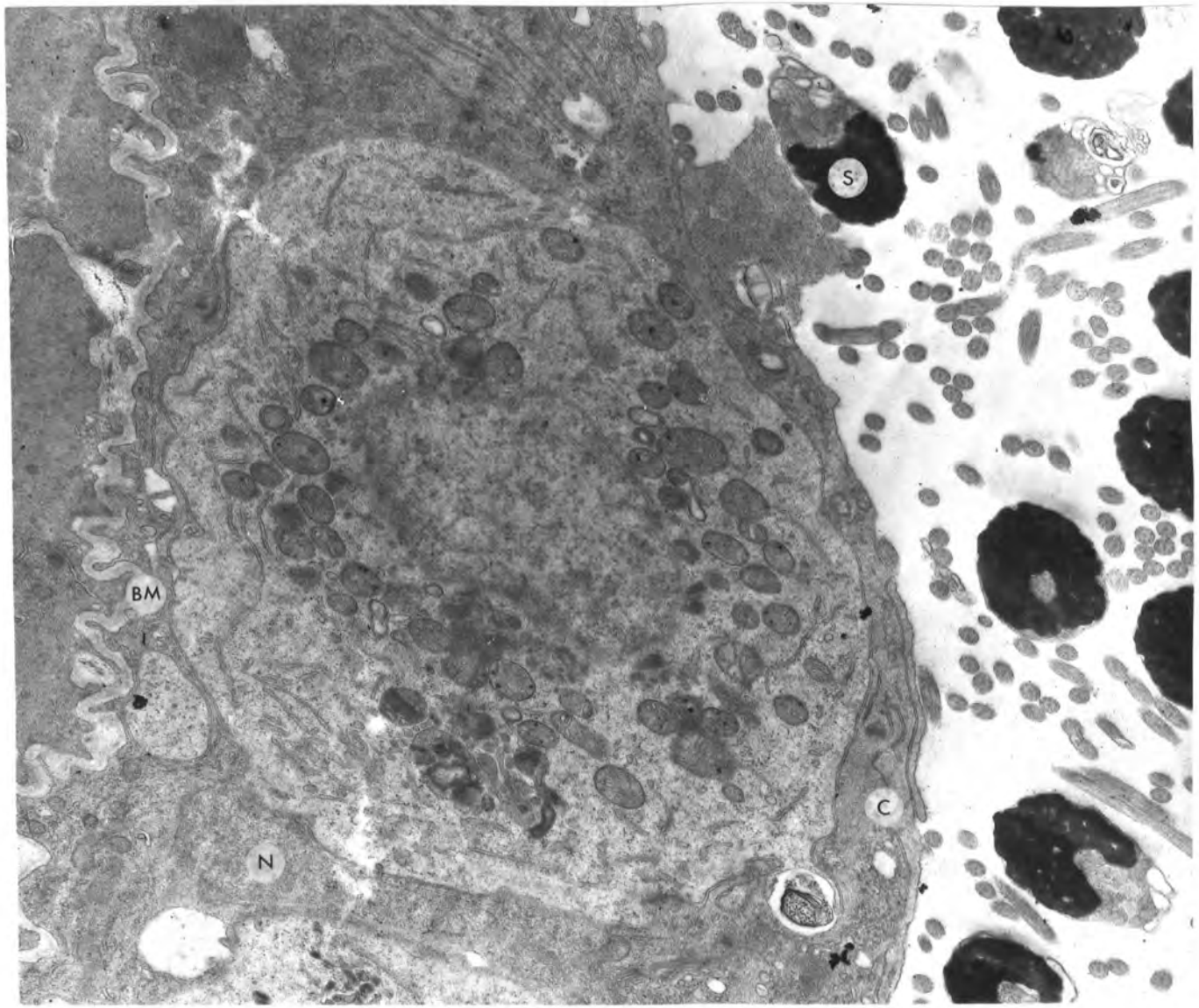

Figure 30 Electron micrograph showing a Sertoli cell in the testis of C. gariepinus. BM = basement membrane of seminiferous tubule, C = cytoplasm of Sertoli cell, N = nucleus of Sertoli cell, S = sperm head. 13 200X.

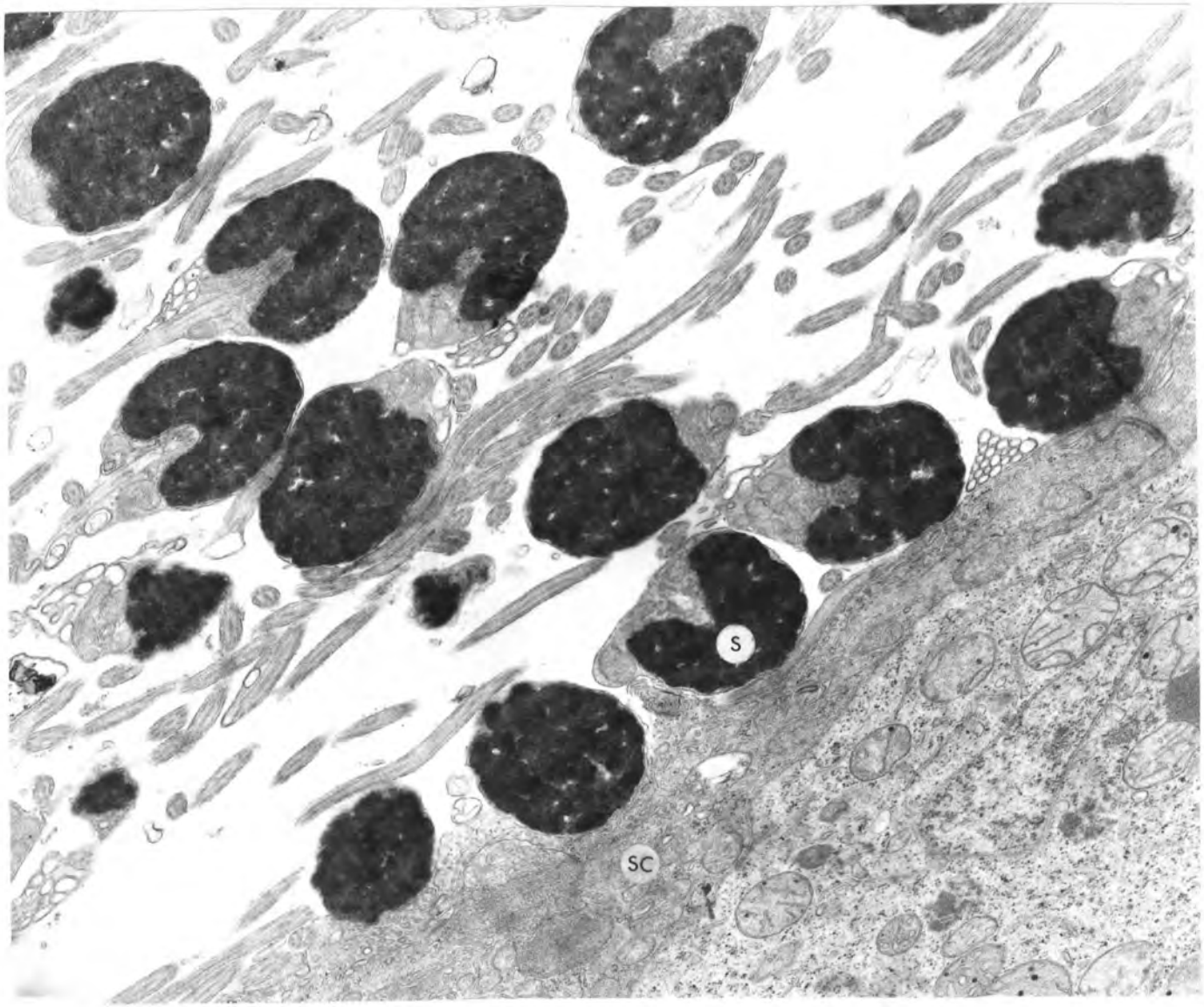

Figure 31 Developing sperm (S) seen in close contact with a Sertoli cell (SC). 18 000X.

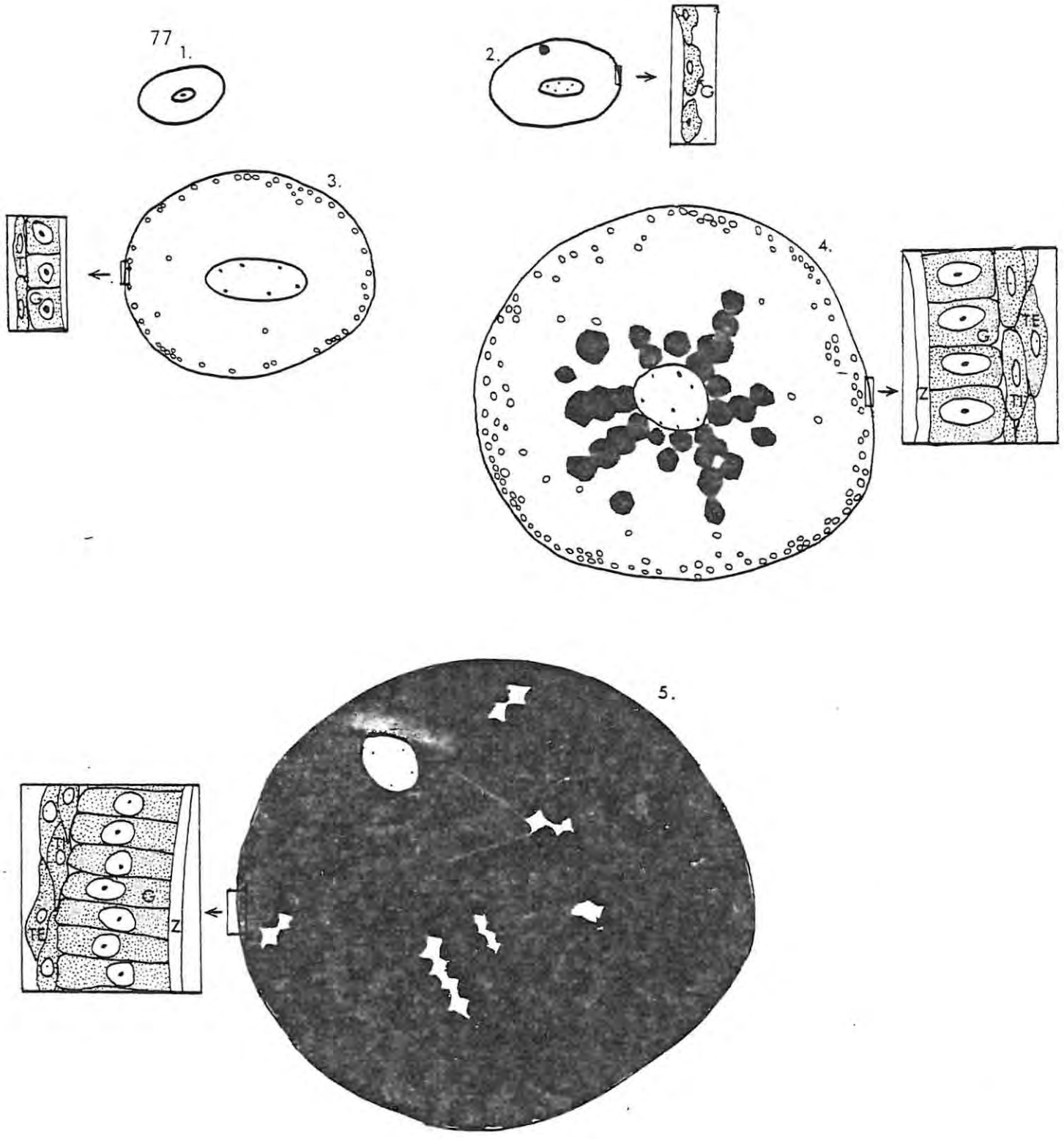

Figure 32. Diagrammatic representation of oocyte developmental stages in a typical teleost ovary. G = granulosa, T = theca, TE = theca externa, TI = theca interna, Z = zona radiata.

1. = primary oocyte, 2. = yolk nucleus stage,
3. = yolk vesicle stage, 4. = yolk globule stage,
5. = maturing ovum.

Ova are formed within the ovary by a cycle of events which begins when cells of the germinal epithelium differentiate into follicle cells and oogonia. Each oogonium then becomes surrounded by a layer of follicular cells, which is known as the granulosa layer. Later in the development of the oocyte another two layers of cells become organised around the outside of the granulosa, and these cells then form what is known as the theca, comprising the theca interna and the theca externa (Barr, 1968; Scott, 1979).

The cycle of events which occur in the ovary of an annually spawning oviparous teleost can be divided into four phases:

(i) Multiplication of oogonia (by mitotic division).
(ii) Transformation of oogonia into oocytes (when the oogonium enters the prophase of the first meiotic division). This is a non-vitellogenic phase.
(iii) Vitellogenesis (the nuclei remain in meiotic prophase).
(iv) Maturation and ovulation (meiosis is completed and the nucleus migrates to the animal pole where it breaks down and a secondary oocyte is formed, together with a first polar body. The second meiotic division converts the secondary oocyte into an ovum, with the formation of a second polar body).

The youngest primary oocytes are thus no larger than oogonia, and have little cytoplasm. As the oocyte developes the amount of cytoplasm increases, and it acquires a marked affinity for stain. At this time the oocyte becomes surrounded by granulosa cells. A yolk nucleus then becomes visible in the cytoplasm, loses its intense affinity for stain, and the cell diameter increases greatly. The nucleus and chromatin gradually become less distinct. Yolk vesicles become visible in the cytoplasm followed by the development of yolk globules, which first appear near the nucleus and radiate outward until the whole oocyte is full of yolk, and the ovum is mature (Hoar, 1969). Figure 32 represents stages of oocyte development in a typical teleost.

At the end of the growth phase there may be a static period before the mature ova are ovulated. At ovulation the follicular membranes rupture and the ovum is released. After ovulation there is a proliferation of granulosa cells in the formation of a post-ovulatory corpus luteum (Hoar, 1969).

Pre-ovulatory corpora lutea or corpora atretica are also a feature of teleost ovaries. These appear when yolk ova become atretic. Corpora atretica are characterized by breakdown of the oolemma and the penetration by phagocytic granulosa cells (Scott, 1979).

The site of oestrogen synthesis in the teleost ovary is a subject of controversy. Granulosa cells, thecal cells and pre- and post-ovulatory corpora lutea have all been proposed (Ball, 1960; Hoar, 1969; Nicholls and Maple, 1972; Nagahama *et al.*, 1976; Hoar and Nagahama, 1978; Scott, 1979). Early workers favoured the suggestion of the corpora lutea in this role (see Ball, 1960; Hoar, 1969 for review), but recent histochemical and ultrastructural evidence indicates that special cells of the thecal layer may be the main steroidogenic cells of the ovary. Granulosa cells are thought to have a role in the transportation of materials to the growing oocyte for the formation of yolk, but they may in addition have a steroid-secretory function (Nicholls and Maple, 1972; Nagahama *et al.*, Hoar and Nagaham, 1978; Scott, 1979).

Whether the corpora lutea secrete steroid hormones remains dubious. Scott (1979) remarks that while it may be that endocrine pre-ovulatory corpora lutea exist in some species, in many it seems certain that they are simply oocytes undergoing resorption. He furthermore states that post-ovulatory corpora atretica are probably not endocrine in function.

RESULTS ON OVARIES OF C. GARIEPINUS AND COMPARISON WITH OTHER SILUROIDS

C. gariepinus is an oviparous fish. The ovaries are paired structures lying in a dorsal position in the coelomic cavity. In immature fish the ovaries are transparent or pink, thin, strap-like structures. As the fish mature the ovaries increase greatly in mass until at maturity they fill most of the coelomic cavity. The ovary wall becomes opaque during the early stages of

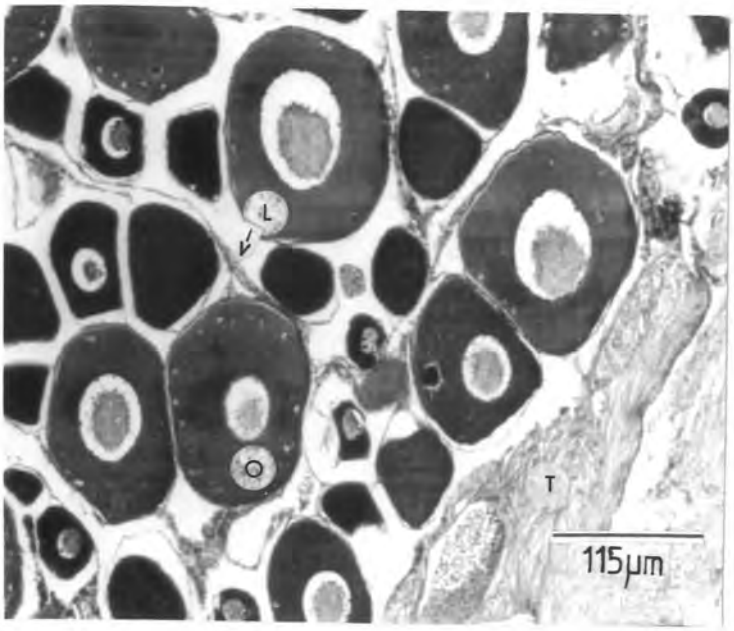

Figure 33 Section through a maturing ovary of C. gariepinus. L = lamellae of germinal epithelium projecting into ovarian cavity, O = developing oocyte, T = tunica albuginea. Masson's trichrome. 5 µm.

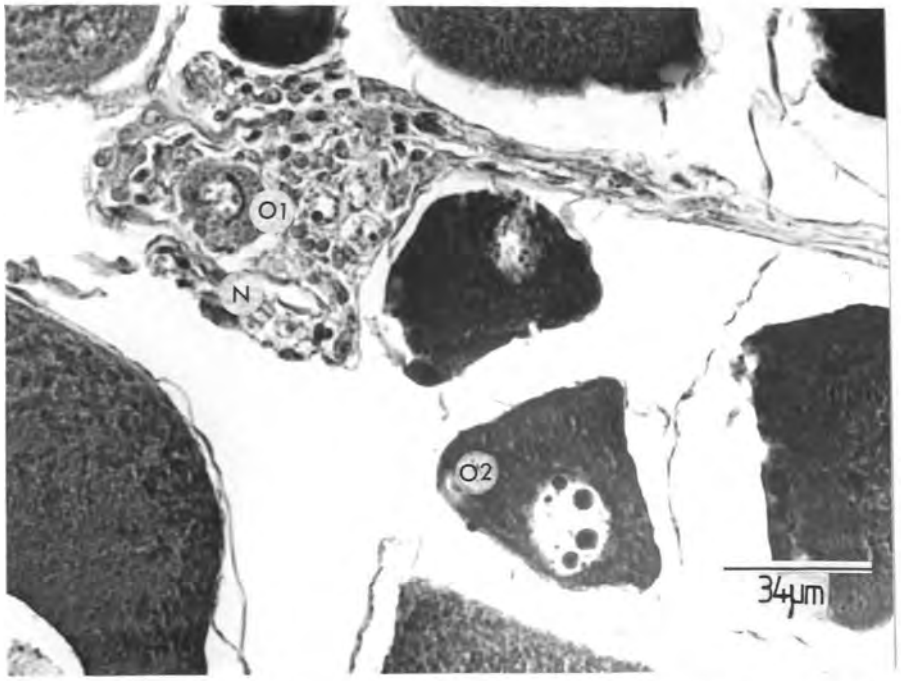

Figure 34 Nests of oogonia (N) in the C. gariepinus ovary. An early stage 1 oocyte (O1) can be seen emerging from this nest. A stage 2a oocyte is also visible (O2). Masson's trichrome. 7 µm.

maturation and then transparent again at maturity, when ova within the ovary can easily be distinguished with the naked eye. The colour of the ovaries ranges from white (immature) through red, reddish brown or orange (Bruton, 1979a and pers. obs.). The vascularisation of the ovaries increases greatly as maturity

Histological structure

The ovary of C. gariepinus, like that of the majority of teleosts, is a hollow sac, the connective tissue covering of which is confluent with the oviduct. The wall of the ovary consists of three layers: - an outer connective tissue layer called the peritoneum, a middle layer known as the tunica albuginea and an inner layer, the germinal epithelium. The tunica albuginea makes up the bulk of the ovary wall and consists of connective tissue, muscle layers and blood vessels. The germinal epithelium consists of a single layer of cuboidal epithelial cells. The germinal epithelium does not line the tunica albuginea closely but forms projections into the ovarian cavity called lamellae. The lamellae greatly enlarge the surface area of the germinal epithelium (Figure 33). Nests of oogonia like those described by Lehri (1968) in C. batrachus ovaries are found at intervals in the germinal epithelium (Figure 34).

