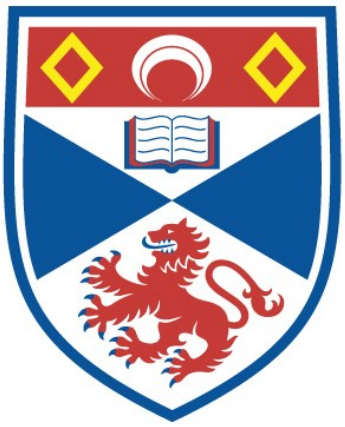
ENDOCRINOLOGY OF THE HEAD-KIDNEY TISSUES IN TELEOST FISH

Nasser A. Al-Asgah

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1977

ENDOCRINOLOGY OF THE HEAD-KIDNEY TISSUES IN TELEOST FISH

Volume I

by

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A Thesis submitted for the

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SUMMARY

This is a study of the structure and functions of the endocrine tissues in the head-kidney of the teleost fish, the homologous tissues to the mammalian adrenal cortex (=adrenocortical tissue) and adrenal medulla(=chromaffin tissue). The study is divided into three main sections:

1. The first section comprises a study of the general morphology, at the anatomical and histological level, of the different types of head-kidney which occur in teleost fish. The range of types is illustrated by studies on twenty-four species, some of which have been previously investigated, and including in particular sixteen marine species from the Red Sea coast of Saudi Arabia collected by the author. No previous studies of the head-kidneys of middle-eastern teleosts have been made, despite their economic importance. From these twenty-four species, and 129 previously studied by Nandi (1962), there is clearly a considerable range in the anatomical configuration of the head-kidneys and the trunk-kidneys = The head-kidney may be separate from the trunk-kidney, completely fused with it, or intermediate in form. Haemopoietic tissue may occupy most of the head-kidney and functional renal elements may be absent, or renal elements may predominate. A complete range of intermediate forms exist.

Melanophore-macrophage complexes occur in the haemopoietic tissue. The distribution of the endocrine tissues is also very variable from species to species. Both the adrenocortical and chromaffin tissues are in some way associated with the main veins draining the head-kidney into the heart. They may exist as sheaths around major veins (either the posterior cardinals, or the ducts of Cuvier), as diffuse masses of

tissue permeated by minor sinusoidal branches of the veins, or as discrete masses of tissue embedded in the haemopoietic tissue. The adrenocortical and chromaffin tissues may lie intermingled together, or they may form separate layers around the vessels, sometimes with the chromaffin closer to the vein lumen, sometimes with the adrenocortical in this position. The adrenocortical tissue and chromaffin tissue may lie separately with the chromaffin tissue posterior. There is little evidence for the association of a particular morphological type with a particular taxonomic group of teleosts, so that each species is best investigated in its own right.

2. The second section of this thesis comprises a detailed study of the morphology of the head-kidney of one particular species, Phoxinus phoxinus (Linnaeus), and a two year study of seasonal variations in the activity of its adrenocortical and chromaffin tissue. Samples of fish were collected from a population in the Walton Reservoir, Scotland, at monthly intervals. The activity of the adrenocortical tissue was assessed by measuring nuclear diameter of the adrenocortical cells, a criterion already widely used for this purpose. The activity of the chromaffin cells was similarly assessed, though the methodology is less well established in this case. The effects were compared of electrofishing followed by anaesthesis and immersion in Bouin's fixative while still under electronarcosis. Both proved to be relatively stress-free methods. The reproductive cycle of both males and females sampled were assessed by measuring the gonadosomatic ratio, and by counting the proportions of different oocyte stages and observing the different spermatocyte stages in gonad sections. The cycle was found to vary a little from year to year, dependent on weather conditions. Nuclear diameter of both adrenocortical and chromaffin cells showed a seasonal variation which was closely correlated with the reproductive cycles; minimum mean nuclear diameter in both cases occurring at the end of

spawning period. A high degree of adrenocortical activity accompanying spawning has been described independently in other teleosts.

3. The third section of this thesis comprises a study of the fine structure of the endocrine tissues of the head-kidney of Phoxinus phoxinus and Salmo gairdnerii. Electron micrographs were prepared using fish caught under stress-free conditions in the Walton Reservoir, and from aquarium-maintained fish. The adrenocortical cells in both species are characterised by having a great many conspicuous mitochondria with tubulo-vesicular internal structure. The nucleus is circular in section, and centrally situated in the cell. There is an extensive smooth endoplasmic reticulum and numerous ribosomes. Microvilli occur on cell surfaces in contact with veins. There is a wide range in the stŭcture of the adrenocortical cells of individual fish; mitochondria range from small, elongated structure, with dark matrix to large, circular structures in which the internal structures eventually breaks down. The cytoplasm as a whole tends to be pale in cells with small dense mitochondria, and dense in cells with large, paler mitochondria. Pale highly vacuolated cytoplasm is associated with cells in which the mitochondria are breaking down; the vacuoles are probably associated with the degenerating mitochondria. In Phoxinus maintained in aquaria for twenty-four hours before killing, the proportion of adrenocortical cells with small mitochondria with dark matrices, .. as compared to fish caught by stress-free methods and fixed immediately. In Salmo, which had been maintained in aquaria for longer periods, the proportion of cells showing mitochondrial degeneration and cytoplasmic vacuolation is higher. It is concluded that small, dark matrix mitochondria are typical of early stages of adrenal activity; dense cytoplasm and an increased number of large, circular mitochondria are typical of maximum activity; and mitochondrial degeneration and vacuolation of

the cytoplasm is typical of exhaustion. Chromaffin cells, not hitherto described in teleost fish, are of the type found in other vertebrates, with many chromaffin vesicles containing varying amounts of granular inclusion. Synaptic contacts occur commonly, apparently all of cholinergic type. In Salmo the chromaffin and adrenocortical cells lie separately, but in Phoxinus the adrenocortical cells form a sheath round the posterior cardinal veins and their main tributaries, and the chromaffin cells lie beyond them, against the haemopoietic tissue. These chromaffin cells communicate with the vein by elongated projections running amongst the adrenocortical cells.

DECLARATION

I declare that this thesis is the result of my own work. Where observations and experiments performed by others are referred to in the text, they have been acknowledged. None of the material in this thesis has been submitted by me for any other degree. Parts of Chapters II and IV are now being submitted for publication.

.....

N.A. AL-ASGAH

(Candidate)

CERTIFICATE

I certify that Mr. Nasser A. Al-Asgah has spent twelve terms at research work on the endocrinology of teleost fish, that he has fulfilled the conditions of ordinance No. 16 (St. Andrews) and that he is qualified to submit the accompanying thesis for the Degree of Doctor of Philosophy.

.....

D.B.C. Scott.
(Supervisor)

July, 1977.

UNIVERSITY CAREER

I began my University career in Riyad, Saudi Arabia in October, 1967. In June 1971, I graduated with grade "Very Good" in zoology and chemistry. I worked in Riyad University as demonstrator in the faculty of Science from 1971 to 1972.

In January 1973, I came to Britain and studied the English language at Swan School of English in Oxford from January to July 1973.

In October 1973, I began research into the structure and function of the endocrine tissues of the head-kidney of teleost fish in the Zoology Department of the University of St. Andrews. From December 1973 to February 1974 I collected fish for my studies on the Red Sea Coast of Saudi Arabia; this work is described in Chapter II. I also made a field study of the minnow, Phoxinus phoxinus (Linnaeus) in the Walton Reservoir, to investigate seasonal changes in head-kidney endocrine tissue in relation to reproduction; this work is described in Chapter III.

The final aspect of the work is a study of the fine structure of the teleost head-kidney; this work is described in Chapter IV.

ACKNOWLEDGEMENTS

I am especially grateful to my supervisor, Dr. D.B.C. Scott, not only for continued encouragement, invaluable suggestions and assistance throughout, but also for introducing me to this field of zoological science and for his unlimited patience.

My thanks go to Walton Reservoir Angling Society for permission to electrofish for minnows, also to the Ministry of Agriculture, Riyadh, Saudi Arabia, for allowing me to use their marine laboratory in Jeddah while I was collecting specimens from Saudi Red Sea coastal waters.

I would also like to express my gratitude to the University of Riyadh, Saudi Arabia who paid all the costs of my trip to the Red Sea and supported this work.

Finally, I would like to thank Professor H.G. Callan for allowing me to carry out this work in his department, and the technical staff for their co-operation, especially J. Mackie for the electronmicroscopic help.

I N D E X

TABLE OF TRANSLITERATION

(For use of interpretation of arabic local names of Red Sea fish)

a	=	ا	ḍ	=	ض
b	=	ب	ṭ	=	ط
t	=	ت	ẓ	=	ظ
th	=	ث	ʿ	=	ع
j	=	ج	gh	=	غ
h	=	ح	f	=	ف
kh	=	خ	q	=	ق
d	=	د	k	=	ك
dh	=	ذ	l	=	ل
r	=	ر	m	=	م
z	=	ز	n	=	ن
s	=	س	h	=	ه
sh	=	ش	w	=	و
ṣ	=	ص	y	=	ى
			Hamza		ء

ḍamma + waw	= u	fatha	= a
fatha + ya	= ai	kasra	= i
fatha + waw	= au	ḍamma	= u
		fatha + alif	= a
		kasra + ya	= i

CHAPTER I

GENERAL INTRODUCTION

This study is intended as a contribution to our knowledge of the morphology and physiology of the head-kidney of teleost fish. The head-kidney was first described in 1845 by Miller. It comprises the anterior part of the kidney, but typically it has no renal function. It consists mainly of haemopoietic tissue, a mass of lymphocytes and supported by sparse connective tissue. The head-kidney is drained by the cardinal veins and associated with these veins are two types of endocrine tissue=

1. The adrenocortical tissue
2. The chromaffin tissue

which correspond with the adrenal cortex and the adrenal medulla of mammals.

There are many morphological variations of this basic pattern in different species of fish, of which only a relatively small number have so far been investigated (Nandi 1962; Chavin 1966; Nandi, 1965; Banerji and Ghosh 1965; Banerji 1973).

The physiological functions of the head-kidney tissues are extensive, and by no means understood. Lymphocytes are mobilised during stress (such as disease) and pass into the general circulation (Slicher et al. 1962).

The adrenocortical tissue secretes corticosteroid hormones (Chester Jones 1957; Idler 1972; Fuller 1974; Fuller, Scott and Fraser 1976 and many others) similar to the mammalian corticosteriods; but perhaps with somewhat different functions (Goswami and Sundararaj 1971b) which are not yet clear. Less research has been carried out on the chromaffin tissue, but it apparently secretes adrenalin and/or noradrenalin (Banerji 1973; Chavin 1966; Coupland 1953). The corpuscles of Stannius which lie posterior to the head-kidney but were once thought to have

properties similar to the adrenocortical tissue, are now considered to be a completely separate system, perhaps concerned with the regulation of mineralocorticoid secretion in the fashion of the juxtaglomerular apparatus of the mammalian kidney.

Only very recently have any studies been made on the fine structure of the head-kidney tissue

In the present work, three main aspects of the subject are considered:-

1. A review of the morphology and histology of the head-kidney of a number of teleost species; in particular of a range of marine species from the Red Sea coast of Saudi Arabia which have not been previously studied.

2. Physiological changes in the head-kidney in relation to the reproductive cycle in a natural population of the european minnow, Phoxinus phoxinus. Associated with this phase of the project is a detailed morphological study of the head-kidney of this species.

3. An electron microscope study of the fine structure of the head-kidney tissues in Phoxinus phoxinus.

CHAPTER II

MORPHOLOGY OF THE HEAD-KIDNEY

A. Introduction

1. Head-kidney

The head-kidney is the name given to the anterior part of the kidney of teleost (and other) fish. In many species it is more or less isolated in one or more lobes from the posterior part of the kidney (the trunk kidney) and contains few or no renal elements (type i Fig. 1). In other species the head-kidney is not externally distinguishable from the trunk-kidney, but it can be histologically identified by the absense of renal elements (type ii Fig. 1). In other species again, renal elements extend to the anterior end of the kidney, and the head-kidney can not be distinguished from the trunk-kidney (type iv Fig. 1). There are many intermediate types (type iii Fig. 1). There was early interest in the embryonic origin of this complex body of tissues and several embryological studies were made about the turn of the century, including the beautifully illustrated work of Giacomini (1902- 1922) who also discovered the adrenocortical and chromaffin tissues, now known to be endocrine, and which he called interrenale anteriore and sistema feocromo, in the head-kidney. It was generally considered that the head-kidney of adult teleosts was homologous with the pronephros, the first functional kidney to develop in the embryo. As more species were studied, it became evident that not all teleosts had a discrete, non-renal head-kidney and in some species functional renal elements extended to the anterior end of the kidney. This discovery led to considerable dispute towards the end of the century as to the exact relationship between pronephros and head-kidney (Balfour 1881, 1882; Parker 1882; Weldon 1884; Calderwood 1891). This line of investigation has now

Fig. 1. Diagram of different types of kidney in teleost fish.

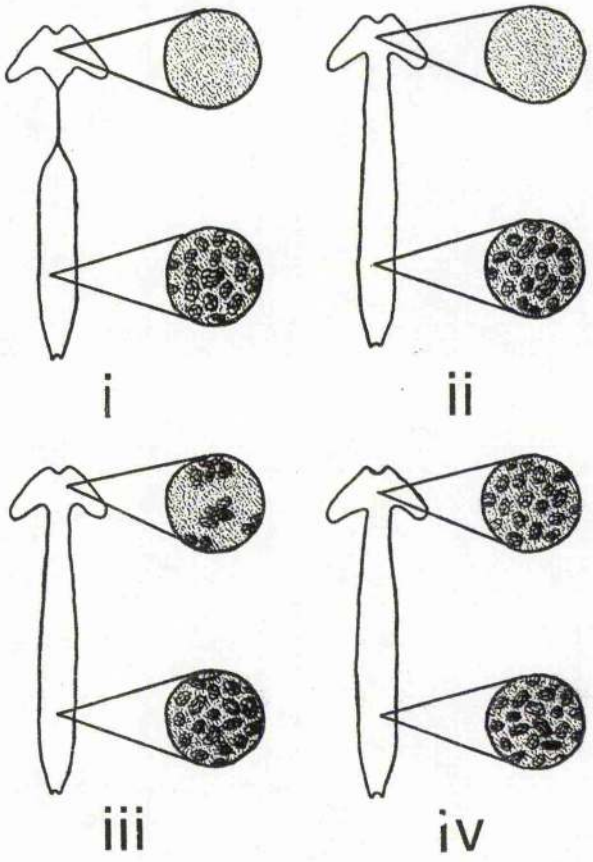
(i) Discrete head-kidney without renal elements.
(ii) Head-kidney continuous with trunk kidney, but containing no renal elements.
(iii) Head-kidney continuous with trunk kidney, and containing varying amounts of renal elements.
(iv) Head-kidney continuous with trunk kidney, and containing many renal elements.

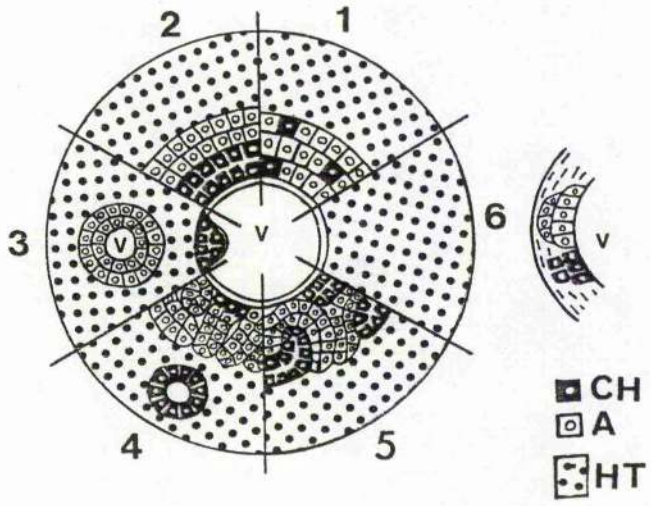
(See text pages 3-4)

Fig. 2. Types of distribution of the associated endocrine tissues in the kidney according to Oguri and Hibiya (1957b).

(See text pages 6-7).

CH = chromaffin cell
A = Adrenocortical cell
HT = haemopoietic tissue
V = vein.


i
ii
iii
iV


2
1
3
6
4
5
V
V
V
CH
A
HT

fallen into desuetude, as interest has been transferred to discovering the functions of the tissues, rather than their homologies. The accepted viewpoint now is that the head-kidney, where it exists as a discrete organ, is homologous with the pronephros. Kerr (1919)has also pointed out that since the posterior kidney of all fishes is derived from the entire caudal portion of the nephrotomic plate, it should be called an opisthonephros, representing both the embryonic mesonephros plus the caudal metanephros of amniote vertebrates. To avoid confusion, in the present work, the terms head-kidney and trunk-kidney will be used, without any implication of embryonic origins.

The discrete head-kidney comprises lymphoid tissue, extensive sinusoids, melanocytes, adrenocortical tissue and chromaffin tissue, plus of course arteries (including the main arterial branches to the gut, which often pass through the head-kidney mass), veins (notably the large posterior cardinal veins which drain the kidneys into the sinus venosus of the heart via the ducts of Cuvier) and nerves. Some renal elements may be present, and the pronephric ducts are often conspicuous. The corpuscles of Stannius generally lie posteriorly in the trunk-kidney, and are only considered incidentally in the present work.

In those species without a discrete head-kidney (plates 5,6,7) renal elements predominate and only the presence of adrenocortical and chromaffin tissues distinguish the head-kidney from the trunk-kidney.

2. Adrenocortical Tissue

"Interrenale anteriore", "Interrenal", "Acidophil cells" and "Suprarenal".

The adrenocortical tissue of teleosts was first described in 1902 (Giacomini 1902 in Anguilla vulgaris Flem; Esox lucius Lin; Cyprinus carpio Lin; Tinca vulgaris Cuv; Leuciscus albus Bp; Leuciscus aula Bp; Barbus plebejus Val;) as distinct epithelial cell-layers along the

walls of the cardinal veins in the head-kidney. Between 1902 and 1922 Giacomini described the histology of the head-kidney tissues in many species (Giacomini 1922). Nandi (1962) has reviewed these and subsequent descriptions of 129 species from fifty-five families, and since 1962 further species have been described (Weatherley 1963; Scott 1963; Roy 1964; Nandi 1965; Banerji and Ghosh 1965; Chavin 1966, Olivereau 1966; Hanke and Chester Jones 1966; Banerji 1973.) Basically, the adrenocortical tissue is associated with the veins in the head-kidney (in the anterior region of the functional kidney, if no head-kidney as such is present.) There are, however, many variations in detail. Nandi 1962 has attempted to bring order to this variety by categorising the distribution of adrenocortical tissue into four main types (Fig. 3A), while admitting that many intermediate types exists:

Type 1. Adrenocortical tissue surrounds the post-cardinal veins or their largest branches.

Type 2. Adrenocortical tissue surrounds small or medium sized branches of the veins, and therefore is rather widely dispersed throughout the anterior parts of the kidney.

Type 3. Adrenocortical tissue is associated with venous sinuses within the anterior kidney tissue; it often forms strands or cords of cells, sometimes scattered through the haemopoietic tissue and sometimes appearing to replace large areas of the latter. Adrenocortical cells do not surround the veins.

Type 4. Adrenocortical tissue forms a solid mass of cells in a localised area.

3. Chromaffin Tissue.

"Sistema Feocromo" "Chromaffin".

The chromaffin tissue of teleosts was first described in 1908 by Giacomini. As a general rule, chromaffin cells are much less abundant than adrenocortical cells, and tend to be concentrated rather in the posterior region of the head-kidney. However, as in the case of the

Fig. 3. A. Types of distribution of adrenocortical tissue according to Nandi (1962).

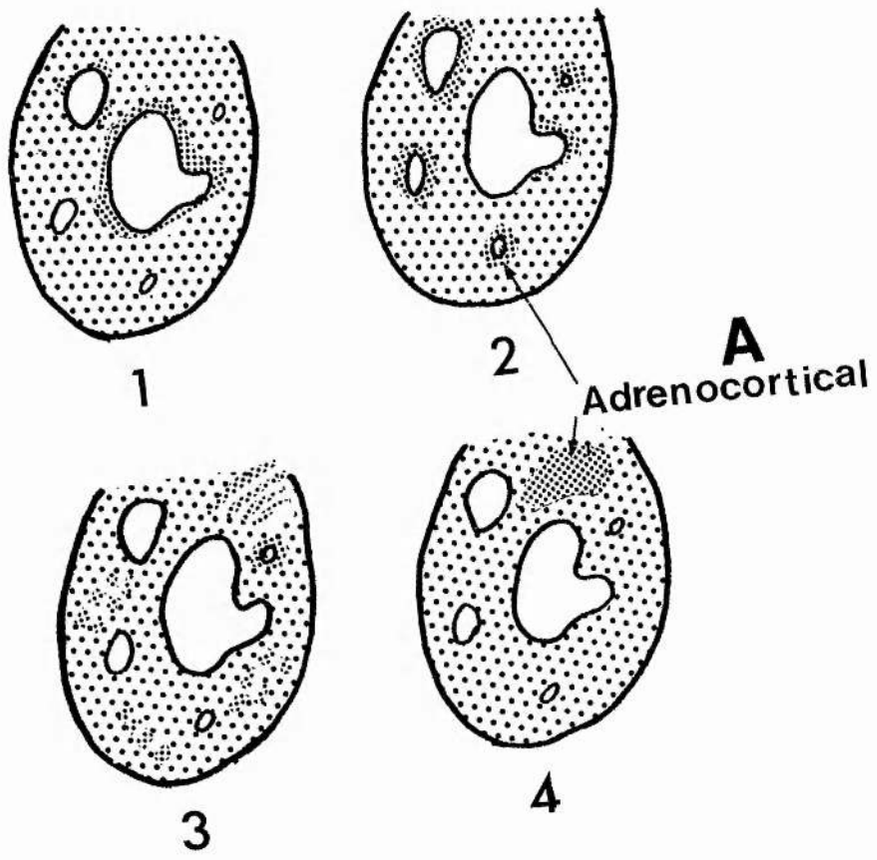
(See text page 5)