Developing oocytes of C. gariepinus can be divided into five phases, easily distinguishable with a light microscope:

1. Chromatin nucleolus stage:

   The oocytes are slightly bigger than oogonia (average diameter 24 µm, n = 40) and consist mainly of a nucleus surrounded by a thin layer of cytoplasm. The cytoplasm has little affinity for stain, and the nucleolus is central.

2. Perinucleolus stage:

   (a) The nucleolus splits into several small nucleoli which are arranged around the periphery of the nucleus near the nuclear membrane. The cytoplasm stains very intensely with basic dyes (e.g. haemotoxylin). Oocytes at this stage are often rather angular in outline, and approximately 130 µm, n = 40) in diameter (Figure 33).

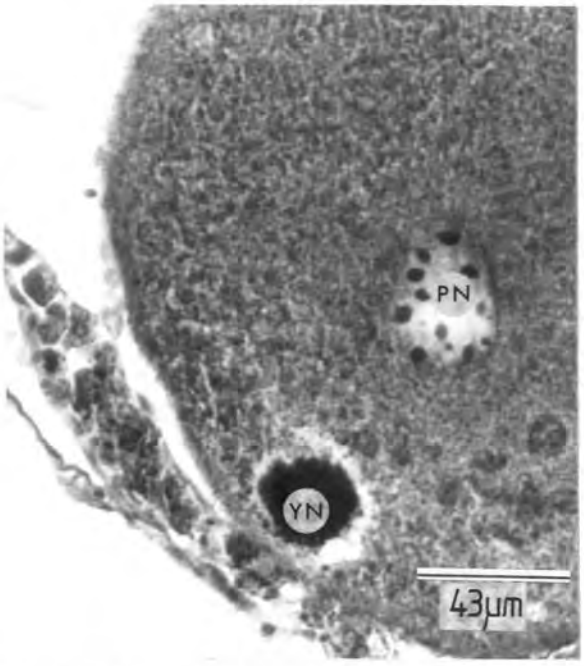

Figure 35 Yolk nucleus (YN) in the cytoplasm of a stage 2 oocyte. Note also perinucleoli (PN). Haematoxylin and eosin. 7 µm.

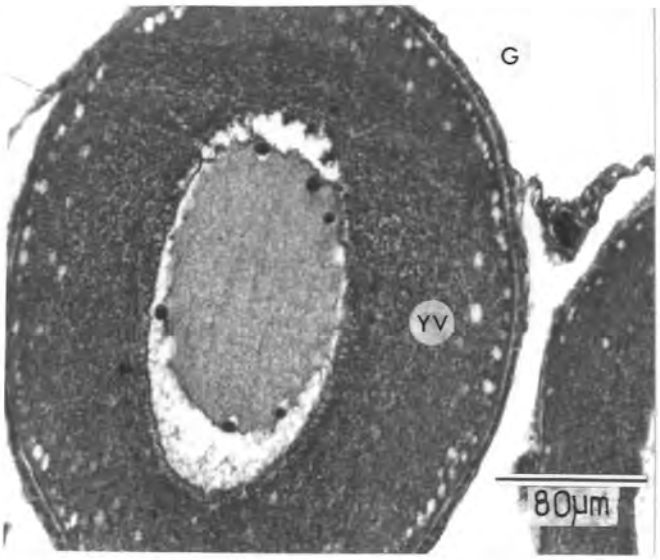

Figure 36 Oocyte at early stage 3 of development, showing formation of yolk vesicles (YV). Note granulose layer (G) becoming evident around periphery of oocyte. Haematoxylin and eosin. 5 µm.

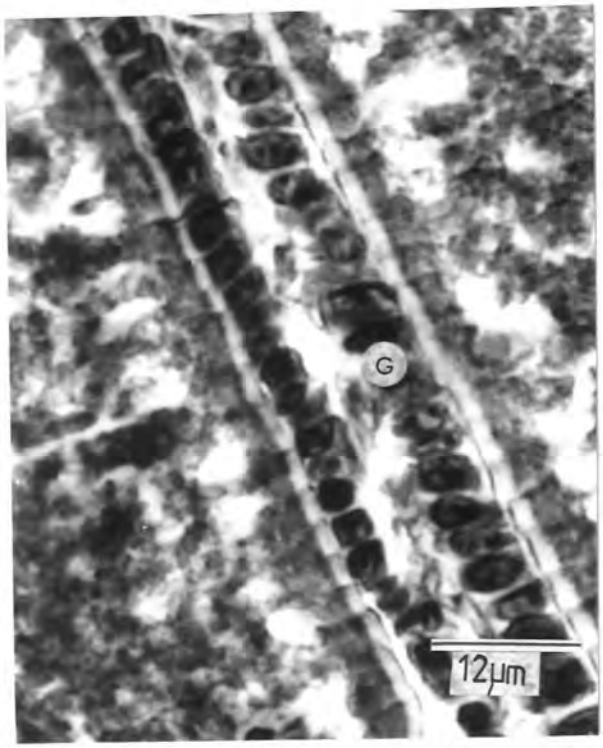

Figure 37 Granulosa cells (G) of stage 3 oocytes. Haematoxylin and eosin. 7 µm.

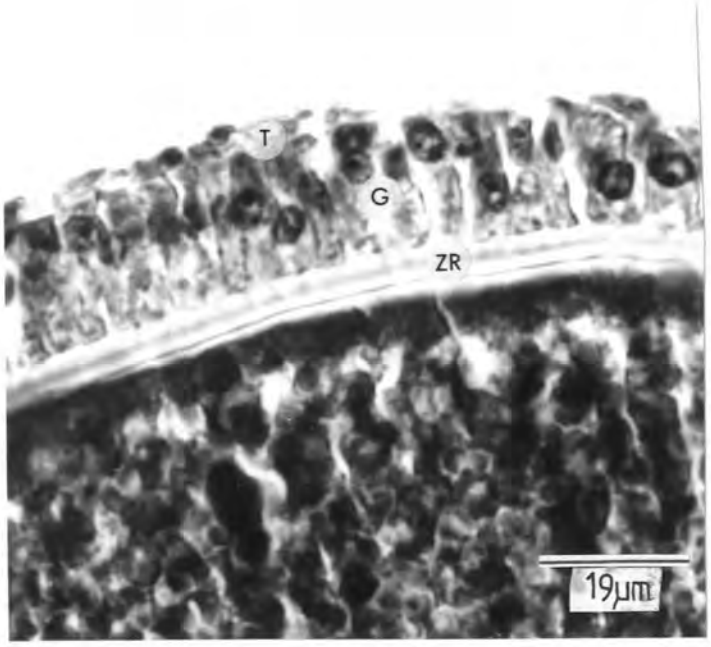

Figure 38 Granulosa cells (G) of stage 4 oocyte. Note columnar shape and central nuclei. A thecal layer (T) is distinguishable external to the granulosa layer. ZR = reticulata. Haematoxylin and eosin 7 µm.

(b) As maturity advances the cytoplasm loses intensity in its affinity for basic dyes and loses its angularity in outline. A yolk nucleus becomes apparent in the cytoplasm. The yolk nucleus is a deeply staining region in the cytoplasm, of questionable functional significance (Figure 35). It appears first near the nuclear membrane, and migrates slowly to the periphery of the cytoplasm where it breaks down. The chromatin component of the nucleus assumes a scattered appearance, indicative of the formation of lampbrush chromosomes, as described by Lehri (1968) in C. batrachus oocytes. The nucleoli remain on the periphery of the nucleus. The granulosa layer becomes evident as a layer of squamous epithelial type cells around the oocyte. Oocytes at this stage are up to 300 μm, (n = 40) in diameter.

3. Yolk vesicle stage:

Yolk vesicles become apparent first in the periphery of the ooplasm, and gradually spread towards the nucleus, until they fill most of the ooplasm. These vesicles remain colourless with most staining techniques (Figure 36). The yolk nucleus may still be present at the beginning of this stage, but soon breaks down. The nucleoli remain around the periphery of the nucleus, and the nucleus loses its 'scattered' appearance.

The granulosa cells become cuboidal or low columnar in shape and a layer of thecal cells becomes arranged external to the granulosa (Figure 37). The zona radiata becomes apparent. Oocytes at this stage are up to 400 μm, (n = 40) in diameter.

4. Primary yolk phase:

Yolk globules begin to form in the cytoplasm. They are seen first in the perinuclear region, and radiate outwards towards the periphery. The nucleoli become more randomly arranged, although some remain near the nuclear membrane. No lampbrush chromosomes are visible. The zona radiata increases in thickness and striations can sometimes be distinguished in this layer. The granulosa cells become highly columnar in shape, with a centrally located, oval nucleus (Figure 38). Two thecal layers, a theca interna and a theca externa can now sometimes

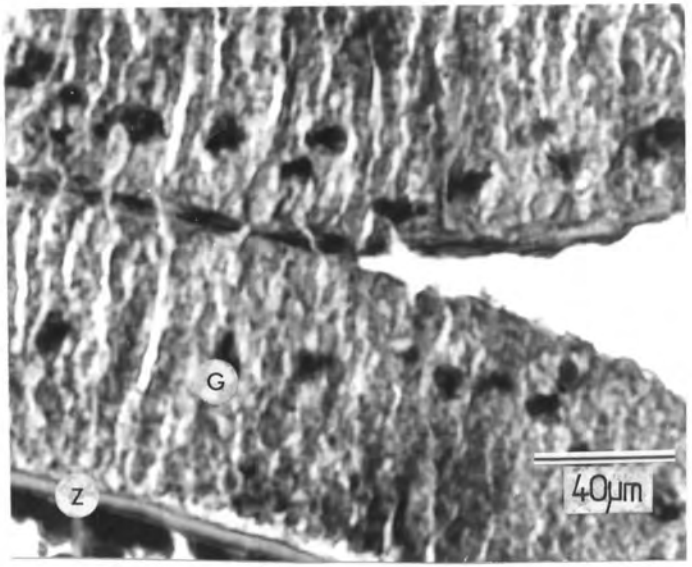

Figure 39 Granulosa cells (G) of stage 5 oocytes. Z = zona reticulata. Haematoxylin and eosin. 7 µm.

be seen. Oocytes at this stage measure up to 60 µm, (n = 40 in diameter.

5. Secondary yolk phase:

The oocytes become completely full of yolk. The yolk globules are large, and tend to fuse together. The nucleus is now relatively small and moves towards the periphery where the nuclear membrane eventually disappears. The radial striations in the zona radiata are more pronounced. Oocytes at this stage measure up to 1 mm diameter.

6. Maturity:

Ripe eggs are spherical in form and full of yolk. Granulosa cells are highly columnar (Figure 39). The radial striations in the zona radiata are clearly visible. Ripe ova measure up to 1,7 mm (n = 40). Stages 1 and 2 are present in ovaries of fish caught at every time of the year. Stage 3 becomes apparent in fish caught in the early spring, and as spring progresses ovaries also contain oocytes in stages 4 and 5. Stage 6 oocytes are only seen in the ovaries of fish caught in the summer months.

Thus, unlike the situation described in males of C. gariepinus, histological sections reveal that gametogenesis in females takes place only in spring and summer, and the female gonads do not contain ripe (or almost ripe) gametes through the winter months. Development of primary oocytes is is retarded until environmental stimuli indicate that the spawning season is approaching. Figure 40 relates the number of oocytes in different stages in the ovary of a fish to the approximate maturity state as calculated from macroscopical examination alone.

Atretic oocytes are commonly observed in histological sections of C. gariepinus ovaries. Atretic oocytes are characterized by hypertrophy and hyperplasia of the granulosa and theca cells which penetrate the interior of the follicle. The yolk becomes liquified and then disappears. In the early stages of atresia the zona radiata becomes folded, and colloid particles appear between this and the granulosa. Later the zona radiata breaks down completely. Figures 41 and 42 show atretic oocytes. Post ovulatory corpora lutea are short-lived and are not apparent in the ovaries several months after spawning, as was described in C. lazera ovaries

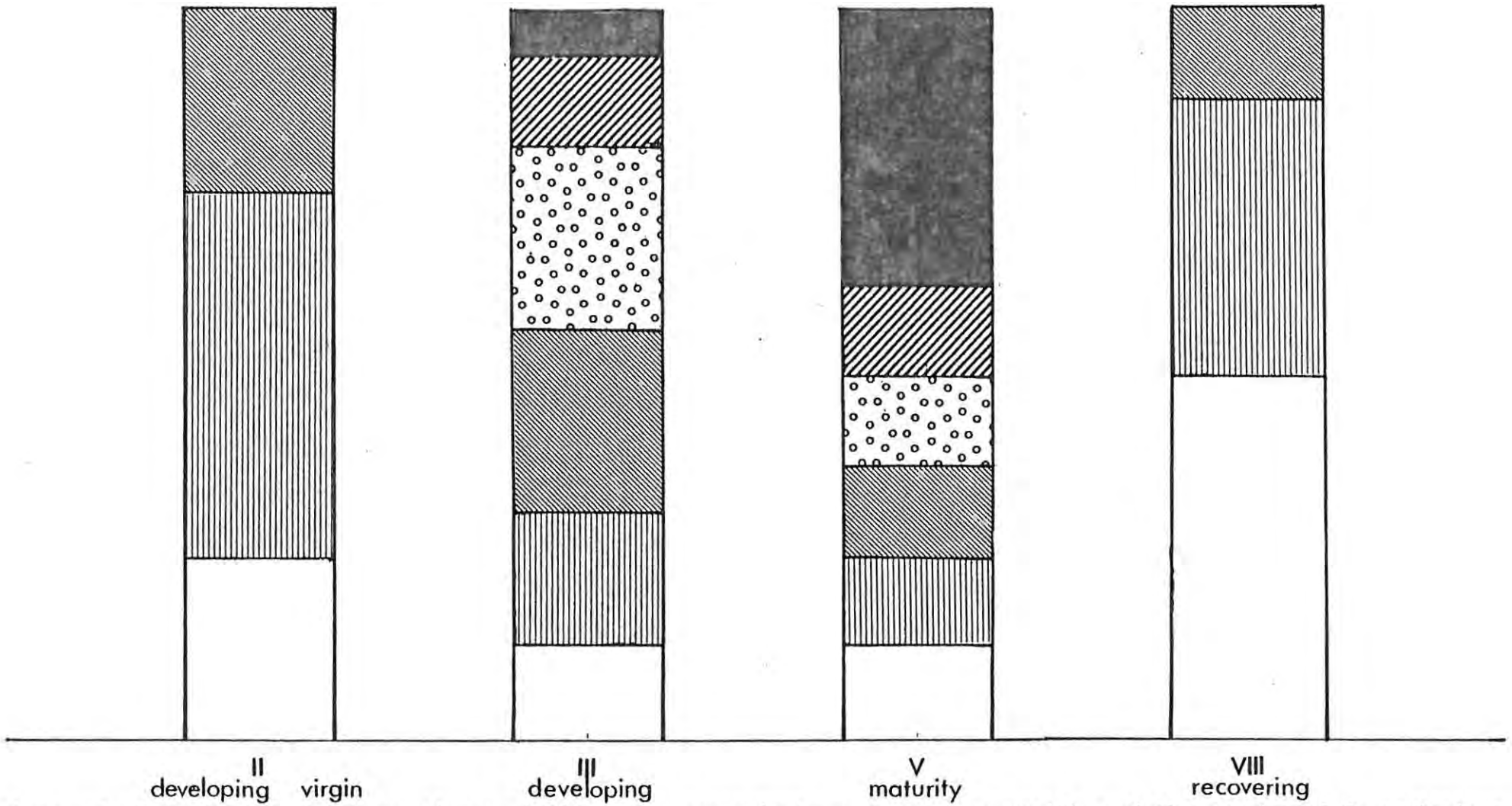

Figure 40 Diagrammatic representation to indicate the approximate percentage composition of the ovaries of C. gariepinus at differemt stages of development. Numbers 11 to V111 represent maturity stages described by Bruton (1979a). Key to shadings: Blank = stage 1 oocyte, vertical lines = stage 2 oocyte, thin diagonal lines = stage 3 oocytes, spots = stage 4 oocytes, thick diagonal lines = stage 5 oocytes, dark stipple = stage 6 oocytes.

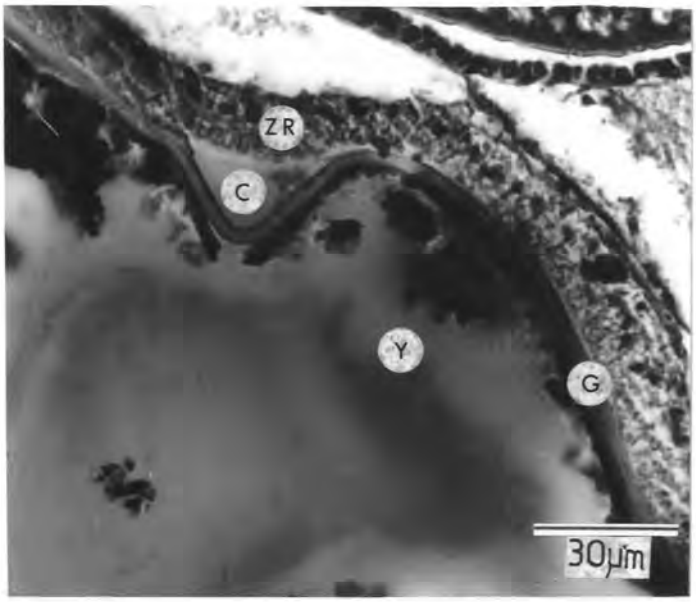

Figure 41 Early stages of atresia. Note the folding of the zona reticulata (ZR) and the appearance of colloid between this and the granulosa layer (G). Note also the liquefaction of the yolk (Y). Haematoxylin and eosin. 7 µm.

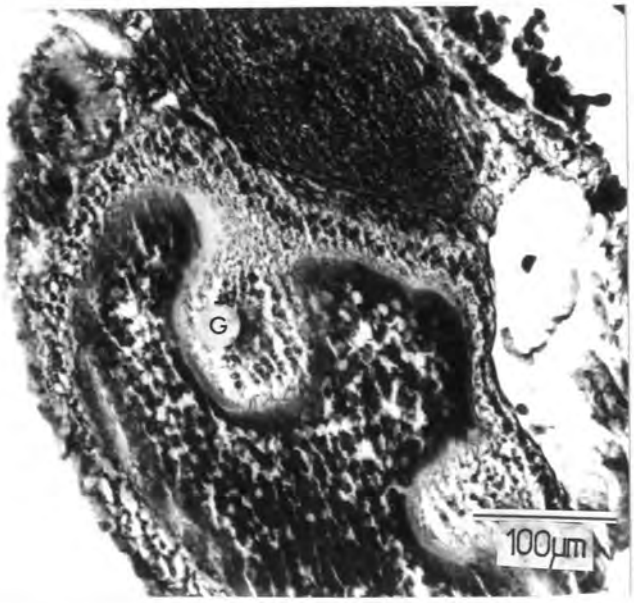

Figure 42 Later stage of atresia. Note the proliferation of the granulosa cells. Haematoxylin and eosin. 7 µm.