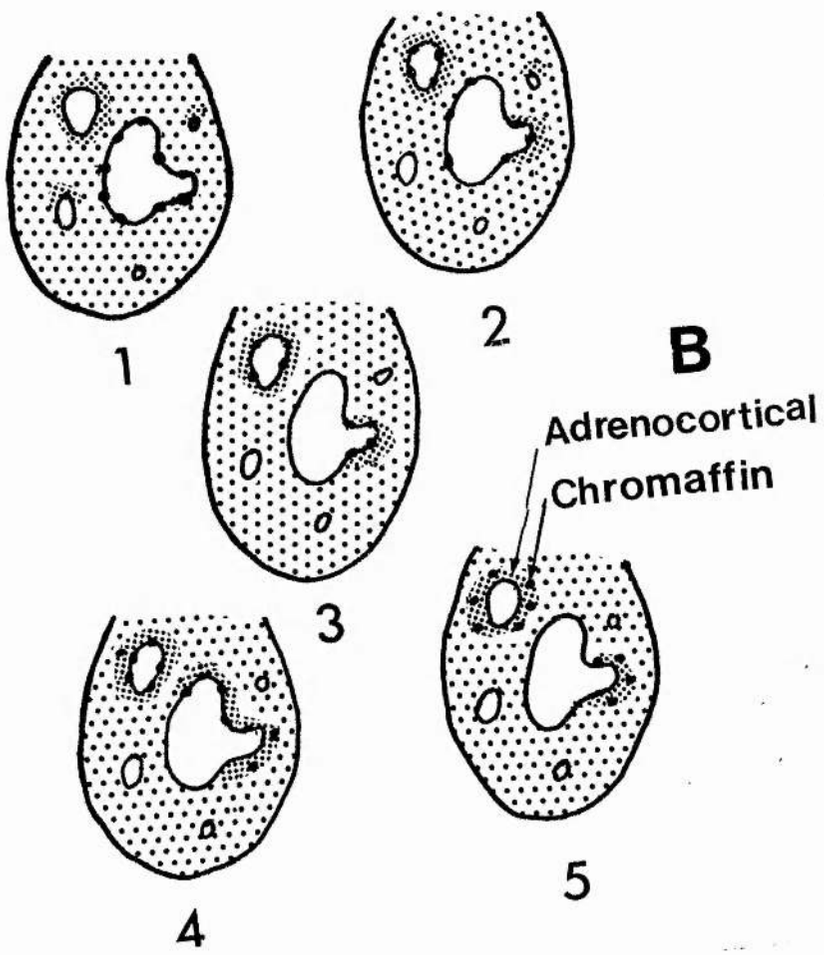
Large stippling = Haemopoietic tissue
Small stippling = Adrenocortical tissue
Unstippled = Veins.

B. Types of distribution of chromaffin tissue according to Nandi (1962)

(See text page 6).

Legend as for Fig. 3A, and chromaffin tissue indicated by largest stippling.


1
2
A
Adrenocortical
3
4


1
2
B
Adrenocortical
Chromaffin
3
4
5

adrenocortical tissue, there is considerable specific variation which Nandi (1962) has simplified into the following five categories (Fig. 3B):

Type 1. Chromaffin cells are embedded in the vein walls; none occurs in the region where adrenocortical tissue is situated.

Type 2. Chromaffin cells are embedded in the vein walls, and occur in the area containing adrenocortical cells as well as in other regions of the anterior kidney.

Type 3. Chromaffin cells are embedded in the vein walls, but only in the region where adrenocortical cells are situated.

Type 4. Chromaffin cells are embedded in the vein walls, and are also interspersed among adrenocortical cells.

Type 5. Chromaffin cells are found only interspersed among adrenocortical cells.

Nandi (1962) categorises adrenocortical and chromaffin distribution separately, though the two tissues are always spatially associated for biochemical reasons.

An attempt to classify head-kidney types based on the association of the two types of endocrine tissues has been made by Oguri and Hibiya (1957b). They visualise six categories (Fig. 2):

1. Type I The main vein in the head-kidney and its small branches surrounded by adrenocortical cells and among them exist the chromaffin cells. (e.g. Carassius auratus, Cyprinus Carpio. Misgurnus anguillicaudatus, Channa argus, Plotosus anguillaris).

2. Type II Chromaffin cells are found within the walls of the main vein and its branches. Adrenocortical cells are found surrounding these veins. (e.g. Parasilurus arotus, Niphon spinosus, Pterois lunulata, Lepidotrigla microptera, Hoplobrotula armata.)

3. Type III Both types of cells are not next each other, but they exist within the head-kidney separately. Chromaffin cells appear in the vein wall in the head-kidney and are not as numerous as adrenocortical cells. The chromaffin cells form two to three layers. Some of them protrude into the vein wall as small groups of cells. (e.g. Salmo gairdnerii irideus.)

4. Type IV Most of the chromaffin cells are similar to those in Type III separated from the adrenocortical cells, but some of them exist among the adrenocortical as very small groups. (e.g. Prionurus microlepidotus, Hexagrammos otakii.)

5. Type V Adrenocortical and chromaffin cells form strands of cells. These strands exist beside each other and sometimes some nerve cells are found among them. (e.g. Paralichthys olivaceus.)

6. Type VI Neither the adrenocortical nor the chromaffin exist in the head-kidney, but both cells are confined within the thick wall of sinous vein near the head-kidney. Adrenocortical cells exist in the thick vein wall as two to three layers. Chromaffin cells exist among the adrenocortical cells or separately within the wall of the vein and sometimes chromaffin cells protrude into the vein lumen as small groups separated from the adrenocortical cells by connective tissue. (e.g. Anguilla japonica.)

4. Vascular supply and innervation:

The primitively paired posterior cardinal veins gather blood from the kidney tissue and run forwards within the kidneys, towards the heart. It is not uncommon, however, for one of the veins to be much larger than the other (e.g. Coregonus lavaretus Plate 4 ; and Phoxinus Fig. 23 Plate 28) in which case blood from one kidney lobe drains across into the other. The posterior cardinal vein/veins pass through the head-kidney to meet the anterior cardinal veins, generally at the anterior end of the head-kidney. The ducts of Cuvier then continue to the sinus venosus of the heart; in those species in which the posterior cardinal veins are asymmetrical, so are the ducts of Cuvier. The posterior cardinal veins may break up into sinusoids as they pass through the head-kidney, or they may remian as simple large vessels.

The main artery (gastromesenteric) serving the organs in the coelomic cavity generally passes close to the head-kidney, or even through it (e.g. Coregonus) and presumably is the source of oxygenated blood to the head-kidney itself.

Sympathetic nerves, derived from segmental spinal nerves, generally

run to the coelomic organs in association with the gastromesenteric artery, forming a conspicous ganglion close to the head-kidney. Presumably the sympathetic innervation of the chromaffin tissue is derived from these nerves.

B. Materials and Methods.

The species used in this section of the work were obtained;

(i) From aquarium dealers

Genus/species	Family
Aphanius dispar	Cyprinodontidae
Brachydanio rerio	Cyprinidae
Rasbora heteromorpha	Cyprinidae
Salmo irideus	Salmonidae
Xiphophorus helleri	Poeciliidae
Phoxinus phoxinus	Cyprinidae

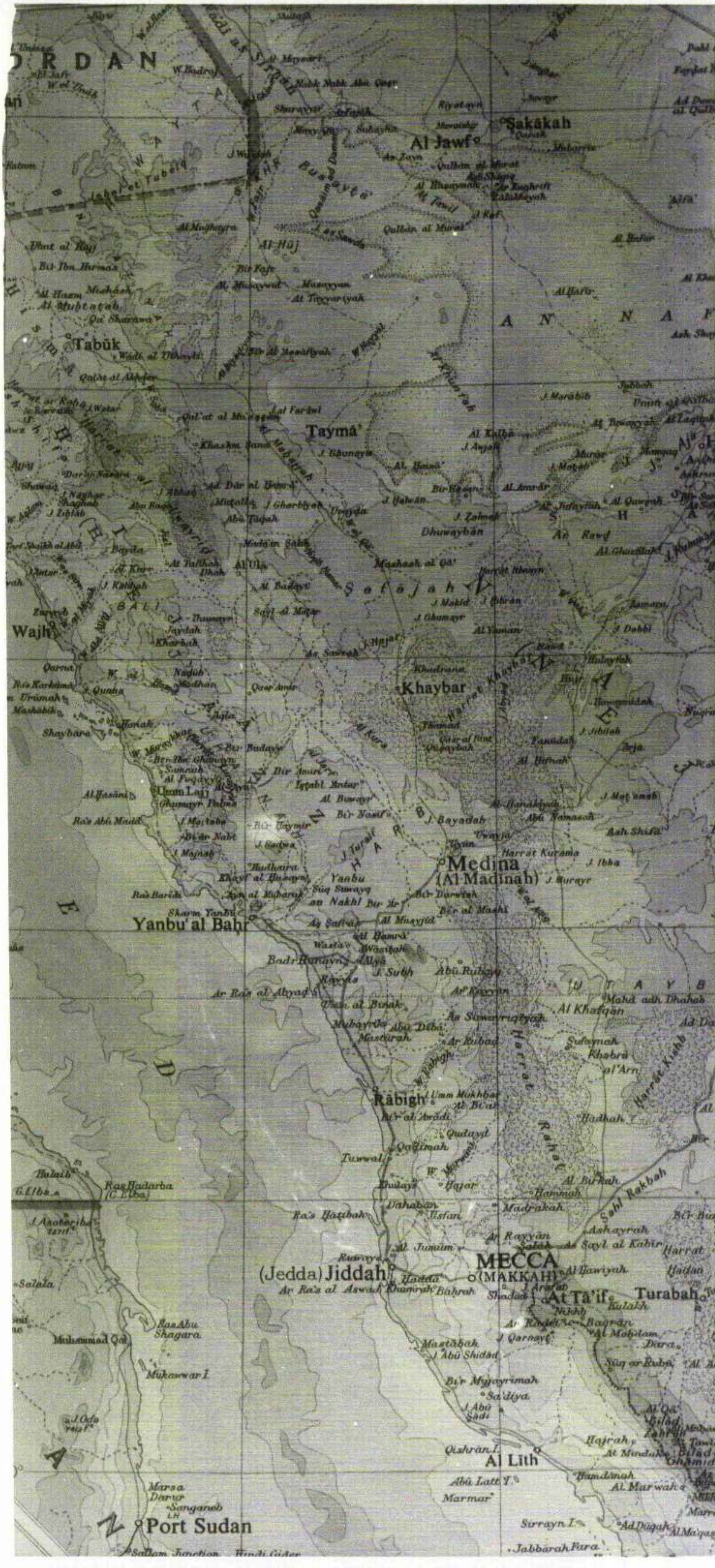
(ii) By the author during an expedition to the Red Sea coast of Saudi Arabia in January 1974 (Fig. 4).

Genus/species	Family
Chanos chanos	Chanidae
Acanthopagrus bifasciatus	Sparidae
Crenidens crenidens	Sparidae
Scarus species	Scaridae
Herklotsichthys punctatus	Clupeidae
Thalassoma amblycephalus	Labridae
Thalassoma lunare	Labridae
Cheilinus diagrammus	Labridae
Variola louti	Serranidae
Cephalopholis miniatus	Serranidae
Monodactylus argenteus	Monodaxtylidae
Gaterin gaterinus	Plectorbynchidae
Lethrinus species	Lethrinidae
Hepsetia pinguis	Atherinidae
Siganus species	Siganidae
Zebrasoma veliferum	Acanthuridae

Fig. 4. Map of part of Red Sea coast of Saudi Arabia, showing location of fishing.

(See text pages 9-10)

Scale line corresponds to 45 Km.



Sakakah
Al Jawf
Tabūk
Taymā'
Al 'Ulā
Wajh
Khaybar
Medina
(Al Madinah)
Yanbu' al Bahr
Rābigh
MECCA
(MAKKAH)
(Jedda) Jiddah
At Tā'if
Turabah
Al Lith
Port Sudan
Halaib
Muhammad Qol

The latter group were mostly obtained by commercial fishermen in their camps south and north of Jeddah, who use nets and traps, which they set in the evening and collect in the morning. Some were purchased from fishermen along the Red Sea shore between Jeddah and Yanbu Al-Bahr (the second Saudi Arabian port on the Red Sea) 300 Km. to the north of Jeddah. Air temperature at the time in Jeddah area was between fifteen and twenty degrees centigrade, water temperature twenty-four and twenty-seven degrees centigrade, in Yanbu Al-Bahr the air temperature was eight to twelve degrees centigrade and there were eleven hours of light per day.

Some of the fish caught from the Jeddah area were brought live from the fishery camps to the marine laboratory at the Ministry of Agriculture in Jeddah, where they were killed, fixed, and dehydrated to seventy per cent ethanol=

Chanos chanos

Achanthopagrus bifasciatus

Scarus species

Variola louti

Monodactylus argenteus

Zebrasoma veliferum

Lethrinus species

The others were killed and fixed in the camps and then taken to the laboratory and dehydrated to seventy per cent ethanol=

Thalassoma lunare

Herklotsichthys punctatus

Cephalopholis miniatus

Caterin caterinus

Hepsetia pinouis

Siganus species

The fish caught along the Red Sea shore from Jeddah to Yanbu Al-Bahr were killed, fixed and dehydrated to seventy per cent ethanol on the spot:

Crenidens crenidens

Thalassoma amblycephalus

Cheilinus diagrammus

(iii) From D.B.C. Scott

Genus/species	Family
Scleropages formosus	Osteoglossidae
Coregonus laveretus	Salmonidae

Fish bought from aquarium dealers in Britain were killed by immersion in a lethally concentrated solution of MS222 (Sandoz). The Red Sea fish were killed by one of three methods= large specimens by concussion or decapitation, small specimens by direct immersion in fixative. All fish were fixed as soon as possible after death, in Bouin's (Aqueous) fixative [picric acid saturated solution in distilled water seventy-five parts; formalin twenty-five parts; glacial acetic acid five parts, v/v]. The abdomen of small fish was slit open to permit rapid fixation of the kidney, and the whole specimen immersed in fixative. In the case of large fish, the kidneys were dissected out and fixed separately. In intermediate cases the head-kidneys were fixed in situ and fixed with the vertebral column and dorsal musculature attached.

After fixation for twenty-four hours the tissues were transferred to thirty per cent ethanol for two hours, fifty per cent ethanol for three hours, and seventy per cent ethanol for storage. Picric acid was removed from the tissue by repeated changes of seventy per cent ethanol. Red Sea fish were transported to Scotland in seventy per cent ethanol.

The tissues were embedded in "Fibrowax" (A.B. Horwell, Ltd.) using a tertiary butyl alcohol/paraffin series =

70% Embedding alcohol { T.B.A. 80 ml. ; 95% Ethanol 200 ml. 24 hrs. ; H_2O dis. 120 ml.

	H_2O dis.	60 ml.	
85% Embedding alcohol	95% Ethanol	200 ml.	4 hrs.
	T.B.A.	140 ml.	
	H_2O dis.		
95% Embedding alcohol	95% Ethanol	180 ml.	3 hrs.
	T.B.A.	220 ml.	
	H_2O dis.		
100% Embedding alcohol	100% Ethanol	100 ml.	3 hrs.
	T.B.A.	300 ml.	

Tertiary butyl alcohol = three changes two, twelve, two hours. Tertiary butyl alcohol/paraffin 50/50=four to six hours. Filtered "fibrowax" = three changes, two twelve, two hours embed in fresh wax.

Serial sections of all specimens were cut at a thickness of five to eight µm. (500 - 1000 sections) on MSE rotary microtome, and sections were routinely stained with Masson's trichrome (Humason 1972).

The morphology of the head-kidney and associated tissues were determined by gross dissection and by reconstructing serial sections as three dimensional drawings. This was achieved by transposing drawings of sections on to isometric graph paper, and superimposing sections in series. (Figs. 5,6,7,9,10).

C. Results

Review of head-kidney types. (Fig. 7)

(a) Type I. Discrete head-kidney anatomically separate from trunk-kidney devoid of renal elements.

(i) Rasbora heteromorpha (Harlequin fish)

Family - Cyprinidae.

/ A species occurring in freshwaters of S.E. Asia, length 35 mm. purchased from aquarium dealers, St. Andrews. / (Fig. 5).

There is a discrete head-kidney, which is connected to the trunk-kidney by a thin strand of renal tissue which runs along the right side of the dorsal aorta. (Plate 1). The head-kidney itself is bilobed with a narrow transverse bridge joining the left and the right lobes about half-way along the length of the head-kidney. The right posterior cardinal vein is large, and runs as a continuous vessel from the trunk-kidney to the right duct of Cuvier. The left posterior cardinal vein is smaller, and breaks up into a capillary network in the left lobe of the head-kidney before re-forming into a discrete vessel, the left duct of cuvier. Venous blood evidently returns to the heart mostly on the right side of the system.

The coeliacomesenteric artery branches off the dorsal aorta immediately anterior to the head-kidney, passing ventrally between the two lobes, and accompanied by sympathetic nerve trunks. The dorsal aorta runs between the lobes of the head and trunk kidneys except at the extreme posterior end of the trunk-kidney, where the lobes fuse to form a median organ. Here the dorsal aorta lies in a groove on the dorsal surface of the kidney.

The adrenocortical tissue is of Nandi's Type I, forming a layer one to three cells in thickness surrounding the posterior cardinal vein, and extending for a short distance along some of the major branches. The adrenocortical cells lie in the vein walls directly

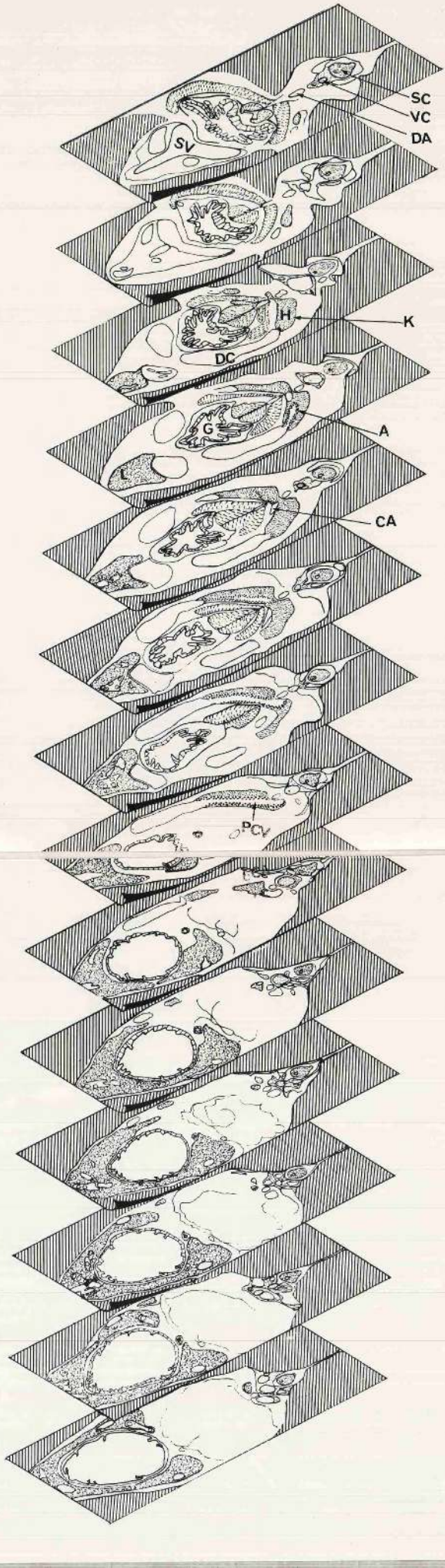
Fig. 5. Isometric drawing of serial transverse sections through specimen of Rasbora heteromorpha,, 72 µm apart. Each section is 7 mm. long by 4 mm. wide. Right side of fish on left of drawing and anterior end at top of page.

(See text page 13)

Legend:

Kidney	K
Gut	G
Liver	L
Adrenocortical	A
Chromaffin	C
Muscle	M
Spinal (nerve) Cord	SC
Duct of Cuvier	DC
Heart	HT
Renal tubules	T
Nerve	N
Posterior Cardinal Vein	PCV
Vertebral Column	VC
Haemopoietic tissue	H
Sinus Venosus	SV

This legend for fig. 5, 6,9,10, & 23.


SC
VC
DA
SV
H
K
DC
G
A
CA
PCV

below the endothelium. The diameter of the nuclei of adrenocortical cells is 4.65 ± 0.12 µm. Chromaffin cells were observed in a very few number of cells with very much irregular nuclei. The chromaffin tissue is of Nandi's Type V. The diameter of the nuclei of chromaffin cells is 5.11 ± 0.29 µm.

(ii) Scleropages formosus (Kelesa)

Family: Osteoglossidae

[A species occurring in freshwaters of Malaysia. Length 40 cm. Supplied by D.B.C. Scott]. Plate 2.

The head-kidney is discrete and consists of two main lobes and a variable number of smaller lobes. The posterior cardinal veins pass through the main lobes. The major part of the lobes consists of lymphoid cells and a very extensive capillary network. Erythrocytes are much more abundant than in typical teleost head-kidneys (Scott 1963, Hanke and Chester Jones 1966).

Adrenocortical tissue is of Nandi's Type IV forming isolated islets of cells interspersed amongst the tissues of the right lobe of the head-kidney. The diameter of the nuclei of adrenocortical cells is 3.65 ± 0.14 µm.

Chromaffin tissue is of single cell form scattered within the vein walls. It is of Nandi's Type I. The diameter of the nuclei of the chromaffin cells is 4.25 ± 0.22 µm.

(b) Type II Head-kidney not discrete, externally indistinguishable from trunk-kidney, but containing no renal element.

(i) Salmo gairdnerii irideus (Rainbow trout)

Family: Salmonidae

[A species occurring in freshwaters of North America and non introduced elsewhere as a game - and food-fish. Length 20 cm. purchased from fish farm, Perth.] Plate 3.