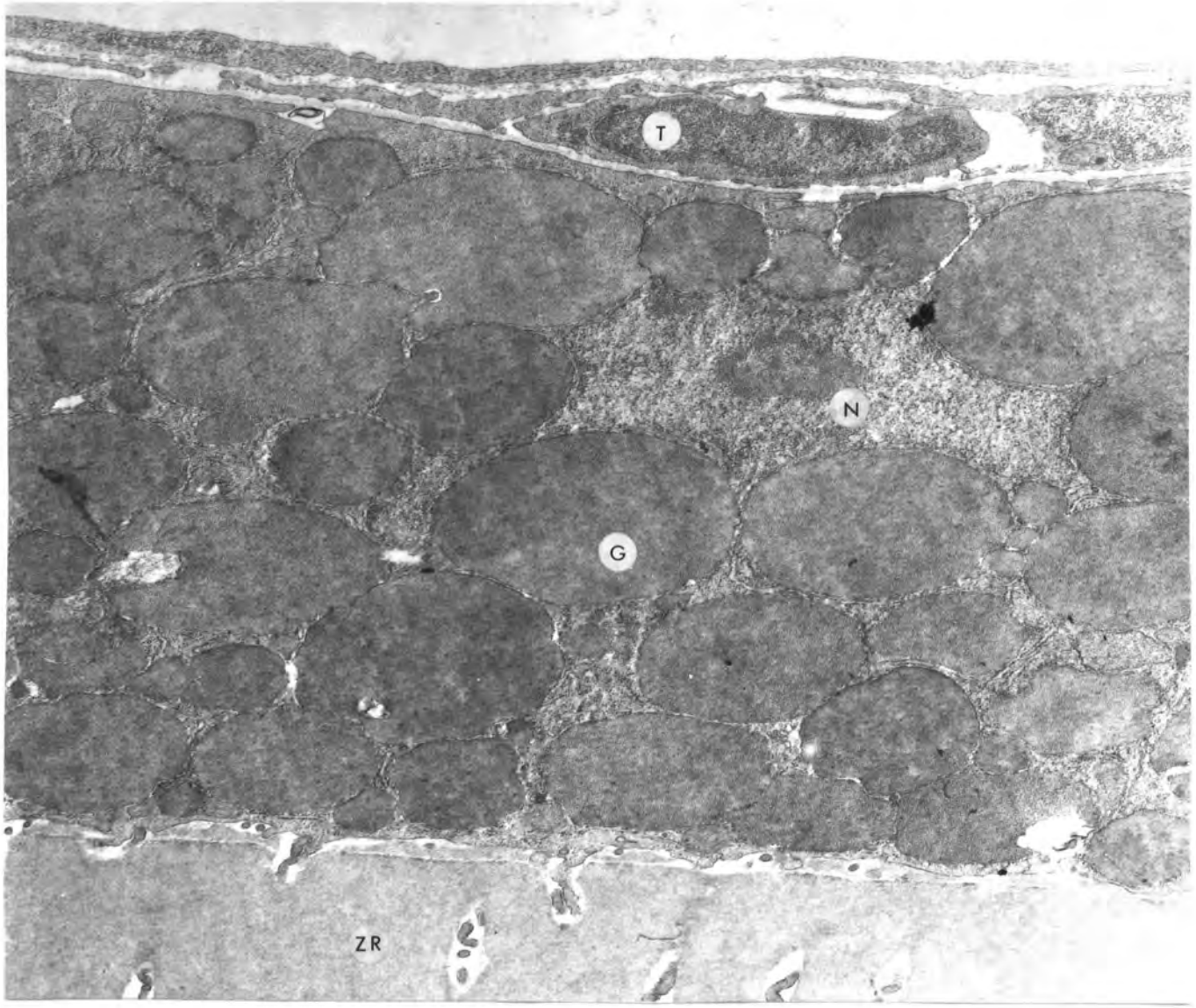

Figure 43 Electron micrograph showing the granulosa layer of a maturing oocyte. Note the large electron dense globules (G) in the cytoplasm. N = nucleus of granulosa cell, T = nucleus of theca cell. 14 300X.

by Rizkalla (1970a).

The histological structure of the ovaries of C. gariepinus was found to be very similar in every way to those described in closely related catfish (e.g. Ghosh and Kar, 1952 and Sundararaj, 1959 for H. fossilis; Rizkalla, 1970a for C. lazera; Lehri, 1968 for C. batrachus and Belsare 1975 for C. batrachus and H. fossilis).

Comparison of C. gariepinus results with Grizzle and Rogers (1976) description of the ovary of the channel catfish I. punctatus shows that the ovarian structure and development in these two less closely related catfish are also very similar. The only notable difference was that atretic follicles were only seen in spent ovaries of I. punctatus, while atretic oocytes were also observed in pre-spawning ovaries in both C. batrachus (Lehri, 1968) and C. gariepinus.

Maturing yolky oocytes (stages 3 - 4) were examined in the transmission electron microscope for steroid secretory characteristics in the cells forming the ovarian follicle.

The granulosa cells were found to contain large numbers of electron dense globule-like inclusions. Nicholls and Maple (1972) described a similar phenomenon in the granulosa cells of both the cichlids Cichlasoma nigrofasciatum and Haplochromis multicolor, but more marked in the former species. These globules appear to be due to an accumulation of material of medium electron density within the cytoplasmic membranes, particularly between the membranes of the endoplasmic reticulum, as the ovary matures (Nicholls and Maple, 1972). In granulose cells of C. gariepinus oocytes, even when not fully mature the cytoplasm was completely filled with these globules, so that even the nucleus was distorted (Figure 43). A large number of mitochondria with lamellar cristae were also present in the granulosa cells. The fine structure of these cells indicates a nutritive function, emphasized by the presence of projections in the form of microvilli running through the zona reticulata towards the cytoplasm of the oocyte. The electron dense material is thought to be storage vessels containing mutrients.

The thecal component of the C. gariepinus ovarian follicle consists of 1 - 3 layers of cells. The innermost layer, always present in stage 3 or 4 oocytes, comprises small flattened cells with elongate nuclei and sparse

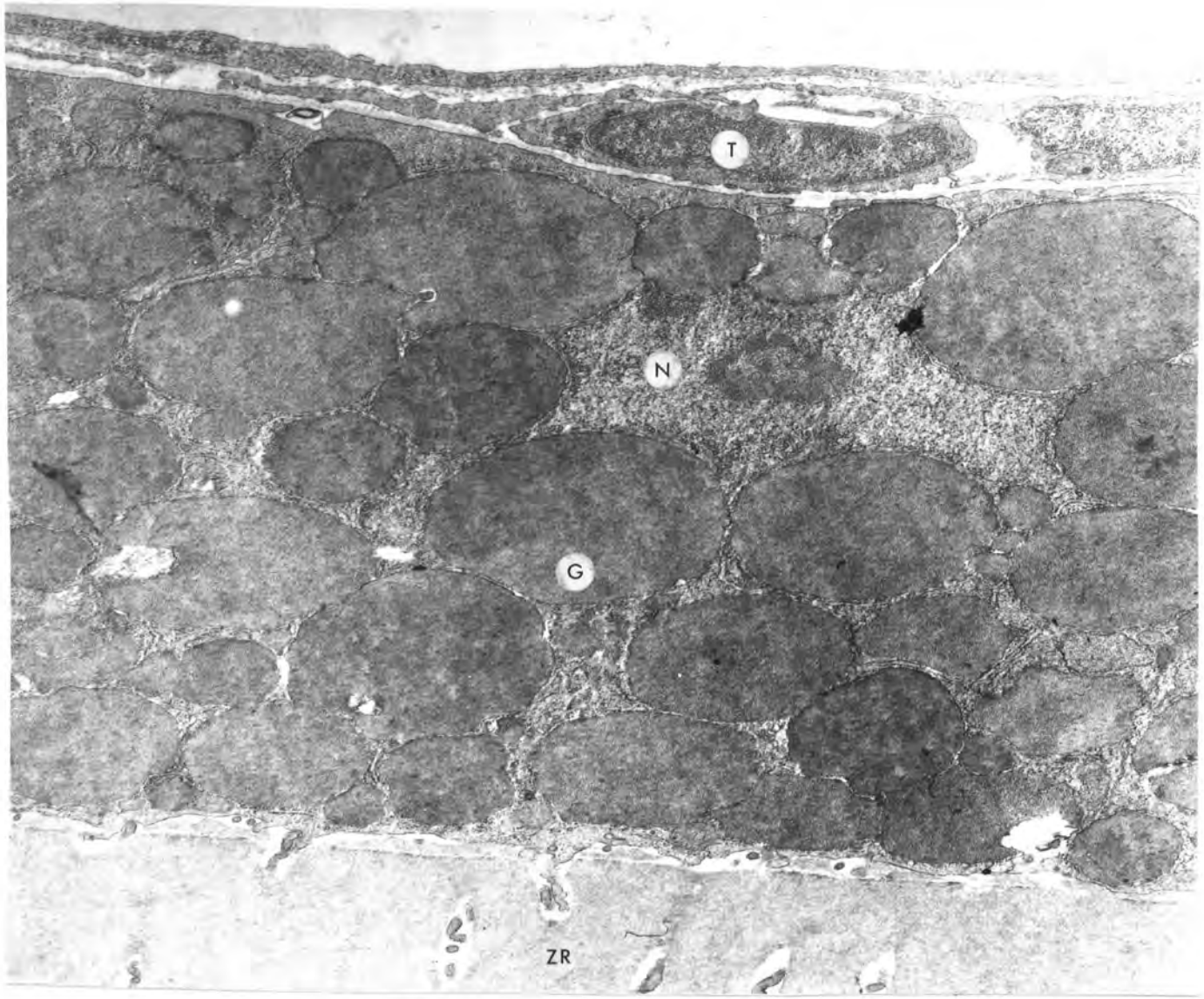

Figure 44 Special thecal cell (ST) from developing oocyte of C. gariepinus. Note large amount of smooth endoplasmic reticulum (ER), and mitochondria (M) with tubular cristae, indicating a steroid-secretory function. G = granulosa layer, TE = projection from a theca externa cell. 35 600X.

cytoplasm, which is nevertheless rich in mitochondria with lamellar cristae. The middle layer, when present, consists of larger cells with a cytoplasm containing very few organelles. Another type of cell was occasionally seen between these follicular cells, which contained large amounts of smooth endoplasmic reticulum. Although the mitochondria of these cells have lamellar cristae, the extent and configuration of the smooth endoplasmic reticulum indicates that these are probably the steroid secretory cells of the C. gariepinus ovary (Figure 44).

THE HEADKIDNEY OR INTERRENAL OF TELEOSTS

Investigations into the teleostean homologue of the mammalian adrenal gland began in the late 19th Century. These early studies were confused by the presence of two different structures in the kidney region which both appear to be secretory. For many years there was disagreement among scientists as to which of these structures was the adrenal homologue (Balfour, 1882; Wheldon, 1885; Diamare, 1895; Vincent, 1898; Giacomini, 1902; 1905; 1908; Vincent and Curtis, 1927). Recent development of histochemical techniques have established without doubt that the tissue described by Giacomini (1902) as the anterior interrenal is the adrenal homologue. The function of the Corpuscles of Stannius, or posterior interrenal, remains in question (Chester Jones, 1957).

There is considerable variation among the teleosts in the position and structure of the anterior interrenal, or, as it is commonly named, the headkidney. Comparative studies on the histology of the interrenal of several different teleost species have been published by Baecker (1928, twelve species); van Overbeeke (1960, eighteen species from twelve families), and Nandi (1962, review of previous literature, plus study of one hundred and twenty nine species from fifty three families). Nandi concludes from his work that there is no correlation between the interrenal morphology and physiology, ecology and taxonomic position of the species studied. He also found no major evolutionary trends and remarks that the morphology of the interrenal gland among species from a single family may be as diverse as it may be among widely separate taxonomic groups.

The headkidney of teleosts is thought to be derived from the pronophros, which in this, unlike the other, vertebrate groups persists into adult life

(Fraser, 1950; Chester Jones, 1957). The gross morphology of the headkidney is extremely variable, although generally it is found anterior to the functional kidney, and in close association with the posterior cardinal veins and their branches. In contrast to higher vertebrates there is no distinct encapsulated gland, which makes extirpation experiments difficult, and accounts to some extent for the difficulties in establishing the position of this tissue. In some fish the headkidney is completely separated from the functional trunk kidney, while in others the interrenal cells are simply found anteriorly in the trunk kidney, from which they may also extend posteriorly (Ogawa, 1968; Lofts and Bern, 1972).

The bulk of the headkidney usually contains no renal elements, but is made up of an organised mass of haemopoietic tissue, which may be lymphoid or myeloid or both (Lofts and Bern, 1972). Dark cell masses known as 'pigment cells with pigment' (Nandi, 1962), or melanophore macrophage centres are also found in the headkidney of most fish.

Adrenocortical tissue consists of epithelial cords or groups of cells scattered in the headkidney often close to the veins. Chromaffin tissue (the homologue of the adrenal medulla of higher vertebrates) is usually found in close association with the posterior cardinal veins and their branches, and is often located close to the adrenocortical tissue. Chromaffin tissue consists of either single cells or groups of cells (Nandi, 1962; Lofts and Bern, 1972).

RESULTS ON HEADKIDNEY OF C. GARIEPINUS AND COMPARISON WITH OTHER SILUROIDS

The literature available on the antomy and morphology of the headkidney of siluroids is scant and confusing. Nandi (1962) in an extensive publication on teleost adrenals mentions only one siluroid, Amiurus catus now known as Ictalurus nebulosus nebulosus (le Sueur). The information provided by Nandi is based on the work of Giacomini (1905), and is not extensive. The interrenal tissue is not mentioned at all, but the chromaffin tissue is said to consist of single cells or cell groups in the walls of the large vessels, corresponding to type 1A of Nandi (1962).

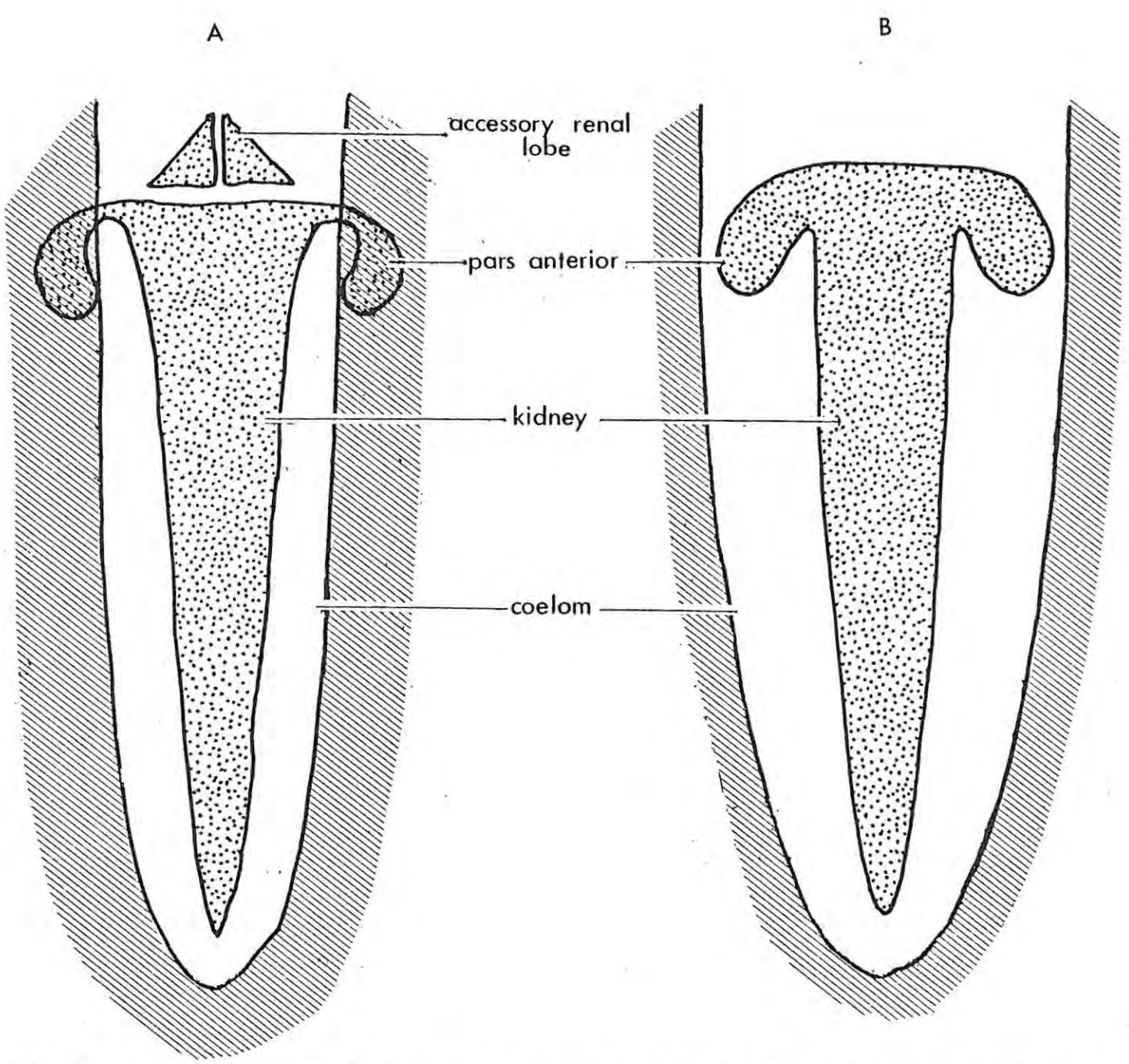

Figure 45 Diagram to illustrate the terms used by Sharma (1971). A represents the tissues of C. gariepinus and H. fossilis, B represents the tissues of N. notopterus and T. fasciatus.

Dixit (1970), Subhedar and Rao (1974) and Shanbag and Nadkarnis (1977) performed experimental work on the interrenal gland of C. batrachus, but little mention is made of the location or morphology of this tissue. Meanwhile Sharma (1971) published his paper entitled "Homology of the so-called Headkidney in certain Indian teleosts". Among the fish he studied were C. batrachus and Heteropneustes fossilis. He refers to two different regions, both of which come under his description of 'headkidney', and names these two regions the accessory renal lobes and the pars anterior of the kidney. The accessory renal lobes lie in front of the septum transversum and are completely separated from the functional kidney. The pars anterior is in two parts on either side of the functional kidney, and corresponds to the extra-coelomic kidney described by Dutta (1923) (Figure 7).

Sharma (1971) shows that in Notopterus notopterus (Pallas) and Trichogaster fasciatus (Bloch.Sch.) thekidneys have a similar structure to those of C. batrachus, with lateral extensions which he again refers to as the pars anterior (Figure 45). In these fish there are no accessory renal lobes and the pars anterior is undoubtedly the functional headkidney. Sharma implies that the pars anterior in C. batrachus and H. fossilis should be homologous with the pars anterior of N. notopterus and T. fasciatus, however, he also implies that the accessory renal lobes, when present, are the functional headkidney. In Dutta's (1923) account of the disposition of the kidney and liver in C. batrachus, he sketched in the accessory renal lobes of Sharma and labelled them headkidney or pronephros.

Sharma's work is extremely confusing as he seems to be unaware of the fact that the headkidney of fish is the site of adrenocortical and chromaffin tissues. This is inexplicable considering his work was published in 1971, by which time the occurrence of endocrine tissues in the teleost headkidney was a well-documented fact. Sharma refers only to the haemopoietic and lymph-producing function of this organ, apart from a fleeting mention of the occurrence in this region of cells that appear to be secretory.

According to Grizzle and Rogers (1976) the headkidney of Ictalurus punctatus is composed of fused bilateral lobes located anterior to the swimbladder. The headkidney is completely separated from the trunk kidney, and would appear to be homologous with the structure which Sharma names the anterior

renal lobe in C. batrachus. Grizzle and Rogers mention no extra-coelomic kidney lobes in I. punctatus and it is therefore assumed that these structures are absent.

It seems probable that in the works of Dixit (1970), Shanbag and Nadkarnis (1977); Qureshi and Sultan (1977) and Rao, Betole and Kondawar (1972) on C. batrachus and Rizkalla (1969) on C. lazera that the so-called headkidney or adrenal gland is the structure which Sharma named the accessory renal lobe.

Due to the confusing and conflicting publications on the position and structure of the interrenal tissues in C. batrachus, C. lazera and other siluroids it was decided that this project should include a histological examination of both the accessory renal lobes and the extra-coelomic kidney and their blood supplies in order to clarify their structure and function.

Location

The left and right kidneys of C. gariepinus are fused along their entire length. The kidney appears as a single, elongated triangular structure extending from posterior to the septum transversum to the region of the urogenital sinus (Figure 7, p. 20). The kidney is deep red in colour and situated against the dorsal surface of the coelomic cavity. The anterior end is considerably broader and thicker than the posterior. The posterior cardinal veins pass through the kidney, the right vein increasing in diameter along the length (posterior to anterior), while the left vein decreases in diameter.

On either side of the main bulk of the kidney tissue and attached to the kidney by a narrow band of tissue known as an isthmus is an extra coelomic lobe of the kidney, comparable to that described by Dutta (1923) and Sharma (1971) in C. batrachus. Sharma named this the pars anterior of the kidney. These extra coelomic kidney lobes are deeply embedded in the musculature of the pectoral region.

Anterior to the septum transversum and swimbladder and enclosed by extensive, thick layers of connective tissue are two small triangular structures. These structures are closely applied to each other, but are separated by mesenteries.