The head-kidney is not externally distinguishable from the trunk-kidney, but its position is indicated by the expansion into lateral wings at the anterior end of the kidney. There are no renal elements in the head-kidney; tubules and glomeruli appear only at the most posterior level of the lateral wings. Pigment cells occur in the haemopoietic tissue.

The posterior cardinal veins are much branched in the anterior part of the kidney. The right posterior cardinal vein is bigger than the left. The adrenocortical tissue is of Nandi's Type II, surrounding the posterior cardinal veins and their branches as small clumps or strands, which sometimes extend into the surrounding tissue. In the posterior part of the head-kidney the adrenocortical cells are lying in the vein wall only, and very few. The diameter of the nuclei of adrenocortical cells is 6.5 ± 0.21 µm.

The chromaffin tissue is of Nandi's Type II, occurring within the walls of the main trunks of the veins only. The diameter of the nuclei of chromaffin cells is 7.48 ± 0.29 µm.

(ii) Coregonus lavaretus (Powan)

Family: Salmonidae.

[A species occurring in freshwaters of Loch Lomond, Scotland. Length 35 cm.] Plate 4.

The kidney extends from the heart region to the posterior end of the body cavity, and lies against the dorsal side of the cavity dorsal to the air bladder, ventral to the vertebral column. The head-kidney is not externally distinguishable from the trunk-kidney. It comprises about the anterior one-tenth of the length of the kidney. No renal elements are found in the head-kidney. The right posterior cardinal vein is much larger than the left. The left lobe of the head-kidney contains numerous small veins rather than one large one.

Adrenocortical tissue is associated with the right posterior cardinal vein and its main branches, though less abundantly, round the veins of the left lobe. The tissue forms only one layer thick round the small veins, but round the larger veins several layers exist. It is intermediate between Nandi's Type I/II. The diameter of the nuclei of the adrenocortical cells is 6.4 ± 0.21 µm.

Chromaffin tissue is of Nandi's Type V, forming groups of cells amongst the adrenocortical cells. These cells are concentrated close to the wall of the main vein. The diameter of the nuclei of chromaffin cells is 7.53 ± 0.25 µm.

(c) Type III. Head-kidney not discrete, externally indistinguishable from the trunk-kidney, and containing some renal elements.

(i) Acanthopagrus bifasciatus (see pp.20-21).

(ii) Lethrinus species (see pp.26-27).

(d) Type IV. No distinct head-kidney, renal elements extend to anteriormost end of kidney.

(i) Brachydanio rerio (Zebra Danio)

Family: Cyprinidae

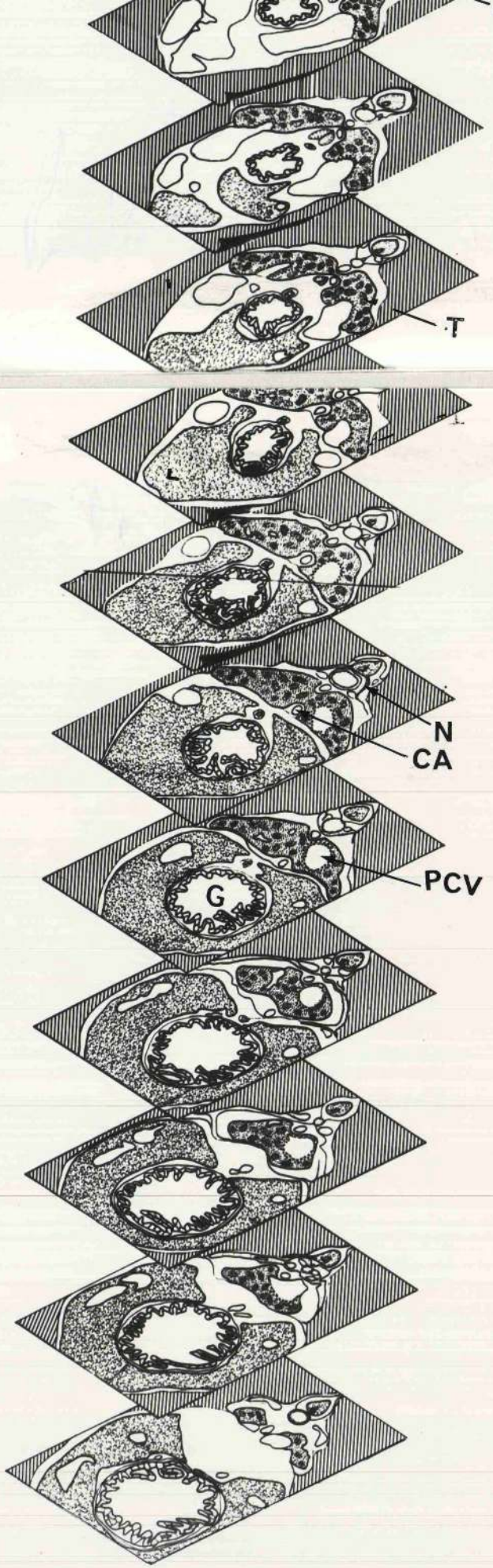
[A species occurring in freshwaters of S.E. Asia. Length 40mm. Purchased from aquarium dealers, St. Andrews.] Fig. 6 Plate 5.

There is no discrete head-kidney, but the kidney in its anterior region extends into two lateral wings, and this is where adrenocortical tissue occurs. Glomeruli, renal tubules and collecting ducts extend throughout the head-kidney. The paired posterior cardinal vein pass through the head-kidney to the ducts of Cuvier. The left posterior cardinal vein drains only the left wing of the head-kidney. The right posterior cardinal vein is larger than the left. It drains the right part of the whole kidney and also receives a medium-sized vein from the left part of the trunk-kidney, at the posterior part of the head-kidney where the left posterior cardinal vein starts.

Fig. 6. Isometric drawing of serial transverse sections through specimen of Brachydanio rerio, 50 µm apart. Each section is 5 mm. long and 4 mm. wide. Right side of fish on right side of drawing and anterior end at top of page.

(See text pages 15-17).

(Legend as for figure 5).


N
T
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PCV
G

The adrenocortical tissue surrounds the posterior cardinal veins, as they pass through the anterior part of the kidney, in a form of a collar one to two cells thick (Nandi's Type I). The diameter of the nuclei of adrenocortical cells is 4.02 ± 0.1 µm.

The chromaffin tissue is represented by a very few pale cells intermingled throughout the adrenocortical collar, as individual cells, Nandi's Type V. The diameter of the nuclei of chromaffin cells is 4.29 ± 0.12µm.

(ii) Xiphophorus helleri (Mexican swordtail)

Family: Poeciliidae

[A species occurring in freshwater streams on the Atlantic slope of Southern Mexico. Length 8 cm.] Fig. 7 Plate 6.

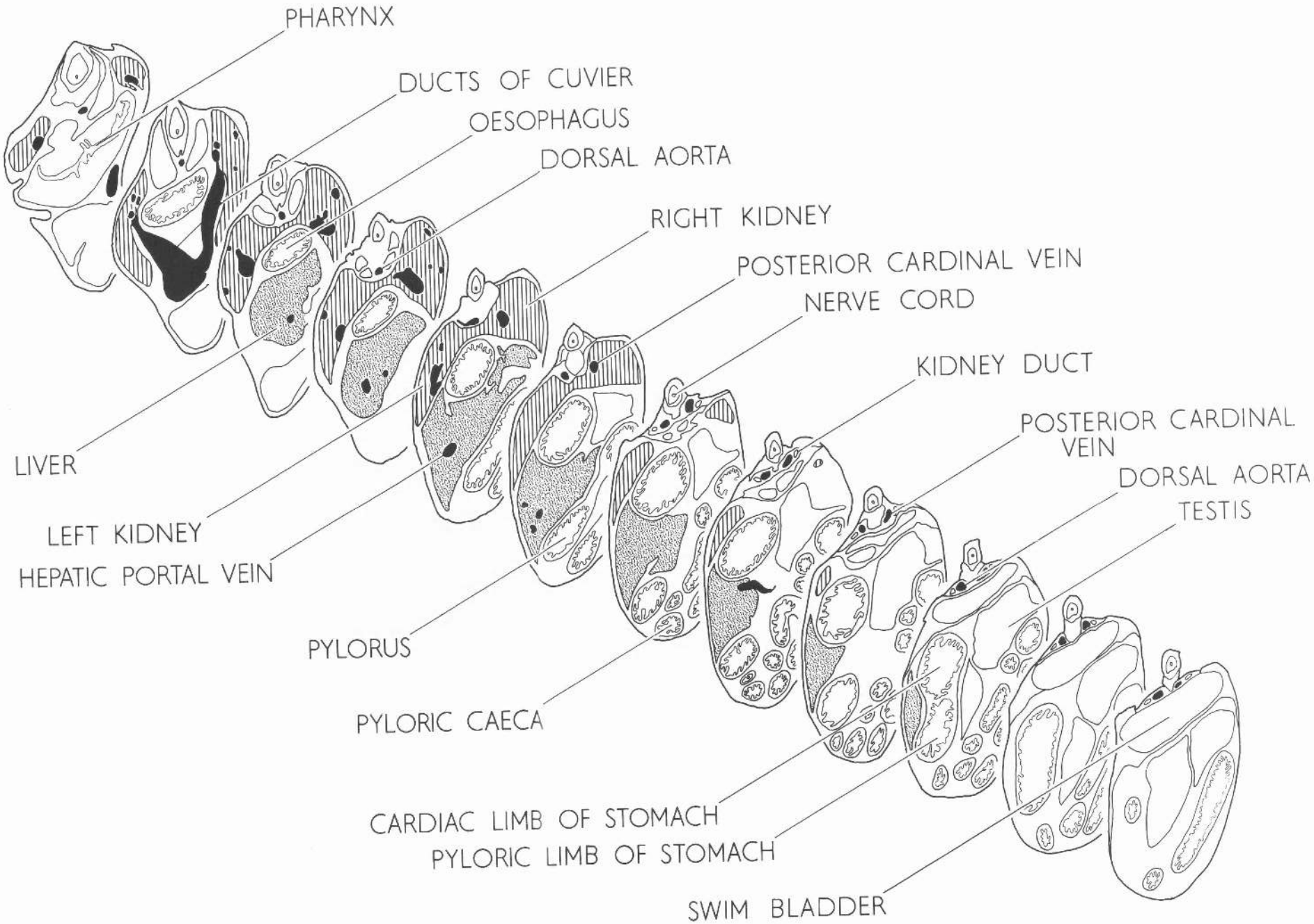
The head-kidney is not externally distinguishable but the kidney in its anterior part divides into two lateral lobes. The renal elements extend throughout the head-kidney which is represented by the two lateral lobes. There is only a single posterior cardinal vein in the posterior part of the body. It runs forward close to the vertebral column on the right side, parallel to the dorsal aorta on the left side. The dorsal aorta might, at first glance, be mistaken for the second of a pair of posterior cardinal veins. Anteriorly, however, the posterior cardinal vein enters the right lobe of the kidney, and the dorsal aorta assumes a median location under the vertebral column. The posterior cardinal vein breaks up into many sinusoids amongst the glomeruli and tubules of the kidney. The left lobe of the kidney receives its blood supply from sinusoids crossing the narrow bridge between right and left lobes, and also from the left anterior cardinal vein, which is one of a pair. Two ducts of Cuvier gather blood from each kidney and run to the sinus venosus of the heart. At the junction of the anterior and posterior cardinal veins are groups of adrenocortical and chromaffin cells.

The adrenocortical tissue is of Nandi's Type I surrounding the

Fig. 7. Isometric drawing of serial transverse sections through specimen of Xiphophorus helleri, 60 µm apart. Liver stippled; kidney striped; blood vessels black.

(Figure prepared by D.B.C. Scott).

(See text pages 17-18).


PHARYNX
DUCTS OF CUVIER
OESOPHAGUS
DORSAL AORTA
RIGHT KIDNEY
POSTERIOR CARDINAL VEIN
NERVE CORD
KIDNEY DUCT
POSTERIOR CARDINAL VEIN
DORSAL AORTA
TESTIS
LIVER
LEFT KIDNEY
HEPATIC PORTAL VEIN
PYLORUS
PYLORIC CAECA
CARDIAC LIMB OF STOMACH
PYLORIC LIMB OF STOMACH
SWIM BLADDER

cardinal veins in a form of a single layer of cells or several layers. The diameter of the nuclei of adrenocortical cells is 3.83 ± 0.09 µm.

The chromaffin tissue is forming groups of cells intermingled with adrenocortical cells. The diameter of the nuclei of chromaffin cells is 4.54 ± 0.26 µm.

(iii) Aphanius dispar

Family: Cyprinodontidae

/ A species occurring in freshwaters of Lebanon. Length 4cm. Purchased from aquarium dealers, Riyadh, Saudi Arabia / Plate 7.

The kidney is composed of the trunk-kidney and the discrete head-kidney which consists of two lateral halves forming two lateral wings arteriorly. These wihgs extend latero-ventrally and approach close to the heart. The head-kidney is connected to the trunk-kidney by a thin sheet of kidney tissue. The head-kidney consists of haemopoietic tissue, renal tubules glomeruli and collecting ducts. Only a single posterior cardinal vein drains the trunk-kidney. It is in fact the right posterior cardinal vein, and enters the right wing of the head-kidney after leaving the trunk-kidney. It passes through the head-kidney and descends to the heart as the right duct of Cuvier. The left duct of Cuvier, which drains the left wing of the head-kidney, forms from many tributary branches in the left wing. It is possible that the venous supply to the left wing of the pronephros comes in the form of small branches from the right wing, or even from the left anterior cardinal vein. The dorsal aorta passes medially between the head-kidney wings, then swings to the left side of the trunk-kidney, parallel to the right posterior cardinal vein on the right side.

The adrenocortical tissue is of Nandi's Type I, associated with the posterior cardinal veins and forming a collar around the veins from

one to two cells thick. The diameter of the nuclei of adrenocortical cells is 3.79 ± 0.14 µm.

The chromaffin tissue forms groups of two to four cells interspersed among the adrenocortical cells, i.e. Nandi's Type V. The diameter of the nuclei of chromaffin cells is 4.77 ± 0.27µm.

2. Red Sea Teleosts

The sixteen species of marine teleosts described below were very kindly identified by Dr. Peter Whitehead of the British Museum of Natural History, London.

(i) Chanos chanos سليمانى (Sulaimāni)

Family: Chanidae

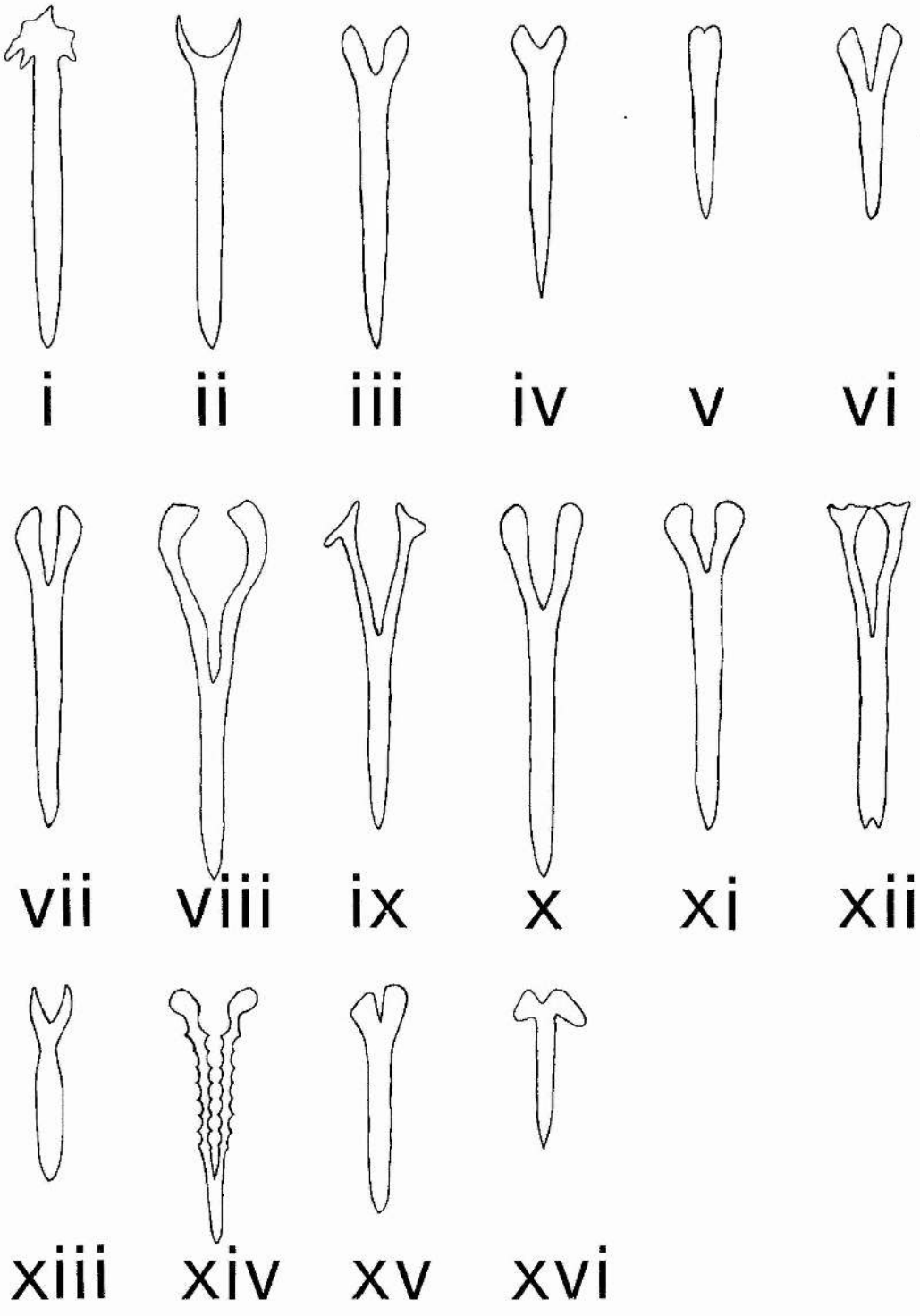
Length 22cm. Figs. 8,9. Plate 8.

The kidney is an elongated organ extending from the heart region to the posterior end of the body cavity. It is thickened in its posterior part, and expanded at its anterior end into a wide region. There is no discrete head-kidney, and renal elements extend throughout.

The adrenocortical tissue is associated with the posterior cardinal veins and their branches, and is sometimes found in the lymphoid tissue, near the veins. It occurs as irregular masses of cells, or in layers two to five cells deep, close to the vein or embedded in its wall. It is intermediate between Nandi's Types I/II. Adrenocortical cells are more abundant on the right side of the kidney than the left. The diameter of the nuclei of adrenocortical cells is 4.36 ± 0.1µm.

Fig. 8. Outline drawings of kidneys of Red Sea teleosts, not all to the same scale, ventral aspect.

No.	Species	Length of kidney cm.
i	Chanos chanos	5
ii	Acanthopagrus bifasciatus	5
iii	Crenidens crenidens	5
iv	Scarus species	4
v	Herklotsichthys punctatus	3
vi	Thalassoma amblycephalus	3
vii	Thalassoma lunare	5
viii	Cheilinus diagrammus	6
ix	Variola louti	5
x	Cephalopholis miniatus	6
xi	Gaterin gaterinus	5
xii	Lethrinus species	5
xiii	Hepsetia pinguis	3
xiv	Siganus species	4
xv	Monodactylus argenteus	3.5
xvi	Zebrasoma veliferum	2.5


i
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iii
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vii
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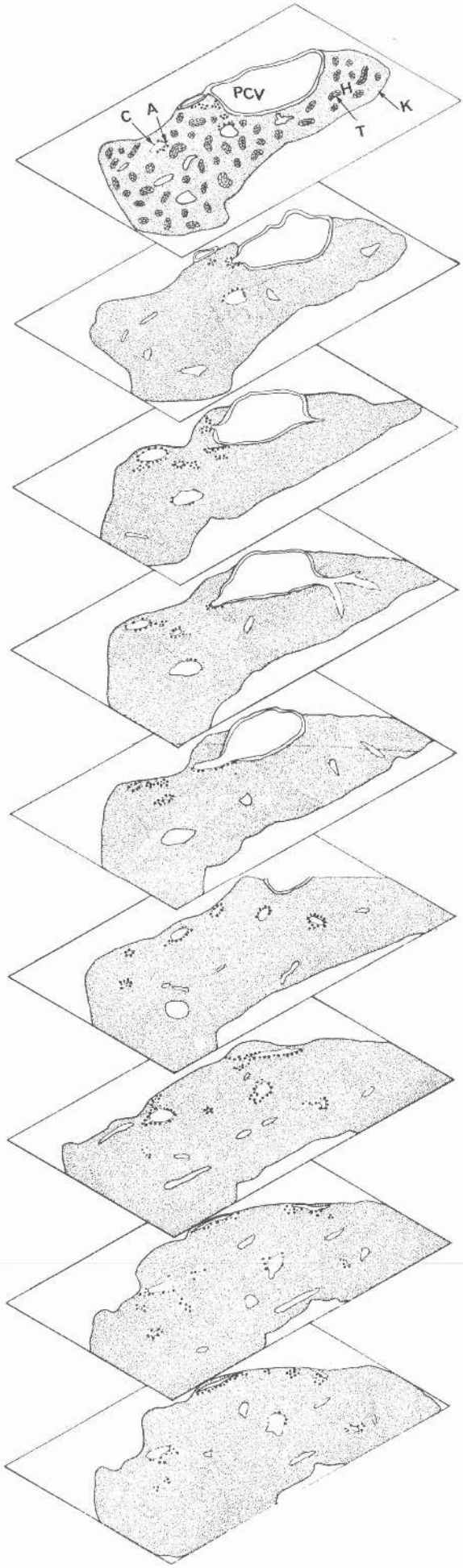
xi
xii
xiii
xiv
xv
xvi

Fig. 9. Isometric drawings of serial transverse sections through anterior region of kidney of Chanos chanos 60 µm apart. Each section is 6 mm. long and 3 mm. wide. Right side of fish on right side of drawing, anterior end at top of page.