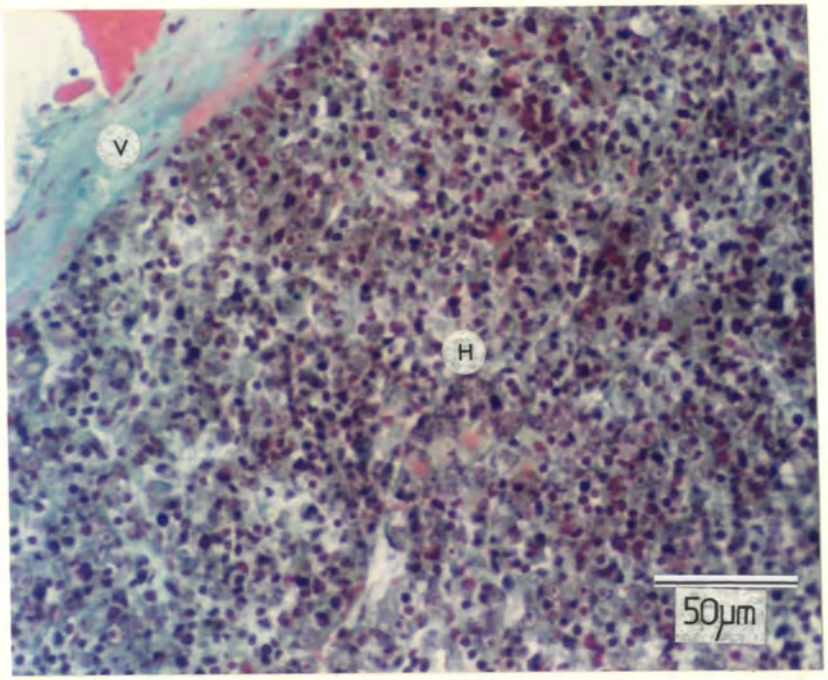

Figure 46 Posterior headkidney tissue of C. gariepinus made up of haemopoietic tissue (H). V = wall of blood vessel. 7 µm.

The longest side of the triangle is parallel to the septum transversum (Figure 7). These structures are paler in colour than the main bulk of the kidney.

Histological sections revealed that the site of the adrenocortical and chromaffin in cells of C. gariepinus is without doubt the tissue corresponding to that named the accessory renal lobe by Sharma in C. batrachus, and it is these structures therefore that are the functional headkidney of this species. The location of the headkidney is unusually far removed from the trunk kidney, as in most teleosts it is either actually confluent with the trunk kidney, or separate from it, but not far removed (Ogawa, 1961). Grizzle and Rogers (1976) remark on the unusual degree of separation of the headkidney from the trunk kidney in I. punctatus (an ictalurid catfish), where the headkidney is anterior and the trunk kidney posterior to a large swimbladder. A similar separation was found in C. gariepinus, although the swimbladder in this species is much reduced. Furthermore, the headkidney of C. gariepinus is proportionately smaller than the trunk kidney compared with I. punctatus.

The histological structure of the extra-coelomic kidney lobes was examined in order to determine their role, if any, in the secretion of adrenal hormones. The structure of this region was found to be very similar histologically to that of the headkidney tissue, containing no renal elements and being made up largely of haemopoietic tissue. No endocrine tissue at all was found in this region (Figure 46).

Sections were also made through the anteriormost part of the trunk kidney, but no endocrine tissue was found in this region, which is composed largely of renal tubules.

Blood supply

Adrenocortical and chromaffin tissues are always found in fairly close association with the posterior cardinal veins and their branches in teleost fish (Nandi, 1962). The posterior cardinal veins of C. gariepinus pass through the kidney, the right vein increasing in diameter along the length (posterior to anterior), while the left vein decreases in diameter. The

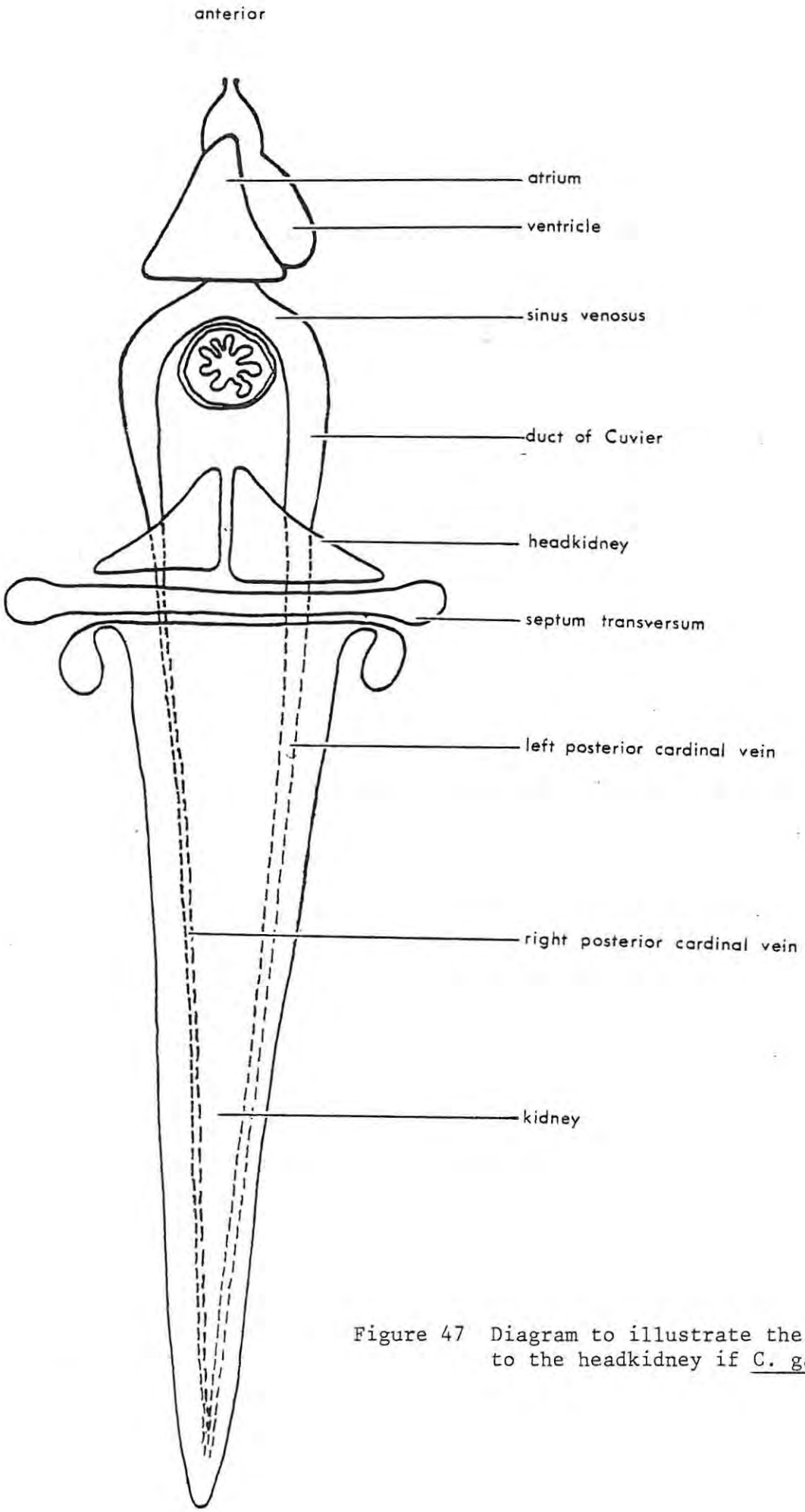

Figure 47 Diagram to illustrate the blood supply to the headkidney if C. gariepinus.

posterior cardinal veins pass dorsal to the bony case enclosing the swim-bladder, and then through the dorsal surface of the headkidneys. They then enter the ducts of Cuvier, which are thin-walled and large-diameter vessels in C. gariepinus. The ducts of Cuvier enter the heart via the sinus venosus (see Figure 47). The posterior cardinal veins do not pass through the extracoelomic kidney lobes, but a small vein which empties into the posterior cardinal vein can be seen draining each lobe.

Histological structure

The left and right headkidneys of C. gariepinus show a different histological structure. The right posterior cardinal vein is very much larger than the left, and as a result of this and the close relationship between the position of the endocrine tissue and the venous supply in teleost adrenals (Nandi, 1962), the majority of the secretory tissue is found in the right headkidney.

The bulk of both right and left headkidneys is composed of haemopoietic tissue, blood vessels and sinusoids and nerve endings. The pigment cells described by Nandi (1962) are not very prominent in C. gariepinus. The adrenocortical tissue is arranged in cords, forming large masses near the walls of the posterior cardinal veins or their larger branches. Individual cells show considerable variation, both in size and shape, ranging from 5 µm to 10 µm in diameter (n = 40) and from rounded or polyhedral to elongate in outline. The nuclei, too, show little consistency in shape and size. They are generally large; some are rounded, some oval and some kidney-shaped. A prominent nucleolus is present in all nuclei and lumps of chromatin are abundant. The cytoplasm is uniformly granular in appearance. Rizkalla's (1969) description of the adrenocortical cells of C. lazera, and Dixit's (1970) description of the interrenal cells of C. batrachus correspond well to the same cells of C. gariepinus.

The cords of adrenocortical tissue are often separated from each other by blood sinusoids. Adrenocortical tissue in C. gariepinus was found to occur near the walls of the posterior cardinal veins and the smaller veins of the headkidney, thus corresponding best with type II of Nandi (1962) (Figures 48 and 49).

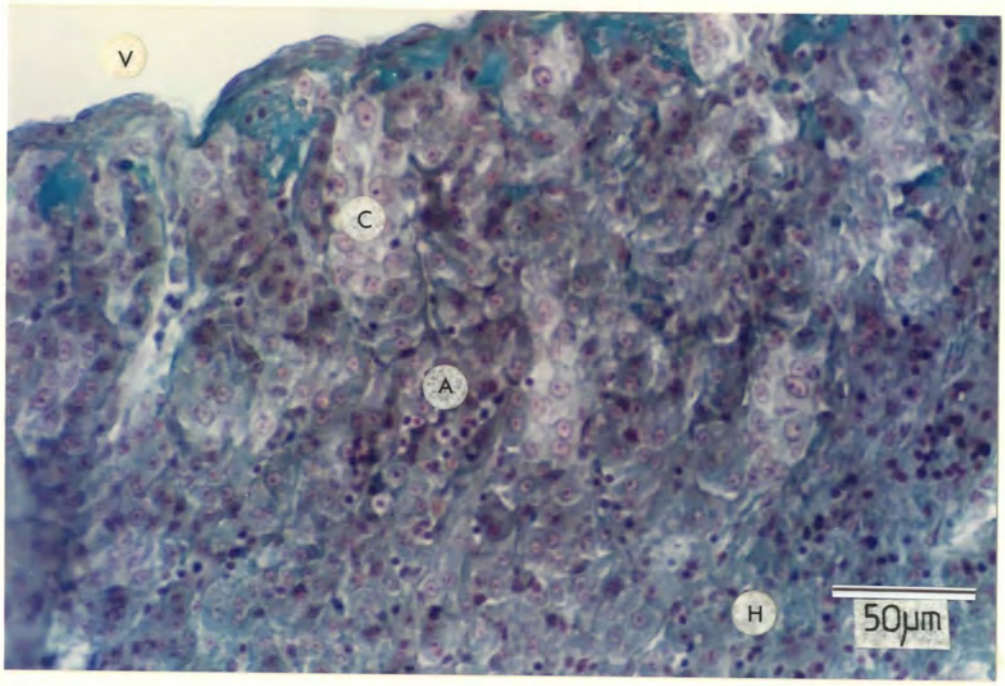

Figure 48 Adrenocortical (A) and chromaffin (C) tissues near the wall of a large vein in the headkidney of C. gariepinus. H = haemopoietic tissue. Masson's trichrome. 5 µm.

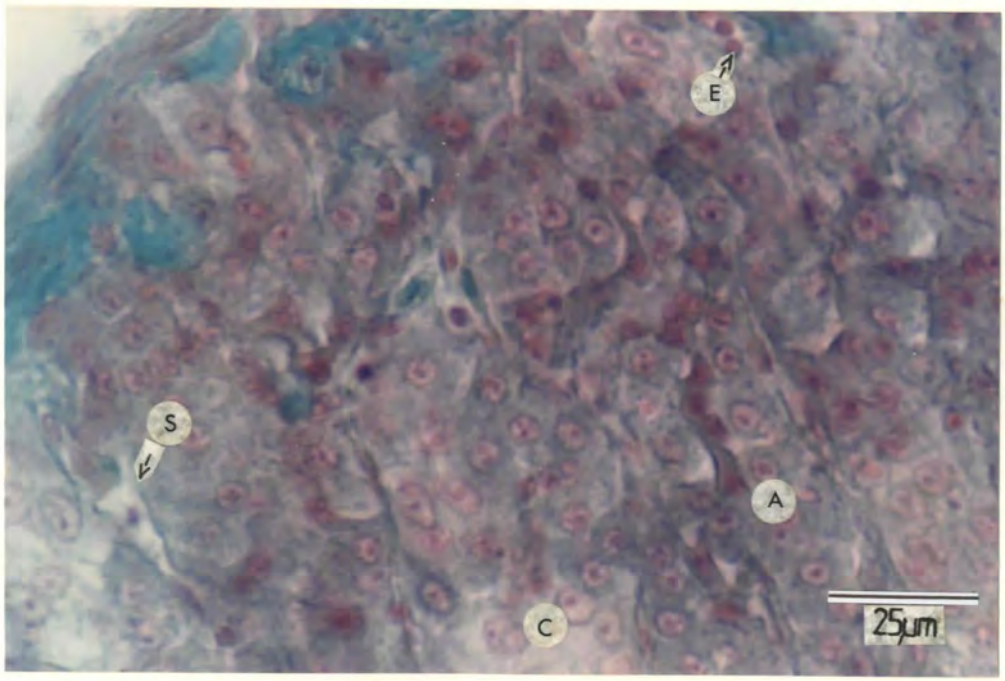

Figure 49 Higher magnification of part of the headkidney shown in Figure 48, to illustrate the cord-like arrangement of the adrenocortical cells (A). C = chromaffin cells, E = erythrocyte, S = blood sinusoid. Masson's trichrome. 5 µm.

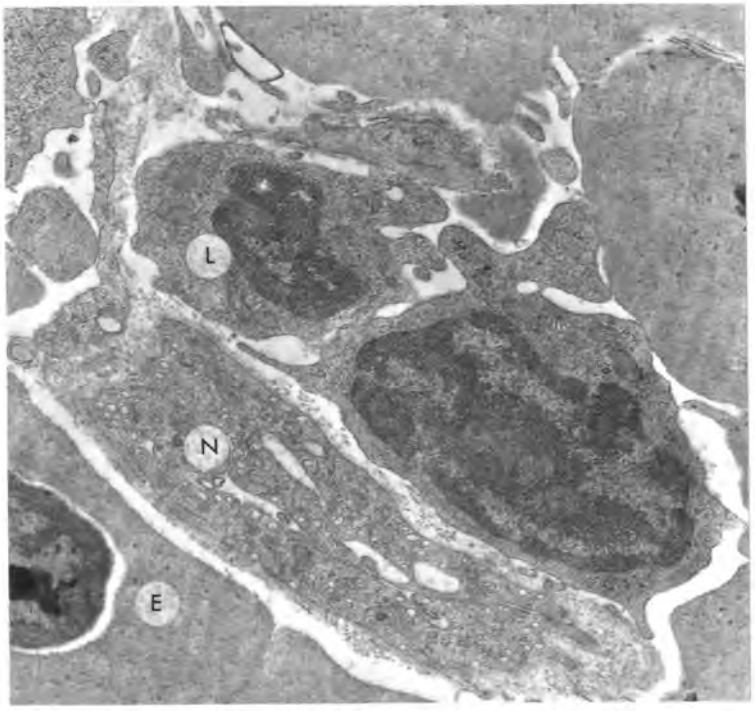

Figure 50 Lymphocytes (L) separated by sinusoids (S) in the headkidney of C. gariepinus. 7500X.

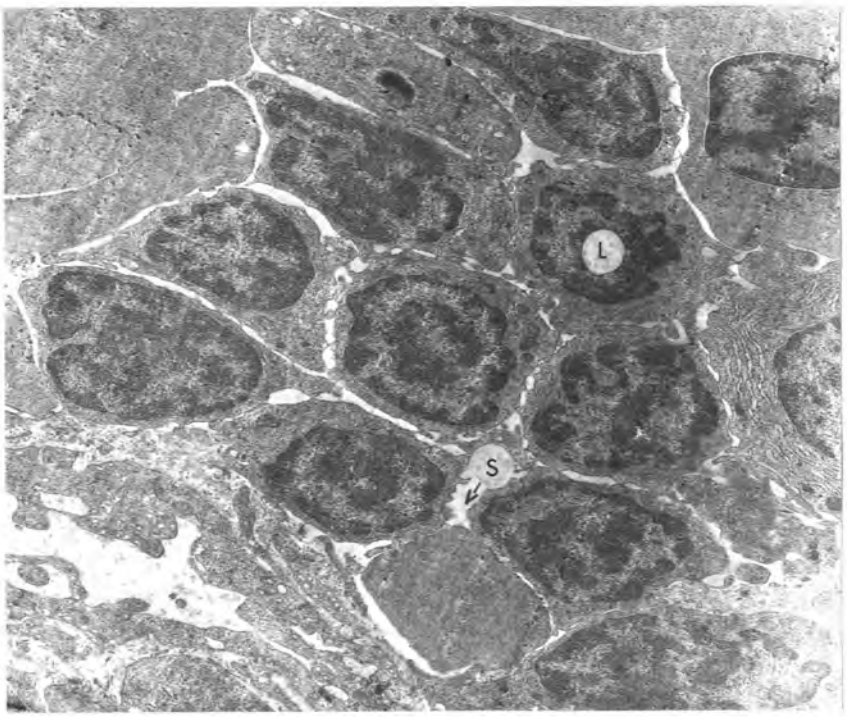

Figure 51 Lymphocytes (L), erythrocytes (E) and nerve fibres (N) in the headkidney of C. gariepinus. 14 000X.

Cells of the chromaffin tissue are larger than adrenocortical cells (average 16 m, n = 40) and colour less intensely pink with Masson's trichrome stain. They are found in small groups, or occasionally singly, interspersed among the cords of adrenocortical cells, but generally fairly close to the veins. They correspond most closely to type V of Nandi, as they are not generally embedded in the walls of the blood vessels (Figure 48). This is in contrast to the findings of Giacomini (1905) or Amiurus catus (Ictalurus nebulosus nebulosus) where the chromaffin cells were embedded in the vein walls, and classified by Nandi (1962) as probable type 1. This reinforces Nandi's statement that great variation in the location of both adrenocortical and chromaffin tissue may occur within a single taxonomic group.

Ultrastructure

Examination of the headkidney tissue of C. gariepinus in the electron microscope helps to clarify the details of the complex structure of this tissue. It is found to consist of large numbers of erythrocytes and lymphocytes arranged in a loose network separated by blood sinusoids and nerve endings (Figures 50 and 51). Interspersed between these lymphocytes and erythrocytes are secretory cells, which are full of small dense granules and large quantities of Golgi apparatus, oval mitochondria with lamellar cristae and rough endoplasmic reticulum (Figure 52). These are suggested to be the chromaffin cells.

Cells which are believed to be adrenocortical have a large amount of smooth endoplasmic reticulum, and round mitochondria with tubular cristae (Figure 53). Smooth endoplasmic reticulum and tubular cristae are both characteristic of steroid secretory cells. In addition it was found that these cells in the headkidney of a fish which had been subjected to 'stress' for several days before killing had an exhausted appearance, with the cristae barely visible within the mitochondria (Figure 54). As the adrenocortical tissue secretes stress hormones this is additional evidence that these are the adrenocortical cells.