(See text pages 19-20).

(Legend as for figure 5. Renal tubules occur in all sections, but are shown in one only).


C
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The chromaffin tissue occurs as small groups of cells, or single cells, interspersed among the adrenocortical cells and are also embedded in the vein walls Nandi's Type IV. They are more abundant in the posterior region of the head-kidney. The diameter of the nuclei of chromaffin cells is 5.0 ± 0.19µm.

(ii) Acanthopagrus bifasciatus ابو كحله (Abū Kuḥlah)

(iii) Crenidens crenidens سمك الجاص (Samak al-Jāṣ (Biṭṭiṭ))

Family: Sparidae

A. bifasciatus Length 21cm. | C. crenidens Length 16.5cm.

Figs. 8, 10. Plate 9 | Plate 10. Fig. 8.

The kidney is divided into two lateral lobes at its anterior end, joined by connective tissue strands. These lobes comprise mainly haemopoietic tissue and represent the head-kidney. In A. bifasciatus the head-kidney contains only a very few renal elements in groups; but in C. crenidens they are much more abundant and scattered throughout the tissue.

The adrenocortical tissue is intermediate between Nandi's Types I and II. It is associated with the posterior cardinal veins and their main branches, forming a mass of tissue round, but not completely encircling them. This mass of tissue is composed of irregular strands of cells separated by large sinusoids and sometimes in tubular arrangement. The strands of adrenocortical tissue occasionally extend into the thick wall of the vein, so that the connective tissue of the vein wall appears between the strands. In A. bifasciatus the diameter of the nuclei of adrenocortical cells is 3.77 ± 0.13 µm. and in C. crenidens is 3.59 ± 0.08 µm.

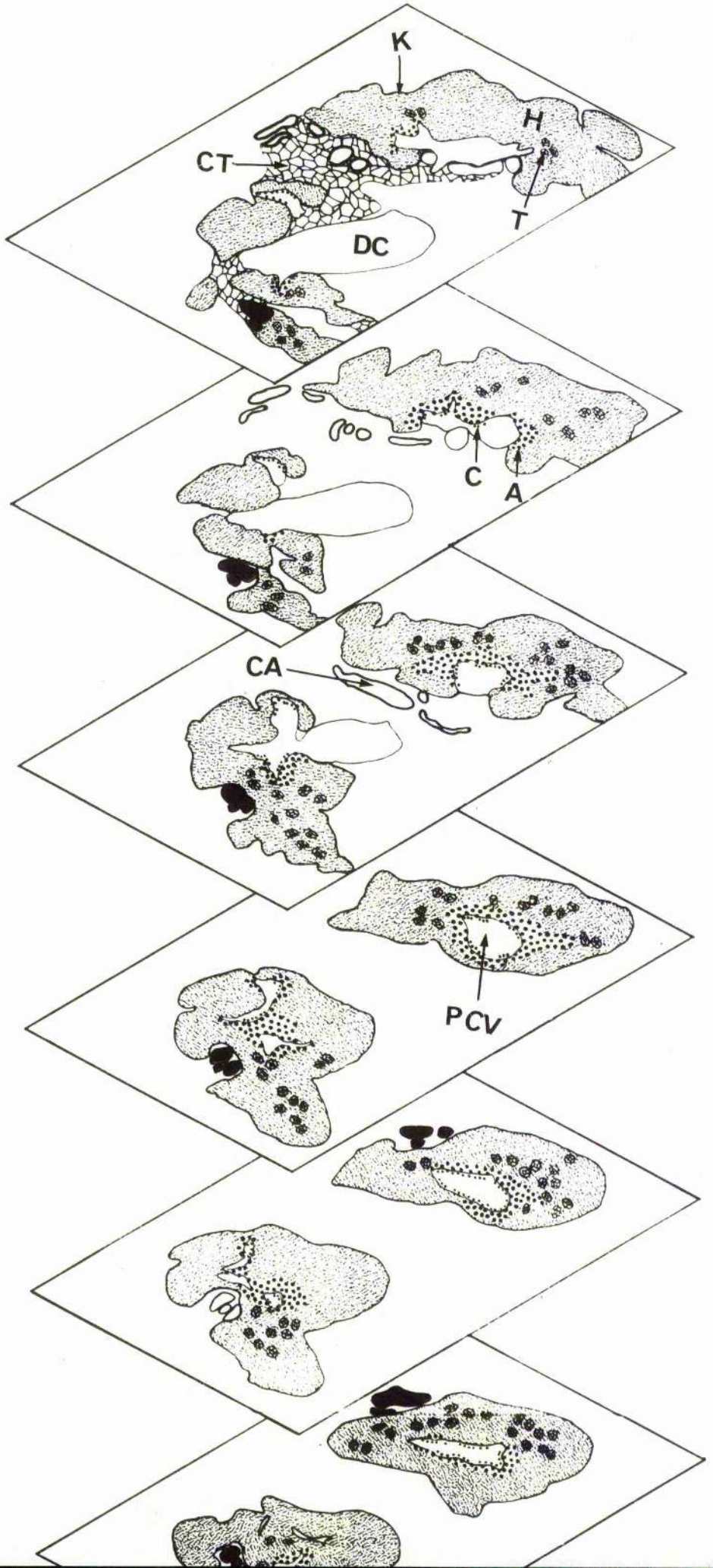
The chromaffin tissue is of Nandi's Type II, embedded in the walls of the posterior cardinal veins and their main branches. In C. crenidens

Fig. 10. Isometric drawings of serial transverse sections through anterior region of kidney of Acanthopagrus bifasciatus, 150 µm apart. Each section is 9 mm. long by 5 mm. wide. Right side of fish on right side of drawing. Anterior end at the top of page.

(See text pages 20-21)

(Legend as for figure 5).

N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chromaffin cells appear in the smaller branches as well. Chromaffin tissue occurs where the veins pass through the mass of adrenocortical tissue as well as in other region of the veins, The chromaffin cells sometimes protrude into the lumen of the vein so it is possible to see chromaffin cells separated by the vein wall from the adrenocortical cells outside it. In A. bifasciatus the diameter of chromaffin cells is 4.38 ± 0.14 µm. and in C. crenidens is 4.61 ± 0.25 µm.

(iv) Scarus species دوالى (Dawālī)

Family: Scaridae

Length 16cm. Plate 11. Fig. 8.

The kidney extends from the heart region to the most posterior end of the body cavity. It thickens towards its anterior end and divides into two lobes at its anterior end. The head-kidney is not externally distinguishable but histologically these two lobes represent it. Renal tubules, glomeruli and collecting ducts extend to the anterior ends of the two lobes. No pigments were observed.

The adrenocortical tissue is associated with the posterior cardinal veins and their major and medium-sized branches, one to three cell-layers in thickness Nandi's Type I/II. They lie in the vein endothelium, protruding into the vein lumen. The diameter of the nuclei of adrenocortical cells is 3.98 ± 0.24 µm.

The chromaffin tissue forms one layer of cells lying separate from the adrenocortical tissue within the vein wall. It is of Nandi's Type II. The diameter of the nuclei of chromaffin cells is 4.92 ± 0.18 µm. The vein wall is very thick, so that the adrenal tissues occupy the inner layers of it but still a thick part of it appears outside them separating them from the rest of the head-kidney tissues.

(v) Herklotsichthys punctatus ساردين (رقطه) (Sardin (Rugṭah))

Family: Clupeidae

Length 10cm. Plate 12. Fig. 8.

The kidney is an elongated organ extending along the dorsal side of the abdominal cavity. It ends anteriorly in the heart region and posteriorly at the end of the cavity. It gradually thickens from the posterior end towards the anterior. The head-kidney is not discrete and can not be recognised externally.

The adrenocortical tissue is of Nandi's Type I, surrounding the posterior cardinal veins and their major branches, forming a collar of one or two cells thick around the vessel. The adrenocortical cells are situated directly under the vein endothelium. The diameter of the nuclei of adrenocortical cells is 2.89 ± 0.13 µm. There is very little connective tissue in the vein wall.

The chromaffin tissue is in the form of a small number of cells scattered through the adrenocortical tissue. It is of Nandi's Type V. The diameter of the nuclei of chromaffin cells is 4.07 ± 0.23 µm.

(vi) Thalassoma amblycephalus مغرد (Mugharrid)

Family: Labridae

Length 9.5cm. Fig. 8. Plate 13.

The kidney occupies the dorsal region of the body cavity, from just posterior to the heart to the posterior end of the cavity. Its anterior third is divided into two lateral halves. The head-kidney cannot be distinguished from the trunk-kidney externally, but it is represented by these two lateral lobes. Histologically, the anterior part of the kidney (in which the adrenocortical tissue lies) is composed of glomeruli, renal tubules, and collecting ducts. The haemopoietic tissue is lymphoid. There are no pigment cells. The

adrenocortical and chromaffin tissues are found in most of the anterior part of the two lateral lobes.

The adrenocortical tissue is intermediate between Nandi's Types I and II, surrounding the posterior cardinal veins and branches. This tissue in the anterior part of the head-kidney forms a mass of tissue covering most of the area and the veins of all sizes run through this mass. The diameter of the nuclei of adrenocortical cells is 3.3 ± 0.14µm.

The chromaffin tissue is of Nandi's Type IV, distributed throughout the adrenocortical tissue and embedded in the vein walls. The chromaffin tissue extends farther posteriorly than the adrenocortical tissue. The diameter of the nuclei of chromaffin cells is 4.2 ± 0.16µm.

(vii) Thalassoma lunare غراب البحر (Ghurāb al-Baḥr)

Family: Labridae

Length 18cm. Fig. 8. Plate 14.

The kidney resembles that of T. amblycephalus but it is longer and has no externally distinguishable head-kidney. The anterior one-quarter of the kidney in this species is divided into two lateral halves which represent the head-kidney.

The adrenocortical tissue and chromaffin tissue are similar to that of T. amblycephalus but they occupy less area in the anterior part of the head-kidney in relation to the size than in T. amblycephalus. The diameter of the nuclei of adrenocortical cells is 3.11 ± 0.2 µm. The diameter of the nuclei of chromaffin cells is 4.3 ± 0.21 µm.

(viii) Cheilinus diagrammus مليص (Malais)

Family: Labridae

Length 25cm. Fig. 8.

The kidney is similar to that of I. amblycephalus. Its anterior half is divided into two halves and these two halves swing away from the midline leaving between them a wide area which is filled by a bundle of muscles. The anterior extremities of these lateral halves represent the head-kidney.

The adrenocortical tissue is of Nandi's Type I, associated with the posterior cardinal veins and their major branches. The diameter of the nuclei of adrenocortical cells is 3.69 ± 0.17µm.

The chromaffin tissue is associated with the posterior cardinal veins and their branches. It lies within the vein walls as individual cells or groups of cells, and extends farther posteriorly than adrenocortical tissue. It is of Nandi's Type II. The diameter of the nuclei of chromaffin cells is 4.71 ± 0.27µm.

(ix) Variola louti لوتى (Louti)

Family: Serranidae

Length 24cm. Fig. 8. Plate 15.