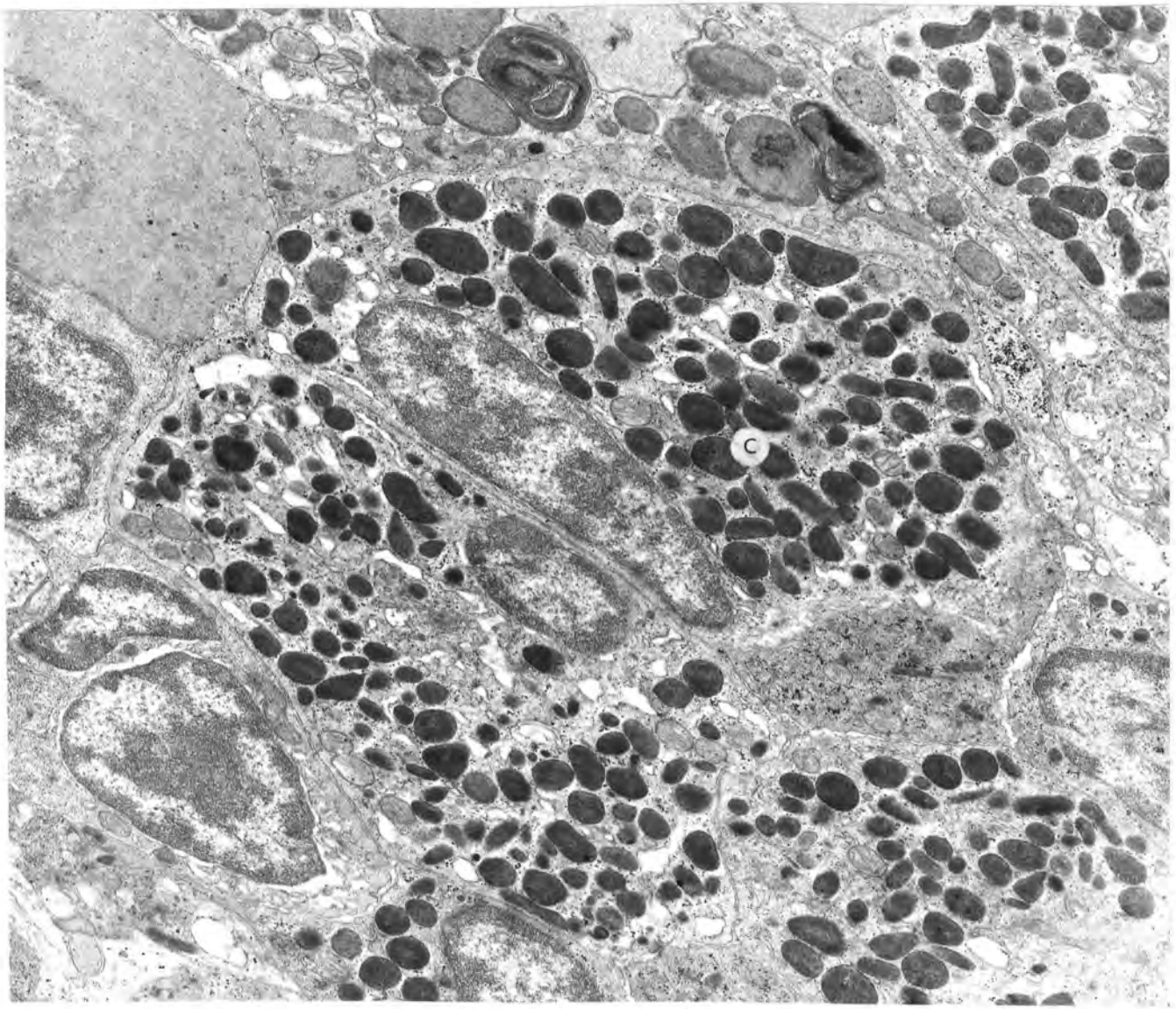

Figure 52 Granular chromaffin cell in the headkidney of C. gariepinus. 14 300X.

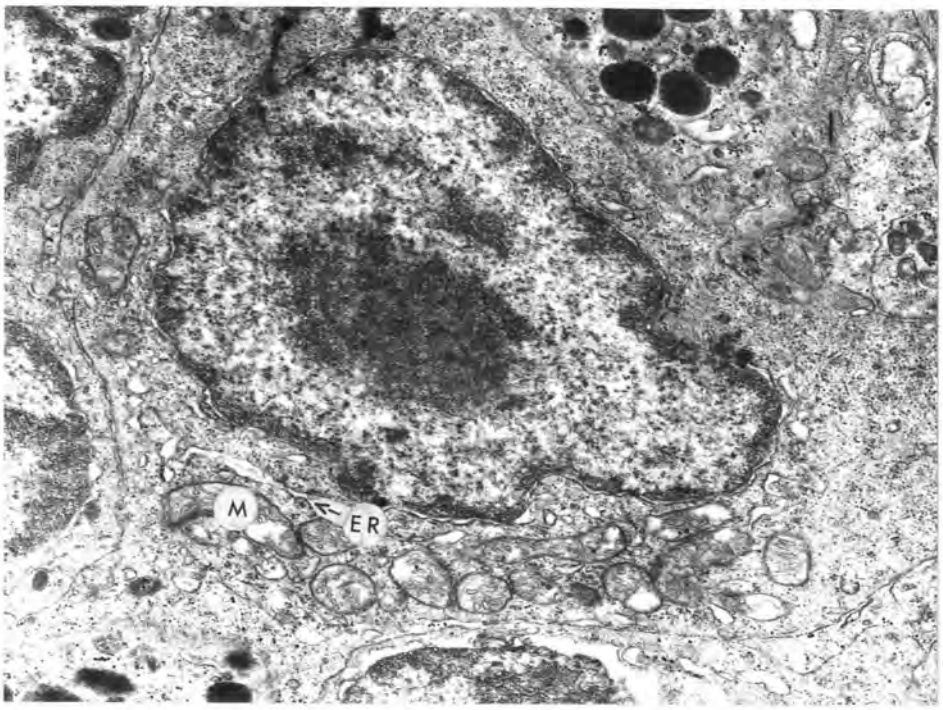

Figure 53 Adrenocortical cell in the headkidney of a normal C. gariepinus. Note large rounded mitochondria (M) with slightly tubular cristae, and smooth endoplasmic reticulum (ER). 20 000X.

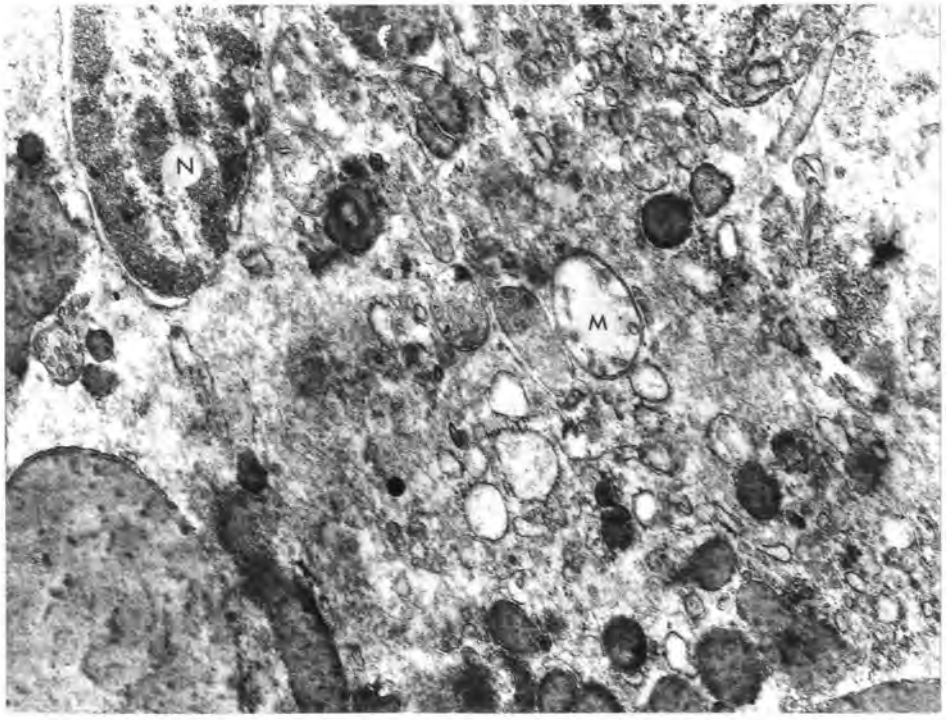

Figure 54 Electron micrograph of adrenocortical cell from a stressed fish. Note large round mitochondria (M), with very few cristae. N = nucleus. 40500X.

THE THYROID OF TELEOSTS

Thyroid tissue is present in all vertebrates, but its gross morphology shows considerable variation (Bentley, 1976). Gudernatsch (1911) first showed that the anatomy of the thyroid tissue of most teleosts was decidedly different from that of other vertebrates. He found that thyroid 'gland becomes rather a misnomer as there is no distinct gland, but rather unconnected follicles found scattered through the loose fatty connective tissue in the pharyngeal region. Gudernatsch's work included descriptions of the thyroid structure of twenty nine species from twenty families (Gudernatsch, 1911). Some teleosts, notably Thunnus thunnus and some salmonids have an encapsulated gland but in most it is diffuse (Olivereau, 1960a).

Generally the distribution of thyroid follicles of teleosts extends below the floor of the pharynx, in the body of the tongue, between the gill arches and posteriorly to behind the origin of the third and fourth branchial arches from the central aorta. The follicles are usually most dense in the region of the ventral aorta and its branches, the afferent branchial arteries. Follicles are often most abundant at the origin of the second branchial arteries, followed in abundance by the origin of the first branchial arteries, and the roots of the third and fourth branches usually have the fewest follicles. A more-or-less dense accumulation is always found around the ventral aorta (Gudernatsch, 1911). Follicles are usually most densely packed near the centre of the region they occupy, and become more scattered towards the periphery. The cephalad and caudad extensions of the thyroid tissue also show considerable variation. Follicles are found anteriorly towards the tip of the tongue and in the hyoid bones, and posteriorly as far as the fourth aortic branch. Dorso-ventral and lateral extensions of thyroid tissue are dependent mainly on the shape and configuration of the pharyngeal floor.

The relationship of the thyroid gland to the ventral aorta and its branches is necessary for the dispersal of hormones. The blood supply to the thyroid is a thyroid artery which arises from the united right and left commisural arteries (derived from the second efferent branchial artery). A thyroid vein which collects blood from the thyroid tissue and also from the musculature below the aorta is also present and empties directly into the

sinus venosus. Capillaries are sometimes seen so close to the follicles that they appear to be embedded between the epithelial cells. A network of capillaries is usually present around each follicle. The follicles are also in close contact with the lymph system (Gudernatsch, 1911).

Many species of fish have what are known as heterotopic thyroid follicles - follicles found in areas outside those mentioned above (Olivereau, 1960a). Heterotopic thyroid follicles have been reported from the eye, spleen, heart and kidney, particularly the headkidney (Baker Cohen, 1959; Olivereau, 1960a).

The thyroid unit of teleosts is thus basically the same as that of higher vertebrates, consisting of a follicle which is a group of epithelial cells surrounding a cavity filled with a glycoproteinaceous secretion. The follicles have a variable appearance in different states of activity. The epithelial cells vary considerably in height, shape and position of the nucleus and the colloid substance found in the central lumen differs in stain affinity, granulation, vascuolization and quantity (Hoar, 1952 Olivereau, 1960; Bentley, 1976).

Small follicles are usually circular in section but larger ones may be elliptical or oval. Follicles closely packed together or close to a bone or muscular structure become flattened by pressure. No communication from follicle to follicle exists (Gudernatsch, 1911).

The epithelial cells of the follicle show shapes from small, flattened squamous type cells, through cuboidal to columnar. Usually all cells in a single follicle are of the same type, but this is not always the case. In the low broad cells the nuclei become oval shaped and lie along the long axis of the cell. In cuboidal to columnar type cells the nuclei is usually round to slightly oval in cross-section and tends to lie at the base of the cell, though this does not always occur. It is thought that the form of the epithelial cells may be in some way connected with age as in very old fishes all epithelial cells tend to be flat (Gudernatsch, 1911).

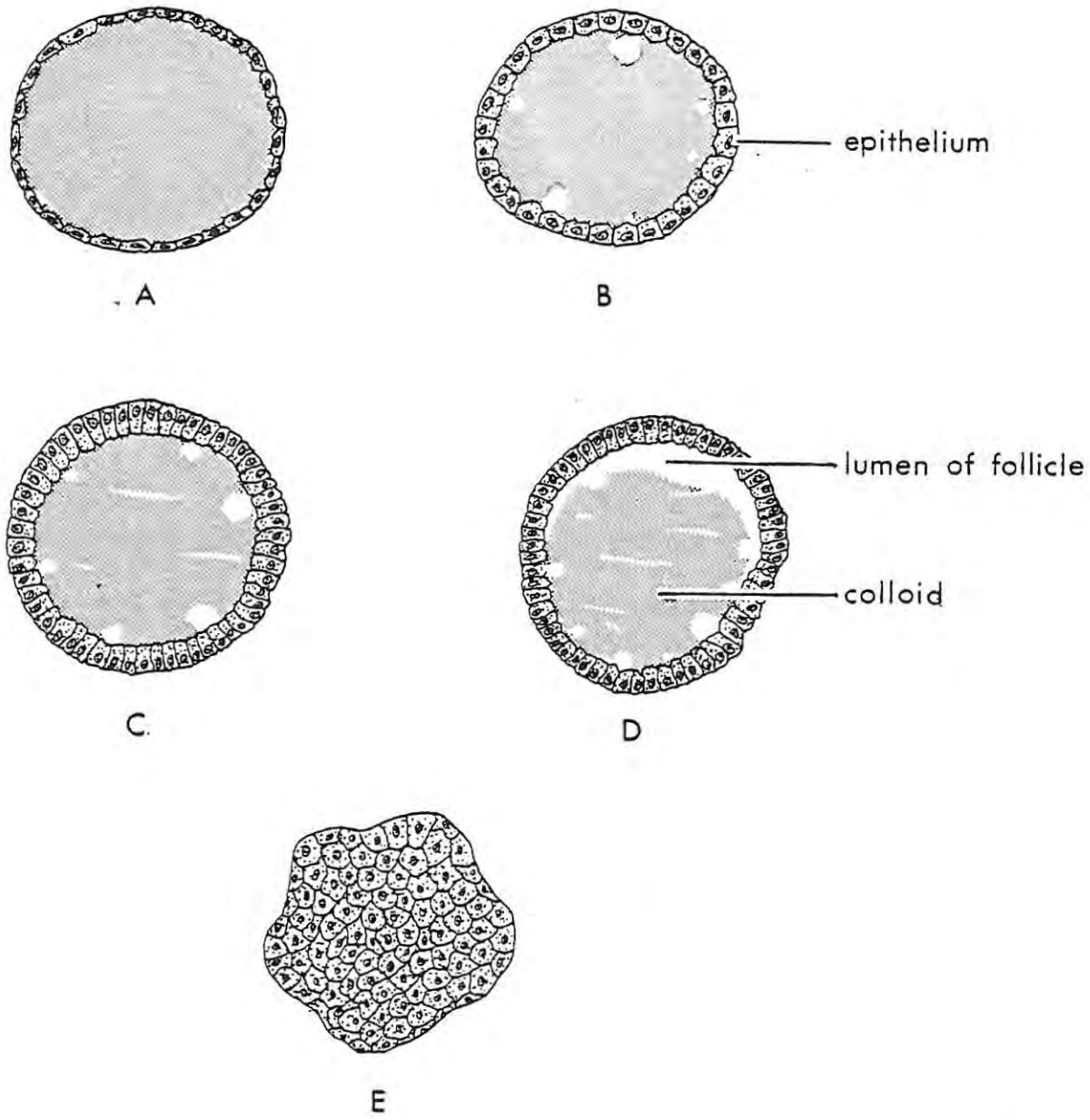

Figure 55 Diagrammatic representation of typical thyroid follicles in different states of activity. A = inactive, B-D = increasing activity, E = hyperplasia. Adapted from Hoar (1957).

The cytoplasm of the epithelial cells is granular. The normal content of the follicles is a colloid secretion which is present in almost all follicles. The structure of the colloid varies, as in some cases it is homogeneous, in others granular. Vacuoles are frequently observed at the periphery of the follicles (Hoar, 1952). These indicate the presence of proteolytic enzymes which hydrolise intrafollicular colloid for the purpose of its passage through the cell wall (Uhlenhuth *et al.*, 1945a and b). The follicles may be full, only partially full, or totally devoid of this colloid.

Low follicular epithelium and an abundance of homogeneous acidophilic colloid are characteristics of a quiescent thyroid (Hoar, 1952; Woodhead, 1975) Stimulation of the gland results in an increase in cell height and vacuolization of the colloid followed by colloid release (Uhlenhuth *et al.*, 1945a and b; Hoar, 1952; Woodhead, 1975). The appearance of typical thyroid follicles in different states of activity is shown in Figure 55.

RESULTS ON THE THYROID OF C. GARIEPINUS AND COMPARISON WITH OTHER SILUROIDS

Gudernatsch's (1911) survey of the thyroids of twenty nine species of teleosts did not include a single catfish.

Research published since 1911 on the anatomy of the siluroid thyroid includes that of Olivereau (1960b) on Uegitglanis zammaronoi and C. buthupogon; Rizkalla (1970b) on C. lazera; Grizzle and Rogers' (1976) brief description of the thyroid of I. punctatus and Srivastava and Sathyanesan (1971) on H. fossilis and C. batrachus. The thyroid gland of C. gariepinus has not previously been studied.

Distribution and blood supply

The thyroid follicles of C. gariepinus were found to be located in the region of the ventral aorta and its branches. They are found in three locations:

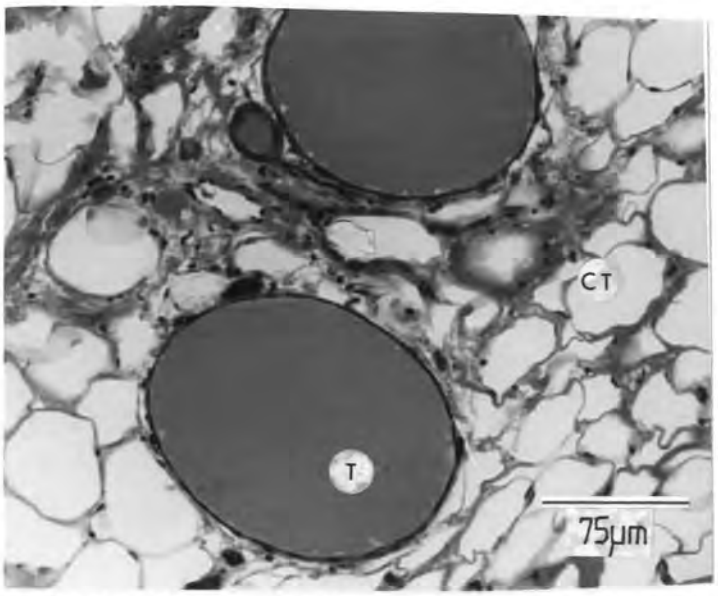

Figure 56 Thyroid follicles (T) in loose fatty connective tissue (CT). Masson's trichrome.

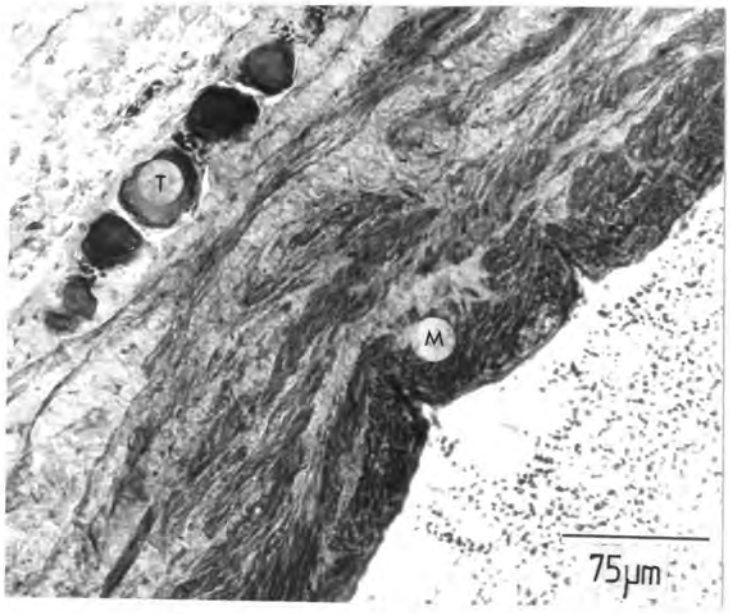

Figure 57 Thyroid follicles (T) in groups near the wall of a blood vessel. Note the linear arrangement. M = muscular wall of blood vessel. Masson's trichrome.

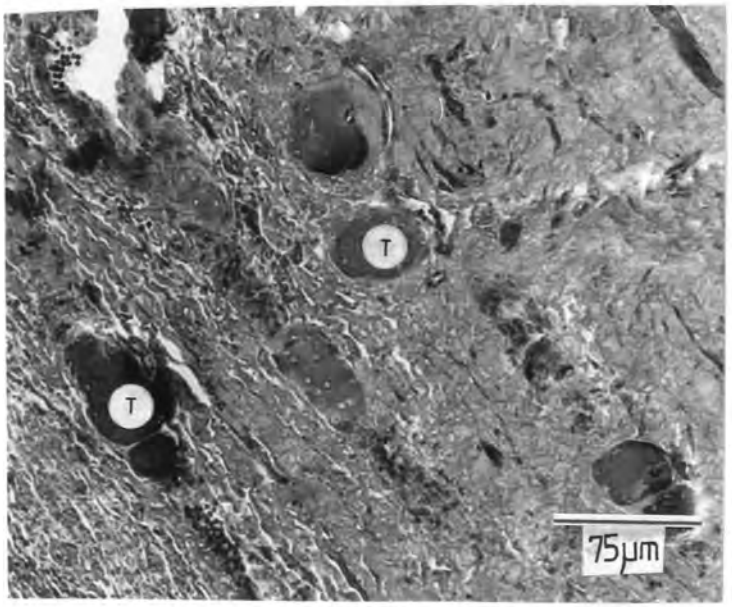

Figure 58 Thyroid follicles (T) embedded in the muscular wall (M) of the ventral aorta. L = lumen of ventral aorta. Masson's trichrome. 5 µm.

1. In the loose, fatty connective tissue surrounding the blood vessels of the anterior pharyngeal region (Figure 56).

2. In concentrated groups near the walls of the veins and arteries. These follicles are usually arranged linearly, parallel to the blood vessels (Figure 57).

3. Embedded in the walls of the ventral aorta and its branches, the afferent branchial arteries (Figure 58).

The relationship between the location of the thyroid follicles and the blood vessels is interesting. In C. buthupogon and U. zammaranoi (a cave-dwelling catfish) the follicles are closely associated with the venous rather than the arterial system of the pharyngeal region (Olivereau, 1960b). Olivereau mentions that this is probably more often the case than has been recorded, due to the thin walls, and small diameter of the veins, making them inconspicuous without careful dissection. According to Rizkalla (1970b) the thyroid follicles in C. lazera are concentrated in the region of the ventral aorta and its branches. He implies (although he does not actually state) that the follicles are found in association with the arterial system. Rizkalla found the highest density of follicles in the connective tissue, and only occasional follicles in the walls of the blood vessels. Similarly Grizzle and Rogers (1976) found in I. punctatus that thyroid follicles are found along the ventral aorta and afferent branchial arteries.

In C. gariepinus some follicles are closely associated with veins, although these follicles are less numerous than those found in association with arteries. These results indicate that although the thyroid follicles are found close to, and even embedded in, the walls of the blood vessels, they are not exclusively associated with either the ventral aorta and its branches on the jugular vein and its tributaries. According to Gudernatsch (1911) the thyroid follicles of fish do not rely on either of the abovementioned vessels for their blood supply due to the presence of a specific thyroid artery and thyroid vein. These blood vessels were not located in C. gariepinus, and it is difficult to visualize how a single artery and vein could supply the widely dispersed follicles. The blood system of the lower pharyngeal region of C. gariepinus is illustrated in

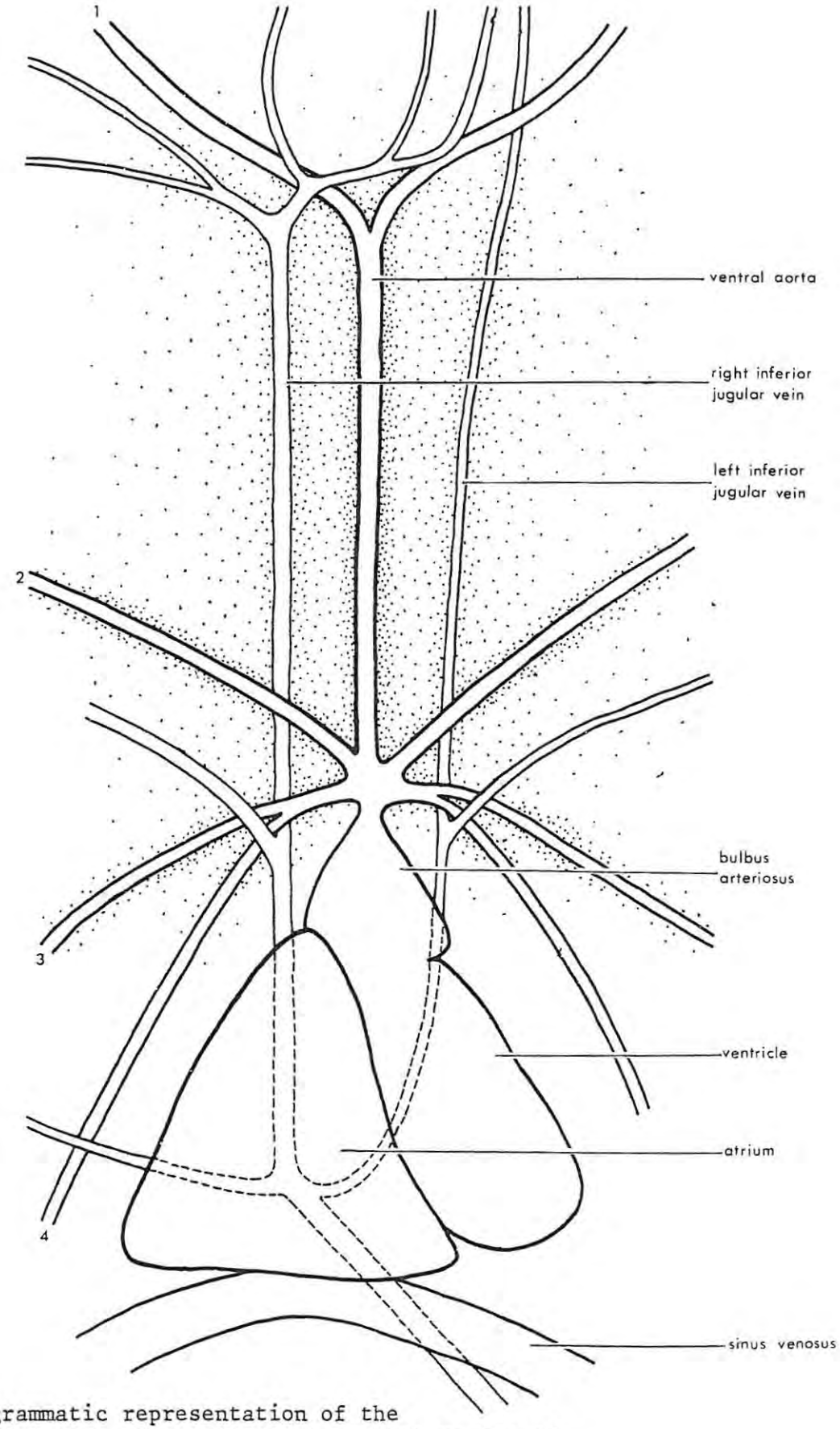

Figure 59 Diagrammatic representation of the blood system of the lower pharyngeal region of C. gariepinus. Light stipple represents areas where thyroid follicles may occur, dark stipple represents areas where thyroid follicles are common.

Figure 59. Electron micrographs reveal how capillaries are found very close to the epithelial cells (Figure 60).

The distribution of thyroid follicles extends anteriorly as far as a few millimetres beyond the final branching of the ventral aorta into the first afferent branchial arteries, and posteriorly to the junction of the bulbus arteriosus with the ventral aorta. Lateral distribution is limited by the gills and the hyoid muscles. No follicles were found embedded in these tissues. The greatest concentration of follicles was found in the area between the first and second afferent branchial arteries. This is a similar distribution to that described by Rizkalla (1970b) on C. lazera, and Olivereau (1960b), on C. buthupogon, but contrasts with Olivereau's (1960b) findings on the blind clariid U. zammaranoi where the greatest concentration of follicles was found at the level of the junction of the bulbus arteriosus and the ventricle. Rizkalla (1970) found a few follicles in the ventricle of the heart of C. lazera. Follicles were also found in the ventricle in both U. zammaranoi and C. buthupogon (Olivereau, 1960b). No follicles were found in the heart of C. gariepinus.

Heterotopic thyroid follicles were not found in C. gariepinus material, although they may have escaped detection. Neither Rizkalla nor Olivereau mention any heterotopic distribution of follicles in the thyroids of the clariids they studied and Grizzle and Rogers report having found none in I. punctatus. It therefore appears that heterotopic follicles are not a common phenomenon in catfish.

Histological structure

The thyroid follicles of C. gariepinus show a wide range of sizes. Follicles located in the loose connective tissue attain sizes of up to 4 mm, but most are in the range of 100 - 300 µm (n = 40). Those found near the walls of the blood vessels are generally smaller (average 40 - 70 µm, n = 40). Follicles embedded in the muscular walls of the blood vessels are very small, not usually reaching more than 50 µm diameter (n = 40). Due to the greater concentration of blood vessels centrally the large follicles tend to be more peripheral than the smaller follicles. This is in contrast to Rizkalla's (1970b) findings on C. lazera, where the central follicles were found to be larger than the peripheral ones.

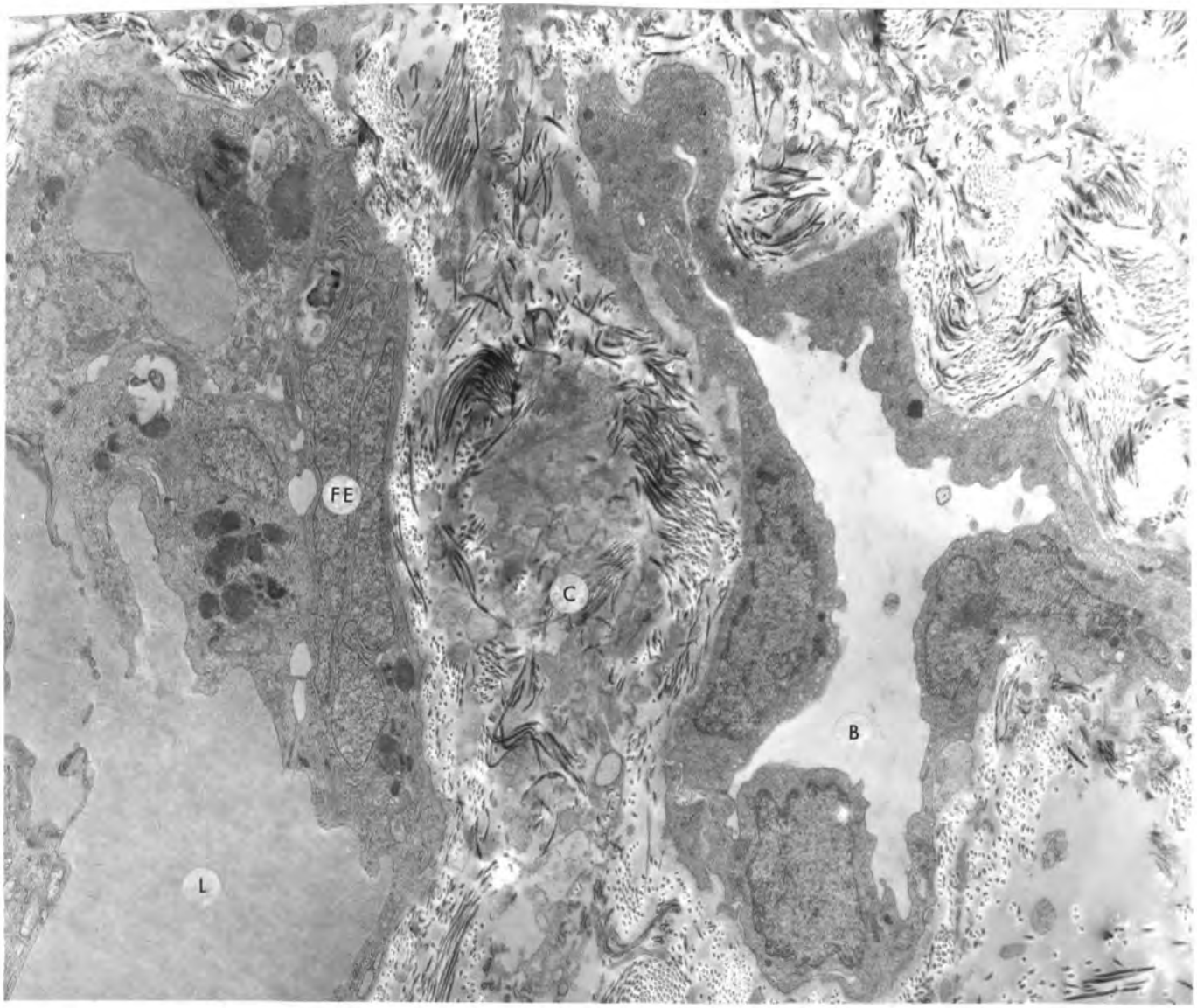

Figure 60 Electron micrograph to show the close relationship between thyroid follicles and blood capillaries (B). C = collagenous connective tissue, FE = follicular epithelium, LF = lumen of follicle, 7000X.

Thyroid follicles of C. gariepinus show some variation in shape, but are generally round to oval. Larger follicles may become distorted. All follicles display a typically vertebrate structure with a group of epithelial cells surrounding a cavity which is usually filled with a colloidal substance. The height of the epithelial cells and the amount and texture of the colloid depends on the activity of the follicle. Electron micrographs show that the shape of the epithelial cells is very variable, and that the nuclei of these cells have irregular outlines. The epithelial cells include large numbers of mitochondria with few cristae, and extensive rough endoplasmic reticulum. Lipid droplets and vacuoles are also common inclusions found in the thyroid epithelial cells (Figure 61). The colloid substance in the centre of the follicle is found to be amorphous.

A considerable degree of variation in activity of follicles in each histological section of the pharyngeal region was often noted (Figure 62). This is significant as studies of thyroid activity often involve measuring some physical parameter of the follicles and the possibility for inaccuracy is great unless a very large number of follicles, from a variety of different locations, are taken into consideration.

No great variation in the state of the follicles corresponding with the maturity of the gonads was observed in C. gariepinus thyroid. Rizkalla (1970b) found no correlation between the activity of the thyroid (based on histological criteria) and the sexual maturity of C. lazera but indicates a seasonal cycle of activity in the thyroid correlated only with the environmental changes. The possibility of a direct relationship between gonad maturity state and thyroid activity in C. gariepinus cannot, however, be ruled out, and warrants further investigation.

Figure 61 Electron micrograph showing part of a thyroid follicle embedded in connective tissue. L = lumen of follicle, E1 and E2 = thyroid epithelial cells, C = collagenous connective tissue, V = vacuole.

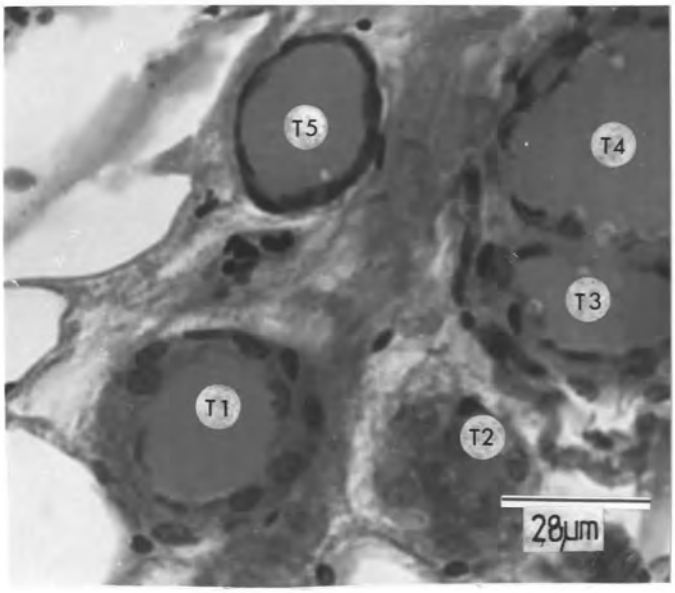

Figure 62 Five closely located thyroid follicles (T1 - T5) in different states of activity. Masson's trichrome. 5 µm.

DISCUSSION

Scott (1979) says of teleost reproductive control 'There is a need for carefully co-ordinated studies, simultaneously from ecological, ethological and physiological viewpoints dealing with a single species deliberately chosen for research into all these aspects'. C. gariepinus was chosen for this, one of the first studies in southern Africa in this field, for various reasons:

1. C. gariepinus is an important food fish in southern Africa, and is readily eaten by many African tribes (Bruton, 1979a and pers. obs.). In some areas interference by man is beginning to threaten the existence of natural populations of C. gariepinus. An example of this can be seen on the Pongolo River in Zululand, where construction of the J.G. Strijdom dam at Jozini is a threat to the existence of the fish in the extensive system of shallow lakes, or pans on the floodplain of the river below the dam. The fish are dependent on normal flooding regimes to initiate spawning, and if this is blocked the fish in the pans will fail to breed; unless reproduction can be artifically controlled, the consequences to the large population of Africans in the area would be severe.

2. C. gariepinus has potential as a suitable fish for aquaculture. Fish farming with clariid fish is economically important in many Eastern countries (e.g. India, Jhingran, 1977). The culture of African Clarias species has recently been initiated, mainly with C. lazera and C. senegalensis in West and Central Africa and Egypt (de Kimpe and Micha, 1974; Micha, 1975; Richter, 1976; Hogendoorn, 1979; Hogendoorn and Vismans, 1980). Preliminary experimental work is now being undertaken on the potential of C. gariepinus as a culture fish in South Africa (van der Waal, 1978; Bruton, pers. comm.; Hecht, pers. comm.) but failure of the fish to reproduce satisfactorily in captivity is a considerable hindrance to these ventures.

3. C. gariepinus is a good experimental fish as it is hardy and easy to keep in ponds in captivity, and is widespread in the wild.

4. C. gariepinus is an annual, flood-dependant spawner, whose reproductive timing is dependant on definite, if unidentified, cues from the environment.

Points 1 and 2 emphasise the great need for studies on the reproductive endocrinology of this species, and points 3 and 4 indicate the suitability of this species for extensive studies as described by Scott (1979) in the passage quoted.

The results presented in this thesis are intended to form a framework onto which an integrated programme of research on C. gariepinus can be built. On their own these results cannot be claimed to make a large contribution to our knowledge of teleost reproductive endrocrinology. They do, however, provide a detailed description of the location, histological structure and ultrastructure of the tissues which are likely to warrant further studies so that subsequent workers can immediately identify tissues they are concerned with, and proceed to more detailed research.

In the course of the description of the secretory tissues of C. gariepinus a comparison has been made between these and their homologues in closely related siluroids. This has underlined the wide degree of variation which can be found in the endocrine tissues of teleosts. The headkidney of I. punctatus, for example, was found to be different both anatomically and histologically from that of C. gariepinus. The distribution of the thyroid follices of C. buthupogon, U. zammaronoi and C. gariepinus were all found to be different, and the structure of the rostral pars distalis acidophils of C. gariepinus was found to be unlike that of C. batrachus.

Less easy to explain are the differences noted between C. gariepinus material and published results on C. lazera. There is strong feeling among taxonomists at present that these two species may in fact be synonymous (Teugels and Bruton, in preparation). It is suggested that these discrepancies in results are due rather to an insufficient number of specimens of C. lazera having been examined to warrant the generalisations that have been made than in actual differences in the tissues . In neither of the publications by Rizkalla (1963, 1970) does he state how many examples were examined, although he does claim that specimens were taken at different seasons of the year. It was found that in some samples of

C. gariepinus material the structure was identical to that described by Rizkalla, for example in the external appearance of the pituitary, but in others the structure was very different. From the results of this investigation it seems likely that the variation in the structure of the pituitaries from C. gariepinus is probably due to seasonal changes in the activity of the tissues of this gland, and perhaps these variations were not picked up in the study on C. lazera. Likewise discrepancies between the histological structure of the rostral pars distalis acidophils of C. lazera and C. gariepinus are probably due to different authors having different opinions of what to describe as follicular-like arrangement of cells, and 'chromophobes', rather than actual differences within the tissues. Similarly the subjectivity involved in the descriptive terms may account for other differences between C. gariepinus tissue and that of other fish, for example the difference in structure of the rostral pars distalis acidophils of C. gariepinus and C. batrachus mentioned earlier. This stresses the fact that histological descriptions are of limited application, and should always be very critically appraised.

An extensive review section has been included in this thesis. This was done in order to enlighten a reader unfamiliar with the subject, and to simplify the task of subsequent workers in this field. It has been emphasised throughout this thesis that results from one species are not necessarily applicable to another. Nevertheless any scientist attempting studies on a new field will need to refer to the literature for background, guidelines and useful trends. The amount of published work in the field of teleost reproductive endocrinology is overwhelming, and as very little attention has been directed to this field in South Africa, none of the libraries hold many of these publications. As a result papers have to be ordered through inter-library loans, often from overseas, a costly and time consuming process. In the course of this work the author was constantly frustrated by this type of drawback and the review section was included so that the most relevant and useful of these many publications might be more easily singled out.

DIRECTIONS FOR FURTHER RESEARCH

An integrated programme of research on the reproductive endocrinology of C. gariepinus has been recommended. Methods which can be included in research of this nature include:

1. Histological methods:
   Statistical assessment of histological changes can be used as an indication of which hormones are active at which times. Methods of histological assessment of hormone activity have been described by many authors (e.g. Barrington and Matty, 1954; Oguri, 1960; Bromage and Sage, 1968; Chan *et al.*, 1975; Ball and Hawkins, 1976; Scott and Currie, 1980; Scott and Rennie, 1980). The accuracy of these methods is always dubious due to the difficulties encountered in selecting a histological criterion which can be accurately and objectively measured. Scott and Rennie (1980) showed that measurement of nuclear diameter (one of the most commonly used criteria) gives a correlation coefficient of only 0,54 when compared with plasma hormone levels as measured using gas-liquid chromatography. Histological methods are useful, however, as they allow a rough assessment of the activity of several different tissues simultaneously, and can therefore be used as a guide to which hormones most warrant investigation using hormone assay methods.

   Refinement of histological type investigations are electron microscope studies and histochemical techniques, which can both be used as tools to indicate the activity or otherwise of the secretory tissues, although quantitative assessments using these methods have not been much exploited by endocrinologists to date.

2. Hormone assay methods:
   Hormone assay methods are more direct and accurate than histological techniques for measurement of hormone activities. Idler (1972) provides a description of some of the methods which can be employed. These include ultraviolet and visible spectrophotometry, gas-liquid chromatography, double-isotope derivative assays, competitive protein binding and radio-immunoassays. Sandor and Idler (1972) discuss the

advantages and shortcomings of these various methods.

3. Extirpation or chemical inhibition of secretory tissues: Endocrinological research often employs methods of extirpation or chemical inhibition of secretory tissues and examination of subsequent changes as compared to controls. Due to the diffuse nature of the secretory tissues of C. gariepinus as has been shown in this thesis, and to the side effects which are often caused by chemical inhibition, the success of these methods would be limited.

4. *In vitro* studies and hormone injection techniques: These methods have been used in studies on some siluroids (Ramaswami and Lakshman, 1959; Goswami and Sundararaj, 1974) but until teleost hormones are generally available for use the validity of results from these experiments will be questionable.

The results of this thesis should be followed by a statistical assessment of gland activity based on histological work. Trends indicated by such studies should ben be verified by biochemical analyses. These results could then be tested, provided teleost hormones were available for use, by *in vitro* studies, extirpation and chemical inhibition experiments. Simultaneous studies should be made on the environmental factors which are the essential cues in stimulating the reproductive cycle, and on the neuroendocrine transducing system. A considerable amount of research is therefore necessary before practical use can be made of these results, but if this is done in its logical order as outlined here, there is hope that in the future it will be possible to control the reproduction of these fish.

Two lines of approach suggest themselves for the actual control of the reproductive cycle. These are:

1. Manipulation at the level of endocrine control, by artificial application (e.g. by injection) of the necessary hormones at the necessary stage in the reproductive cycle.

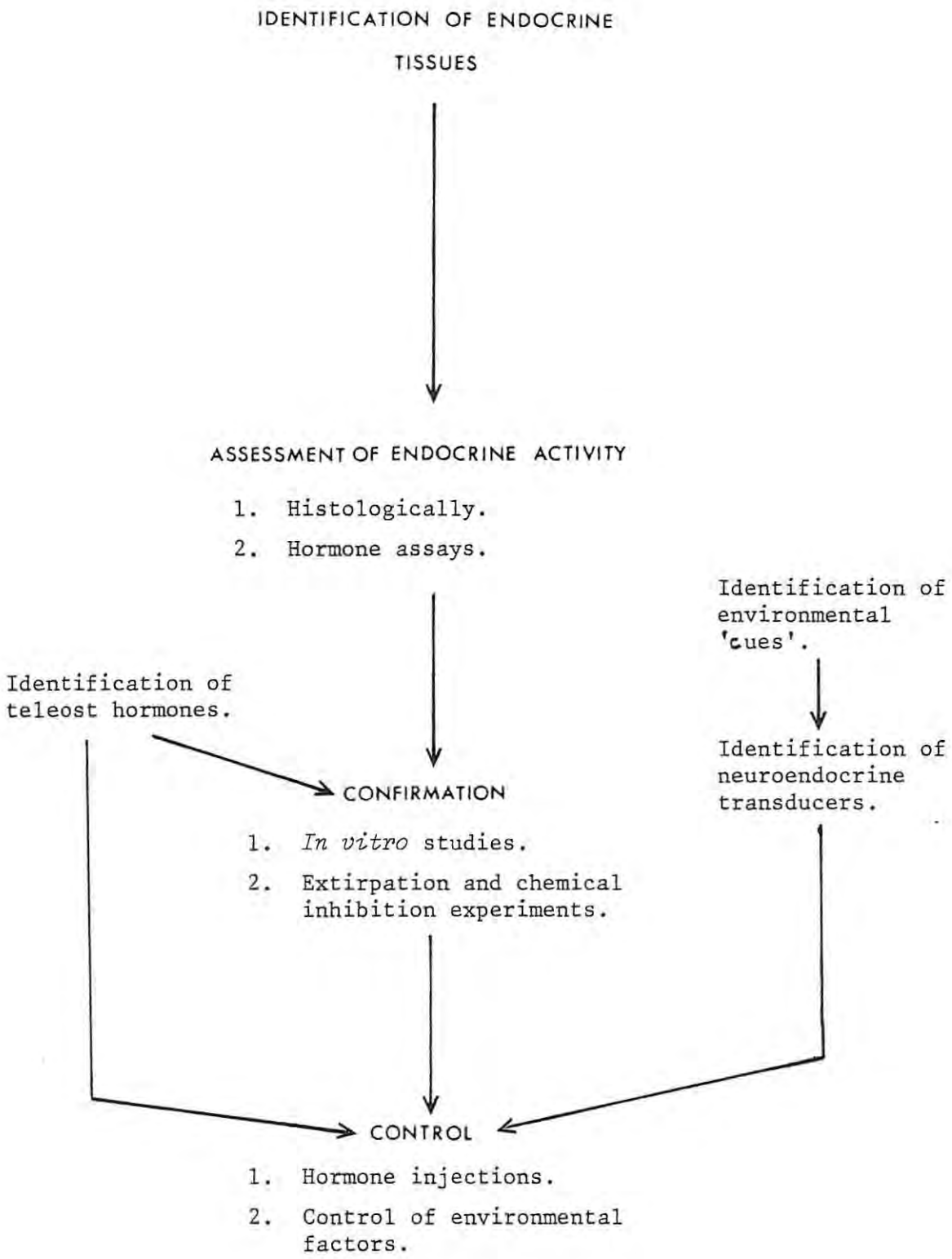

Figure 63 Diagrammatic representation of a suggested programme for integrated research on the control of reproduction in C. gariepinus.

2. Manipulation at the level of environmental control, by artifically applying the necessary 'cues' which are normally supplied by the environment.

Figure 64 represents a suggested programme for integrated research on the control of reproduction of C. gariepinus.

APPENDIX

I. BOUIN'S HOLLANDE SUBLIMATE.

Distilled Water	200 ml
Cupric acetate	5 g
Picric acid	8 g
Formalin	20 ml
Mercuric chloride, saturated aqeous	20 ml

II. ALBUMEN. STOCK SOLUTION

To make a stock solution, the white of an egg is beaten until well mixed, but not stiff. It is then poured into a tall cylinder and left for several hours. The liquid from the bottom is then separated from the scum at the top. The liquid is then mixed with an equal volume of glycerol. A drop or two of formalin added to the solution acts as a preservative.

REFERENCES

Baecker, R. (1928). Uber der Nebennieren der Teleostier. *Z. Mikrosk.-anat. Forsch.* 15: 204-273.

Baker-Cohen, K.F. (1959). Renal and other heterotopic thyroid tissue in fishes. In *Comparative endocrinology*: 283-301. Gorbman, A. (Ed.). New York: John Wiley.

Balfour, F.M. (1882). On the nature of the organ in adult teleosteans and ganoids which is usually regarded as the headkidney or pronephros. *Q. J. microsc. Sci.* 30: 12-22.

Ball, J.N. (1960). Reproduction in female bony fish. *Symp. zool. Soc. Lond.* 1: 105-135.

Ball, J.N. and Baker, B.I. (1969). The pituitary gland. *In Fish physiology.* 1: 1-110. Hoar, W.S. and Randall, D.J. (Eds.). London: Academic Press.

Ball, J.N., Chester Jones, I, Forster, M.E., Hargreaves, G., Hawkins, E.F. and Milne, K.P. (1971). Measurements of plasma cortisol levels in the eel Anguilla anguilla in relation to osmotic adjustments. *J. Endocr.* 50: 75-96.

Ball, J.N. and Hawkins, E.F. (1976). Adrenocortical (interrenal) responses to hypophysectomy and adenohypophyseal hormones in the teleost Poecilia latipinna. *Gen. comp. Endocr.* 28: 54-70.

Barr, W.A. (1968). Patterns in ovarian activity. In *Perspectives in endocrinology*: 164-238. Barrington, E.J.W. and Jorgensen, C.B. (Eds.). London: Academic Press.

Barrington, E.J.W. and Matty, A.J. (1952). Influence of thiourea on reproduction in the minnow. *Nature Lond.* 170: 105-106.

Barrington, E.J.W. and Matty, A.J. (1954). Seasonal variation in the thyroid gland of the minnow, Phoxinus phoxinus, with some observations on the effect of temperature. *Proc. zool. Soc. Lond.* 124: 89-95.

Barrington, E.J.W. and Matty, A.J. (1955). The identification of thyrotropin secreting cells in the pituitary gland of the minnow (Phoxinus phoxinus). *Q.J. microsc. Sc.* 96: 193-201.

Bear, I.J. and Thomas, R.G. (1964). Nature of argillaceous odour. *Nature, Lond.* 201: 993-995.

Bell-Cross , G. (1976). *The fishes of Rhodesia.* Salisbury: Trustees of the National Museum and Monuments of Rhodesia.

Belsare, D.K. (1965). Vascular supply of the pituitary of Channa punctatus. *Nature, Lond.* 206: 211.

Bentley, P.J. (1976). *Comparative vertebrate endocrinology.* Cambridge: University Press.

Bowmaker, A.P. (1973). Potamodromesis in the Mwenda River, Lake Kariba. *Geophysical monograph series.* 17: 159-164.

Bretschneider, L.H. and de Wit, D. 1947). *Sexual endocrinology of the non-mammalian vertebrates.* Amsterdam: Elsevier Publications Co.

Bromage, N.R. and Sage, M. (1968). The activity of the thyroid gland of Poecilia during the gestation cycle. *J. Endocr.* 41: 303-311.

Bruton, M.N. (1978). The habitats and habitat preferences of Clarias gariepinus (Pisces: Clariidae) in a clear coastal lake (Lake Sibaya, South Africa). *J. Limnol. Soc. sthn.Afr.* 4(2): 81-88.

Bruton, M.N. (1979a). The breeding biology and early development of Clarias gariepinus (Pisces: Clariidae) in Lake Sibaya, South Africa, with a review of breeding in species of the subgenus Clarias (Clarias). *Trans. zool. Soc. Lond.* 35: 1-45.

Bruton, M.N. (1979a). The food and feeding behaviour of Clarias gariepinus (Pisces Clariidae) in Lake Sibaya, South Africa, with emphasis on its role as a predator of cichlids. *Trans. zool. Soc. Lond.* 35: 47-114.

Cambray, J.A. and Jubb, R.A. (1977). Dispersal of fishes via the Orange-Fish Tunnel, South Africa. *J. Limnol. Soc. sthn. Afr.* 3(1): 33-35.

Chan, S.T.H., O, W.S. and Hui, W.B. (1975). The interrenal gland and ACTH and prolactin cells in the adenohypophysis of Monopterus, and their roles in osmoregulation. *Gen. comp. Endocr.* 27: 95-110.

Chardon, M. (1968). Anatomie comparée de l'appareil de Weber et des structures connexes chez les Siluriformes. *Annls. Mus. r. Afr. cent.* Ser. 8, 169: 1-281.

Chavin, W., Olivereau, M. and Bowman, B.N. (1962). Abstract in *Amer. Zool.* 2: 512.

Chester Jones, I. (1957). *The adrenal cortex*. Cambridge: University Press.

Chester Jones, I., Chan, D.K.O., Henderson, I.W. and Ball, J.N. (1963). The adrenocortical steroids, adrenocorticotropin and the corpuscles of Stannius. In *Fish Physiology*. 2: 321-376. Hoar, W.S. and Randall, D.J. (Eds.). London: Academic Press.

Christensen, A.K. (1965). The fine structure of testicular interstitial cells in guinea pigs. *J. Cell. Biol.* 26: 911-935.

Clay, D. (1977). Biology of the tropical catfish (Family: Clariidae) with special emphasis on its suitability for culture (including a bibliography of the clariidae and related topics). *Fisheries and Marine Service Manuscript Report* no. 1458, 68 pages.

Clay, D. (1979). Sexual maturity and fecundity of the African catfish Clarias gariepinus with an observation on the spawning behaviour of the Nile catfish Clarias lazera. *Zool. J. Linn. Soc.* 65: 351-365.

Courrier, R. (1921). Sur l'existence d'une glande interstitielle dans le testicule des poissons. *C. r. Séanc. Soc. Biol.* 85: 939-941.

Craig-Bennett, A. (1931). The reproductive cycle of the three-spined stickleback, Gasterosteus aculeatus. *Phil. Trans. roy. Soc. Lond.* (B) 219: 197-279.

Crass, R.S. (1964). *Freshwater fishes of Natal*. Pietermaritzburg: Shuter and Shooter.

De Bont, A.F. and Maes, H. (1965). Relation entre le frai du Labeo altivelis Peters et la conductivité des eaux. *Hydrobiologia* 8 (3-4): 283-292.

Dehuisser, E. (1975). *Bijdrage tot een morfologisch-systematische studie van Clarias vissen uit West en Centraal Afrika*. M.Sc. Thesis, University of Ghent, Belgium.

DeKimpe, P. and Micha, J-C. (1974). First guidelines to the culture of Clarias lazera in Central Africa. *Aquaculture*. 4: 227-248.

De Vlaming, V.C. (1974). Environmental and endocrine control of teleost reproduction. In *Control of sex in fishes*: 13-83. Schreck, C.B. (Ed.). Virginia: Sea Grant Publications.

Dixit, V.P. (1970). Histophysiological studies of the interrenal gland in Clarias batrachus (Linn.). *Acta. anat.* 77: 310-318.

Dodd, J.M. (1960). Gonadal and gonadotropic hormones in lower vertebrates. In *Marshall's physiology of reproduction*. 1(2): 417-582. Parkes, A.S. (Ed.). London: Longmans.

Dodd, J.M. (1975). The structure of the vertebrate ovary. A. The ovary of non-mammalian vertebrates. In *The Ovary*. 1: 219-263. Zuckerman, S. and Weir, B. (Eds.). New York: Academic Press.

Dutta, S.K. (1923). On a peculiar distribution of the liver and kidney in the fish genera Clarias and Saccobranchus. *J. Proc. Asiat. Soc. Bengal.* 19: 110-120.

Fraser, E.A. (1950). The development of the vertebrate excretory system. *Biol. Rev.* 25: 159-187.

Fuller, J.D., Scott, D.B.C. and Fraser, R. (1976). The reproductive cycle of Coregonus lavaretus (L.) in Loch Lomond, Scotland, in relation to the seasonal changes in plasma cortisol concentration. *J. Fish. Biol.* 9: 105-117.

Gabe, M. (1953). Sur quelques applications de la colorations par la fuchsine-paraldehyde. *Bull. microsc. appl.* 3: 153-162.

Gaigher, I. (1977). Reproduction of the catfish (Clarias gariepinus) in the Hardap dam, South West Africa. *Madoqua* 10(1): 55-59.

Ghosh, A. and Kar, A.B. (1952). Seasonal changes in the gonads of the common Indian catfish, Heteropneustes fossilis (Bloch.). *Proc. zool. Soc. Beng.* 5: 29-50.

Giacomini, E. (1902). Sulla esistenza della sostanza midollare nelle capsule surrenali dei Teleostei. *Monitore zool. ital.* 13: 183-189.

Giacomini, E. (1905). Contributo alla conoscenza del sistema delle capsule surrenali dei Teleostei sulla sostanzo middelare (organi soprarenali o tessuto cromaffine) di Amiurus catus L. *Rc. Sess. Accad. Sci. Int. Bologna* 9: 183-189.

Giacomini, E. (1908) Il sistema interrenale e il sistema cromaffine (sistema ferocromo) nelle anguille adulte, nelle cieche, e nei leptocefali. *Mems. R. Acad. Sci. Inst. Bologna, Cl. Sci. Fis.*, ser 6, 5: 407-441.

Gorbman, A. (1959). Problems in the comparative morphology and physiology of the vertebrate thyroid gland. In *Comparative endocrinology*: 266-282. Gorbman, A. (Ed.). New York: John Wiley.

Goswami, S.V. and Sundararaj, B.I. (1971a). *In vitro* maturation of oocytes of the catfish Heteropneutes fossilis (Bloch.). Effects of mammalian hypophysial hormones, catfish pituitary homogenate, steroid precursors and metabolites, and gonadal and adrenocortical steroids. *J. exp. Zool.* 178: 456-478.

Goswami, S.V. and Sundararaj, B.I. (1971b). Temporal effects of ovine luetinising hormone and deoxycorticosterone acetate on maturation and ovulation of oocytes in the catfish, Heteropneustes fossilis (Bloch.), an *in vivo* and *in vitro* study. *J. exp. Zool.* 178: 457-466.

Goswami, S.V. and Sundararaj, B.I. (1974). Effects of C-18, C-19 and C-21 steroids on *in vitro* maturation of oocytes of the catfish Heteropneustes fossilis (Bloch.). *Gen. comp. Endocr.* 23: 282-285.

Green, J.D. (1951). The comparative anatomy of the hypophysis with special reference to its blood supply and innervation. *Am. J. Anat.* 88: 225-311.

Grizzle, J.M. and Rogers, W.A. (1976). Anatomy and histology of the channel catfish. *U.S. Dept. of Commerce, NOAA, Nat. mar. Fish. Ser. comm. Fish. Res. & Dev.* project 2 - 187 - R.

Groenewald, A.A., van J. (1957). The results of a survey of the fish population of the Vaal River during the period April-December 1956. *Rep. Dep. nat. Conserv. Transvaal.* (1957).

Groenewald, A.A. van J. (1964). Observations on the food habits of Clarias gariepinus (Burchell), the South African freshwater barbel. (Pisces: Clariidae) in the Transvaal. *Hydrobiologia* 23: 287-291.

Gudernatsch, J.F. (1911). The thyroid gland of the teleosts. *J. Morph.* 21: 709-782.

Hane, S.; Robertson, O.H.; Wexler, B.C., and Krupp M.A. (1966). Adrenocortical response to stress and ACTH in Pacific salmon (Oncorhynchus tshawytscha and steelhead trout (Salmo gairdnerii) at successive stages in the sexual cycle. *Endocrinology* 78: 791-800.

Heidenhain, M. (1915). Uber der Mallorysche bindege-websfarbung mit karmin und azokarmin als vorfarben. *Z. wiss. Mikr.* 32: 361-372.

Herlant, M. (1960). Etude critique de deux techniques nouvelles destinées à mettre en evidence le differentes catégories cellulaires présentes dans la glande pituitaire. *Bull. microsc. appl*: 10, 37-44.

Hoar, W.S. (1952). Thyroid function in some anadromous and landlocked teleosts. *Trans. roy. Soc. Can.* 46: 39-53.

Hoar, W.S. (1966). Hormonal activities in the pars distalis of cyclostomes, fish and amphibia. In *The pituitary gland.* 1: 242-294. Harris, G.W. and Donovan, B.T. (Eds.). London: Butterworth.

Hoar, W.S. (1969). Reproduction. In *Fish physiology.* 3: 1-72. Hoar, W.S. and Randall, D.J. (Eds.). London: Academic Press.

Hoar, W.S. and Nagahama, Y. (1978). The cellular sources of steroids in teleost gonads. *Annls. Biol. anim. Biochim. Biophys.* 18 (4): 893-898.

Hogendoorn, H. (1979). Controlled propagation of the African catfish, Clarias lazera (C. and V.) Reproductive biology and field experiments. *Aquaculture.* 17: 323-333.

Hogendoorn, H. and Vismans, M.M. (1980). Controlled propagation of the African catfish, Clarias lazera (C. and V.). 11. Artificial reproduction. *Aquaculture.* 21: 39-53.

Holl, A.E. (1966). Some notes on the breeding of barbel, Clarias gariepinus. (Burchell) in Rhodesia. *Limnol. Soc. sthn. Afr. Newsletter.* 7: 38-41.

Holl, A.E. (1968). Notes on the spawning behaviour of the barbel, Clarias gariepinus (Burchell) in Rhodesia. *Zoologica Afr.* 3: 185-188.

Humason, G.L. (1972). *Animal tissue techniques.* New York: W.H. Freeman & Co. 3rd edition.

Idler, D.R. (1972). *Steroids in non-mammalian vertebrates.* New York: Academic Press.

Jhingran, V.G. (1977). *Fish and Fisheries of India.* Delhi: Hindustan Publishing Corporation.

Jubb, R.A. (1967). *Freshwater Fishes of South Africa.* Cape Town: Balkema.

Jubb, R.A. (1978). Sharp-tooth catfish, Clarias gariepinus in the Great Fish River, Eastern Cape. *Piscator,* 32 (102): 24-27.

Katz, Y. and Eckstein, B. (1974). Changes in steroid concentration in blood of female Tilapia aurea (Teleostei: Cichlidae) during initiation of spawning. *Endocrinology.* 95: 936-937.

Kaul, S. and Vollrath, L. (1974). The goldfish pituitary. 1. Cytology. *Cell. Tiss. Res.* 154: 211-280.

Kerr, T. (1942). A comparative study of some teleost pituitaries. *Proc. zool. Soc. Lond.* (A) 112: 37-56.

Knowles, F. and Vollrath, L. (1966a). Neurosecretory innervation of the pituitary of the eels Anguilla and Conger. 1. The ultrastructure of the neuro-intermediate lobe under normal and experimental conditions. *Phil. Trans. roy. Soc. Lond.* (B) 250: 311-327.

Knowles, F. and Vollrath, L. (1966b). Neurosecretory innervation of the pituitary of the eels Anguilla and Conger. 11. The structure and innervation of the pars distalis at different stages of the life-cycle. *Phil. Trans. roy. Soc. Lond.* (B) 250: 329-342.

Knowles, F. and Vollrath, L. (1966c). Cell types in the pituitary of the eel Anguilla anguilla L. at different stages in the life-cycle. *Z. Zellforsch. mikrosk. Anat.* 69: 474-479.

Kolmer, W. and Scheminzky, F. (1922). Finden sich zwischenzellen nur bei den höheren wirbeltieren? *Pflügers Arch. ges. Physiol.* 194: 352.

Lagler, K.F., Bardach. J.E., Miller, R.R. and Pasino, D.R.M. (1977). *Ichthyology*. New York: John Wiley & Sons.

Lake, J.S. (1967). Rearing experiments with five species of Australian freshwater fishes. 1. Inducement to spawning. *Aust. J. mar. Freshwat. Res.* 18: 137-153.

Leatherland, J.F. (1972). Histophysiology and innervation of the pituitary gland of the goldfish, Carassius auratus L. A light and electron microscope investigation. *Can. J. Zool.* 50: 835-844.

Lehri, G.K. (1966). The pituitary gland of the catfish, Clarias batrachus. *Copeia* (1966) 4: 810-818.

Lehri, G.K. (1967). The annual cycle in the testis of the catfish, Clarias batrachus L. *Acta anat.* 67: 135-154.

Liley, N.R. (1969). Hormones and reproductive behaviour in fishes. In *Fish physiology*. 3: 73-116. Hoar, W.S. and Randall, D.J. (Eds.). New York Academic Press.

Lofts, B. (1972). The Sertoli cell. *Gen. comp. Endocr.* (Suppl.) 3: 636-648.

Lofts, B. and Bern, H.A. (1972). The functional morphology of steroidogenic tissue. In *Steroids in non-mammalian vertebrates*. Idler, D.R. (Ed.). New York: Academic Press.

Lofts, B., Pickford, G.E. and Atz, J.W. (1966). Effects of methyl testosterone on the testes of a hyposectomised cyprinodont fish, Fundulus heteroclitus. *Gen. comp. Endocr.* 6: 74-88.

Love, R.M. (1970). *The chemical biology of fishes*. New York. Academic Press.

MacConnaill, M.A. (1947). Staining of the central nervous system with lead haematoxylin. *J. Anat.* 8: 371-372.

Marshall, A.J. and Lofts, B. (1956). The Leydig-cell homologues in certain teleost fishes. *Nature, Lond.* 177: 704-705.

Matty, A.J. (1957). Occurrence of periodic acid-Schiff positive material in the pituitary of the parrot fish, Pseudascarus guacamaia. *Nature, Lond.* 180: 1055.

Matty, A.J. (1966). Endocrine glands in lower vertebrates. *Int. Rev. gen. exp. Zool.* 2: 43-136.

Micha, J-C. (1975). Sythèse des essaies de réproduction, d'alevinage et de production chex un silure Africain: Clarias lazera Val. *Bull. fr. Piscic.* 256: 78-87.

Millonig, G. (1961). Advantages of a phosphate buffer for osmium tetroxide solutions in fixation. *J. Appl. Phys.* 32: 1657.

Mulder, P.F.S. (1971). *'n Ekologiese studie van hengelvisfauna in die Vaalriviersisteem met spesiale verwysing na Barbus kimberleyensis Gilchrist & Thompson*. Proefskrif, Randse Afrikaanse Universiteit. 118 pages.

Murray, J.L. (1975). *Selection of zooplankton by Clarias gariepinus*. M.Sc. Thesis, University of Rhodesia.

Nagahama, Y., Chan, K. and Hoar, W.S. (1976). Histochemistry and ultrastructure of pre- and post-ovulatory follicles in the ovary of a goldfish, Carassius auratus. *Can. J. Zool.* 54: 1128-1139.

Nair, P.V. (1965). Studies on the male reproductive system of some siluroid fishes. Parts 1 and 2. *Indian zootom. Mem.* 9: 1-3.

Nandi, J. (1962). The structure of the interrenal gland of teleost fish. *Univ. Calif. Publs. Zool.* 65: 129-211.

Nawar, G. (1959). Observations on the seminal vesicles of the Nile catfish, Clarias lazera. *Ann. Mag. nat. Hist.* 13 ser. 2 (19): 444-448.

Nicholls, 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. mikrosk. Anat.* 128: 317-335.

Ogawa, K. (1961). Comparative study on the external shape of the teleostean kidney with relation to phylogeny. *Scient. Rep. Tokyo Kyoiku Diagaku B.* 10: 61-88.

Oguri, M. (1960). Studies on the adrenal glands of teleosts. VI. On the interrenal tissue of the chum salmon, Oncorhynchus keta (Walbaum) migrating up river to spawn. *Bull. Jap. Soc. scient. Fish.* 26: 981-984.

Olivereau, M. (1954). Hypophyse et glande thyroide chez les poissons. Etude histophysiologique de quelques correlations endocriniennes en particulier che Salmo salar L. *Annls. Inst. oceanogr. Paris* 29: 95-296.

Olivereau, M. (1960a). Quelques aspects anatomiques de la glande thyroide des poissons. *Annls. Soc. r. zool. Belg.* 90(2): 83-98.

Olivereau, M. (1960b) Etude anatomique et histologique de la glande thyroide d'Uegitglanis zammaranoi Gianferri, poisson aveugle et cavernicole, et comparason avec un Clariidae voisin, Clarias buthupogon. A. Dum. *Annls. Soc. r. zool. Belg.* 90: 99-106.

Olivereau, M. (1967). Observations sur l'hypophyse de l'anguille femelle en particulier lors de la maturation sexuelle. *Z. Zellforsch. mikrosk. Anat.* 80: 286-306.

Olivereau, M. (1970). Coloration de l'hypophyse avec l'hématoxylin au plomb (H.Pb.): Donnees nouvelles chez les téléostéens et comparaison avec les resultats obtenus chez d'autre vertébrés. *Acta zool.* 51: 229-249.

Olivereau, M. (1975). Histophysiologie de l'axe hypophyso-corticosurrenalien chez le saumon de l'Atlantique (cycle en eau douce). *Gen. comp. Endocr.* 27: 9-27.

Olivereau, M. (1976). Les cellules gonadotropes hypophysaire du saumon de l'Atlantique: Unicité ou dualité? *Gen. comp. Endocr.* 28: 82-95.

Olivereau, M. (1977). Données récentes sur la controle endocrinien de la réproduction chez les téléostéens. *Investigacion Pesq.* 41(1): 69-94.

Olivereau, M. and Olivereau, J. (1968). Effets de l'interrénalectomie sur la structure histologique de l'hypophyse et de quelques tissues de l'anguille. *Z. Zellforsch. mickrosk. Anat.* 84: 44-58.

Ozon, R. (1972a). Androgens in fishes, amphibians, reptiles and birds. In *Steroids in non-mammalian vertebrates*: 328-389. Idler, D.R. (Ed,). New York: Academic Press.

Ozon, R, (1972b). Estrogens in fishes, amphibians, reptiles and birds. In *Steroids in non-mammalian vertebrates*. 390-413. Idler, D.R. (Ed.). New York: Academic Press.

Pearse, A.G.E. (1953). *Histochemistry - Theoretical and Applied.* London: Churchill.

Perks, A.M. (1969). The neurohypophysis. In *Fish physiology*. 2: 111-205. Hoar, W.S. and Randall, D.J. (Eds.). London: Academic Press.

Peter, R.E. (1973). Neuroendocrinology of Teleosts. *Amer. Zool.* 13: 743-755.

Pickford, G.E. and Atz, J.W. (1957). *The physiology of the pituitary gland of fishes.* New York: Zoological Society.

Pott, R.M. (1969). *The fish life of the Pongola River before the closure of the J.G. Strijdom dam.* M.Sc. Thesis, University of Witwatersrand.

Purves, H.D. (1966). Cytology of the adenohypophysis. In *The pituitary gland.* 1: 147-332. Harris, G.W. and Donovan, B.T. (Eds.). London: Butterworth.

Qasim, S.Z. and Qayum, A. (1961). Spawning frequency and breeding seasons of some freshwater fishes with special reference to those occurring in the plains of northern India. *Indian J. Fish.* 8: 24-43.

Qureshi, T.A. and Sultan, R. (1977). Histophysiological studies on the interrenal gland of a catfish, Clarias batrachus (Linn.). *Z. mikrosk. - anat. Forsch.* 91(3): 433-452.

Ramaswami, L.S. and Lakshman, A.B. (1959). Action of mammalian hormones on the spawning of catfish. *J. scient. ind. Res.* (C) 18(10): 185-191.

Rao, P.D.P., Betole, U.K. and Kondawar, V.V. (1972). Changes in the pituitary-interrenal axis after gonadectomy in the catfish Clarias batrachus (Linn.). *Acta zool. Stockh.* 53(2): 135-145.

Reynolds, E.S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell. Biol.* 17, 208-212.

Richter, C.J.J. (1976). The African catfish, Clarias lazera (C. and V.), a new possibility for fish culture in tropical regions? *Misc. papers, Landbouwhoogschool Wageningen, The Netherlands.* 13: 51-70.

Rizkalla, W. (1963). The pituitary gland of the teleost Clarias lazera (Cuv. et Val.). *Proc. zool. Soc. U.A.R.* 1: 127-146.

Rizkalla, W. (1969). Studies on the adrenal glands and corpuscles of Stannius in the teleost Nile fish, Clarias lazera (C. and V.). *Acta vet. Hung.* 19: 343-350.

Rizkalla, W. (1970a). Studies on the gonads of the teleost Nile fish Clarias lazera, (C. and V.), with special reference to their endocrine tissues. *Acta vet. Hung.* 20(1): 1-12.

Rizkalla, W. (1970b). Studies on the thyroid gland of the Nile fish, Clarias lazera, (C. and V.) *Acta vet. Hung.* 20: 129-138.

Robertson, O.H. (1958). Accelerated development of testis after unilateral gonadectomy with observations on the normal testis of rainbow trout. *Fish. Bull.* 127: 9-30.

Robertson, O.H. and Wexler, B.C. (1962a). Histological studies on the pituitary gland of the rainbow trout, Salmo gairdnerii, accompanying sexual maturation and spawning. *J. Morph.* 110: 157-170.

Robertson, O.H. and Wexler, B.C. (1962b). Histological changes in the pituitary gland of the Pacific salmon (genus Oncorhynchus) accompanying sexual maturation and spawning. *J. Morph.* 110: 171-179.

Romeis, B. (1940). Innersektretorische drüsen II. Hypophyse. In *Handbuch der Mikroskopischen Anatomie des Menschen* 6(3): van Mollendorf, W. (Ed.). Jena: J. Springer.

Sage, M. (1973). The evolution of thyroidal function in fish. *Am. Zool.* 13: 899-905.

Sage, M. and Bern, H.A. (1971). Cytophysiology of the teleost pituitary. *Int. Rev. Cytol.* 31: 339-368.

Sandor, T. and Idler, D.R. (1972). Steroid methodology. In *Steroids in non-mammalian vertebrates.* Idler, D.R. (Ed.). New York: Academic Press.

Schreibman, M.P., Leatherland, J.F. and McKeown, B.A. (1973). Functional morphology of the teleost pituitary gland. *Am. Zool.* 13: 719-742.

Scott, D.B.C. (1979). Environmental timing and the control of reproduction in teleost fish. *Symp. zool. Soc. Lond.* 44: 105-132.

Scott, D.B.C. and Currie, C.E. (1980). Social hierarchy in relation to adrenocortical activity in Xiphophorus helleri Heckel (Teleostei, Atherinomorpha). *J. Fish. Biol.* 16: 265-277.

Scott, D.B.C. and Rennie, S.E. (1980). Nuclear diameter as a criterion of adrenocortical activity in a teleost fish Coregonus lavaretus (L.). *J. Fish. Biol.* 17: 83-90.

Shanbag, A.G. and Nadkarnis, V.B. (1977). Histological and histochemical studies on the interrenal tissue and chromaffin cells in two freshwater teleosts, Channa striatus (Bloch.) and Clarias batrachus (Linnaeus). *Zool. Anz.* 198: 109-114.

Sharma, S. (1971). The homology of the so-called headkidney in certain Indian teleosts. *Ann. Zool. Agra* 7(2): 19-40.

Singh, T.P. (1969). Observations on the effects of gonadal and adrenocortical steroids on the thyroid gland in hypophysectomised catfish, Mystus vittatus. *Gen. comp. Endocr.* 12: 556-560.

Sokol, H.W. (1955). Experimental demonstration of thyrotropic and gonadotropic activity in the adenohypophysis of the guppy, Lebistes reticulatus (Peters). *Anat. Rec.* 122: 451.

Srivastava, S. and Sathyanesan, A.G. (1971). Structure of the thyroid of some teleosts having accessory respiratory organs. *Z. mikrosk.-anat. Forsch.* 83(2): 237-245.

Stendell, W. (1914). Die hypophysis cerebri. *Oppel's Lehrbuch der vergl. mikrosk. Anat.* 8S: 1-62.

Subhedar, N. and Rao, P.D.P. (1974). Effects of some corticosteroids and metapirone on the corpuscles of Stannius and the interrenal gland of the catfish, Heteropneustes fossilis. *Gen. comp. Endocr.* 23: 403-414.

Sundararaj, B.I. (1958). The seminal vesicles and their seasonal changes in the Indian catfish, Heteropneustes fossilis. *Copeia* (1958):287-297.

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.

Sundararaj, B.I. and Sehgal, A. (1970a). Short and long term effects of imposition of total darkness on the annual ovarian cycle of the catfish Heteropneustes fossilis. *J. interdiscipl. cycle Res.* 1: 291-301.

Sundararaj, B.I. and Sehgal, A. (1970b). Responses of the pituitary and ovary of the catfish, Heteropneustes fossilis (Bloch.) to accelerated light regimes of a decreasing followed by an increasing photoperiod during the post-spawning period. *Biol. Reprod.* 2: 435-443.

Sundararaj, B.I. and Nayyar, S. (1967). Effects of exogenous gonadotropins and gonadal hormones on the testis and seminal vesicles of hypophysectomised catfish, Hetereopneustes fossilis. (Bloch.). *Gen. comp. Endocr.* 17: 73-82.

Sundararaj, B.I. and Vasal, S. (1973). Photoperiod regulation of the reproductive cycle in the catfish, Heteropneustes fossilis. (Bloch.) in '*Endocrinology*' *Proc. IV Int. Cong. Endocr. Washington D.C. 1972 Int. Congr.* Ser. 273: 180-184. Amsterdam: Excerpta Medica.

Sundararaj, B.I., Vasal, S. and Halberg, F. (1973). Circannual rhythmic ovarian recrudescence in the catfish Heteropneustes fossilis. *Int. J. Chronobiol.* 11: 362-363.

Tinley, K.L. (1964). Fishing methods of the Thonga-tembe in north-eastern Zululand and southern Mozambique. *Lammergeier.* 3: 9-39.

Uhlenhuth, E., Schenthal, J.E., Thompson, J.U., Mech, K.F. and Algire, G.H. (1945a). Colloid content and cell height as related to secretory activity of the thyroid gland. 1. In normal thyroids of Triturus torosus. *J. Morph.* 76: 1-29.

Uhlenhuth, E.; Schenthal, J.E.; Thompson, J.U. and Zwillig, R.L. (1945b). Colloid content and cell height as related to the secretory activity of the thyroid gland. 11. The activated thyroid of Triturus torosus. *J. Morph.* 76(2): 45-35.

van der Waal, B.C.W. (1972). *'n Ondersoek na aspekte van die ekologie, teelt en produksie van Clarias gariepinus (Burchell 1822).* M.Sc. Thesis, Rand Afrikaans University.

van der Waal, B.C.W. (1974). Observations on the breeding habits of Clarias gariepinus (Burchell). *J. Fish. Biol.* 6: 23-27.

van der Waal, B.C.W. (1978). Some breeding and production experiments with Clarias gariepinus (Burchell) in the Transvaal. *S. Afr. J. Wildlife Res.* 8: 13-17.

van Oordt, P.G.J.W. (1968). The analysis and identification of the hormone producing cells of the adenohypophysis. In *Perspectives in endocrinology*: 405-467. Barrington, E.J.W. and Jorgensen, C.B. (Eds.). London: Academic Press.

van Overbeeke, A.P. (1960). *Histological studies on the interrenal and phaeochromic tissue in the Teleostei.* Amsterdam: van Munster's Drukkerijen.

Vincent, S. (1898). Contributions to the comparative anatomy and histology of the suprarenal capsules. The suprarenal bodies in fishes and their relation to the so-called headkidney. *Trans. zool. Soc. Lond.* 14: 41-84.

Vincent, S. and Curtis, F.R. (1927). A note on the teleostean adrenal bodies. *J. Anat.* 62: 110-114.

Viswanathan, N. and Sundararaj, B .I. (1974). Seasonal changes in the hypothalamo-hypophyseal-ovarian system in the catfish Heteropneustes fossilis (Bloch.).

Weldon, W.F.R. (1885). On the suprarenal bodies of the vertebrata. *Q.J. microsc. Sci.* 25: 137-150.

Wingstrand, K.G. (1966). Comparative anatomy and evolution of the hypophysis. In *The pituitary gland.* 1: 242-294. Harris, G.W. and Donovan, B.T. (Eds.). London: Butterworth.

Woodhead, A.D. (1975). Endocrine physiology of fish migration. In *Oceanography and marine biology.* 13: 287-382. Barnes, H. (Ed.). London: Allan and Unwin.