The anterior one-third of the kidney is divided into two lateral halves which comprise the head-kidney. These two lateral lobes end anteriorly at the heart region in triangular projections. There is no external difference between head and trunk-kidney. Histologically, the head-kidney is composed of haemopoietic tissue, very few renal tubules and collecting ducts, various sized clumps of yellow-brown pigment, and adrenocortical and chromaffin tissue.

The adrenocortical tissue is intermediate between Nandi's Types II and III, consisting of irregular cords of cells associated with the posterior

cardinal veins branches and also of cords or strands of cells running through the surrounding tissue. Expanded sinusoids are visible between the cords. The tubular arrangements were observed. The diameter of the nuclei of adrenocortical cells is 3.32 ± 0.13 µm.

The chromaffin tissue is only rarely observed. It comprises individual cells, embedded in the vein walls. The diameter of the nuclei of chromaffin cells is 4.24 ± 0.08µm.

(x) Cephalopholis miniatus (ناجل) ابوعدس (Abū ʿAdas (Nājil))

Family: Serranidae

Length 20cm. Fig. 8. Plate 16.

The kidney is bilobed anteriorly. The head-kidney is not externally distinguishable, but consists entirely of haemopoietic tissue, with a very limited number of renal tubules. It contains variously-sized clumps of yellow-brown pigment.

The adrenocortical tissue is intermediate between Nandi's Types I and III, forming clumps and tubules associated with the largest and medium-sized veins. In some areas the adrenocortical clumps comprise relatively large masses of tissue, penetrated by blood capillaries. The diameter of the nuclei of adrenocortical cells is 3.4 ± 0.14 µm.

The chromaffin tissue is associated with the largest branches of the posterior cardinal veins in the form of individual cells embedded in the vein walls where adrenocortical tissue exists and also with small branches where adrenocortical cells are absent. The diameter of the nuclei of chromaffin cells is 4.45 ± 0.2 µm.

(xi) Gaterin gaterinus قطرين (Gaṭerin)

Family: Plectorhynchidae

Length 17cm. Plate 17. Fig. 8.

The kidney is bilobed. Its two lobes anteriorly end dorsally to the heart. The head-kidney is not externally distinguishable but it is represented by the anterior parts of the two halves of the kidney. Each lobe is composed of two zones, an inner and an outer. The inner zone contains many renal elements and the outer zone consists of haemopoietic tissue and adrenocortical tissue. The posterior cardinal veins run between these two zones.

The adrenocortical tissue is concentrated in the outer zone of the head-kidney lobe in association with the main vein Nandi's Type I. Adrenocortical cells also occur on the inner wall of the vein, but only where the renal elements are not close to the vein itself. The adrenocortical tissue forms mass of tissue containing sinusoids and blood capillaries. The diameter of the nuclei of adrenocortical cells is $2.43 \pm 0.12 \mu m$.

The chromaffin tissue is easy to distinguish, as palely-staining cells embedded in the walls of the posterior cardinal veins and associated also with the small branches, Nandi's Type II. Sometimes the chromaffin cells protrude into the vein lumen. The diameter of the nuclei of chromaffin cells is $3.79 \pm 0.22 \mu m$.

(xii) Lethrinus species سمك الشعور (Samak Al-Shuʿūr)

Family: Lethrinidae

Length 12.5 cm. Plate 18. Fig. 8.

The kidney is anteriorly divided into two lateral halves. These two lobes are thin but they end in the heart region in triangular projections and leave a space between them which is occupied by small

muscle bundle. The two triangular ends meet in the mid-line anteriorly by one of their anglas. The head-kidney, which is represented by the triangular regions and a little further back along the two halves, is not externally distinguishable. The greater part of the anterior lobes, however, consist of haemopoietic tissue, amongst which are groups of renal tubules, glomeruli, and collecting ducts. These renal elements are associated with the veins. No pigment cells were observed.

The adrenocortical tissue is of Nandi's Type I, forming a mass of tubules associated with the posterior cardinal veins. In some areas this mass is expanded into the haemopoietic tissue. The adrenocortical mass is penetrated by blood capillaries. The diameter of the nuclei of adrenocortical cells is 3.71 ± 0.08 µm.

The chromaffin tissue is of Nandi's Type II associated with the posterior cardinal veins and their branches. The chromaffin cells are embedded in the vein walls at different depths and sometimes they protrude into the vein lumen. They occur in the same regions as the adrenocortical tissue and also in isolation. The diameter of the nuclei of chromaffin cells is 4.15 ± 0.19 µm.

(xiii) Hepsetia pihquis كشكوشه (Kashkūshah)

Family: Atherinidae

Length 10cm. Fig. 8. Plate 19.

The kidney is divided anteriorly into two lateral wings, separated from each other by muscle. The wings consist of lymphoid tissue, some glomeruli, tubules and collecting ducts. One or two clumps of pigment were observed in the left wing.

The wings are joined together by two junctions one anterior to the other. The first one unites them dorsally with the dorsal aorta,

which swings to the left side and runs through connective tissue in a channel in the ventral side of the left wing. The second junction starts ventrally to the dorsal aorta. After the posterior junction is complete the kidney increases in width. The head-kidney is represented by these two lateral wings and a little farther back. Anteriorly the head-kidney ends in the heart region and the anterior cardinal vein runs through the right wing for a short distance before joining the right posterior cardinal vein just at the ventral surface of the wing. Only the right posterior cardinal vein was observed, and it runs through the right wing of the kidney and receives a medium-sized vein from the left side of the kidney which passed to the right through the anterior junction referred to above.

The adrenocortical tissue is associated with the anterior cardinal vein as it passes through the right wing of the head-kidney. It does not encircle the vein, but forms an irregular collar. Nandi's Type I. The diameter of the nuclei of adrenocortical cells is 3.73 ± 0.13 µm.

The chromaffin tissue is associated with the right posterior cardinal vein Nandi's Type I. It forms groups of two to four cells or individual cells embedded in the vein wall. The chromaffin cells extend with the vein to the extreme posterior part of the head-kidney. The diameter of the nuclei of chromaffin cells is 4.69 ± 0.21 µm.

(xiv) Siganus species سيجان (Sigan)

Family: Siganidae

Length 13.6cm. Fig. 8. Plate 20.

The kidney is almost entirely divided into two lateral halves. These lobes are joined together at the extreme posterior part of the

kidney and end anteriorly in two lobes. These two lobes represent the head-kidney. Histologically, the head-kidney consists mainly of haemopoietic tissue, though some renal elements are also present in groups, similar to that of Acanthopagrus bifasciatus and Lethrinus species.

The adrenocortical tissue is intermediate between Nandi's Types I and II, situated alongside the main posterior cardinal veins and branches, The main vein is not entirely surrounded by adrenocortical tissue. The adrenocortical tissue forms a mass of tissue at the junction of the branches with the main vein. This mass is composed of irregular cords penetrated by blood capillaries. The diameter of the nuclei of adrenocortical cells is 3.28 ± 0.08 µm.

The chromaffin tissue is of Nandi's Type II, associated with the posterior cardinal veins and their branches; both in association with the adrenocortical tissue, and separately. The diameter of the nuclei of chromaffin cells is 4.63 ± 0.28 µm.

(xv) Monodactylus argenteus ابوقرص (Abū Qurṣ)

Family: Monodactylidae

Length 16cm. Fig. 8. Plate 21.

The kidney is divided anteriorly into two lateral wings, and in the posterior part it forms a single mass of tissue extending to the posterior end of the body cavity. The head-kidney is not externally different from the trunk-kidney, but these two wings represent the part of the kidney which contains the adrenal tissues. Histologically, these wings are composed of haemopoietic tissue and renal elements, but some haemopoietic areas are free of any renal elements. The right posterior cardinal vein has a very thick wall after it comes out of the right wing and it continues close to the ventral surface of the wing

for a short distance. Inside its connective tissue sheathe, which is connected to the kidney, are some groups of adrenocortical cells. The right posterior cardinal vein drains the right side of the whole kidney and receives a vein from the left side when the two lateral wings join together to form the trunk-kidney. The left posterior cardinal vein drains the left side of the kidney not only the left half of the head-kidney but also the trunk-kidney.

Adrenocortical tissue is of Nandi's Type I, associated with the posterior cardinal veins and their main branches. It is embedded in the vein walls in the form of single or groups of two to five cells. The diameter of the nuclei of adrenocortical cells is 3.8 ± 0.17 µm.

Chromaffin tissue is embedded in the vein walls where the adrenocortical cells are present and in the posterior part of the head-kidney where the adrenocortical cells are absent. Nandi's Type II. The diameter of the nuclei of chromaffin cells is 4.21 ± 0.17 µm.

(xvi) Zebrasoma veliferum ابوتنباك (Abū Tinbāk)

Family: Acanthuridae

Length 13cm. Fig. 8. Plate 22.

The kidney is bilobed anteriorly. The two lobes are flat and the left is wider than the right. The rest of the kidney is in the form of extended mass of tissue lying ventrally to the notochord. The head-kidney is not discrete, but histologically it is represented by the two flat lobes and a little farther back. It contains renal elements in the same proportions as in the trunk-kidney.

Adrenocortical tissue is associated with the posterior cardinal veins. The adrenocortical cells are found lying in the vein walls closely applied to the endothelium. They form a single irregular layer

of one to two cells thick, Nandi's Type I. The diameter of the nuclei of adrenocortical cells is 3.61 ± 0.09 µm.

Chromaffin tissue is of Nandi's Type II, individual cells amongst the adrenocortical cells in the anterior part of the head-kidney and groups of two to five cells lying in the vein walls in the posterior part of the head-kidney where there is no adrenocortical tissue. Chromaffin cells are more abundant in the posterior part of the head-kidney than in the anterior part. The diameter of the nuclei of chromaffin cells is 4.28 ± 0.24 µm.

CHAPTER III

PHYSIOLOGICAL ASPECTS OF THE HEAD-KIDNEY

1. Introduction.

(a) Endocrine activity of the head-kidney tissues. Intensive study of the physiological activity of the head-kidney tissues dates from the 1950's, when corticosteroid production by the head-kidney was confirmed (Chester Jones 1957; Chester Jones, Phillips and Holmes 1959; Idler, Schmidt and Ronald 1962; Phillips and Chester Jones 1957; Phillips 1959; Phillips, Holmes and Bondy 1959; Phillips and Mulrow 1959). Histochemical studies identified the adrenocortical cells as steroidogenic. Since then, experimental and observational studies by many workers (reviewed by Idler (1972), Chester Jones, Chan Henderson and Ball (1969) and Chester Jones (1976) have shown that the head-kidney hormones are involved in a wide range of activities - osmoregulation and ionic balance, intermediary metabolism, resistance to stress (perhaps including social stress: Erickson 1967) and some aspects of reproduction, in particular the final maturation and ovulation of oocytes. (Goswami and Sundararaj 1971a).

The general aim of this part of the project is to investigate the relationship (if any) between adrenocortical activity and the reproductive cycle.

(b) Reproduction and adrenocortical tissue.

Many studies have shown some correlation between adrenocortical activity and the reproductive cycle in teleosts (Stanworth 1953; Hane, Robertson 1959; Robertson and Wexler 1959; Robertson, Krupp, Favour, Hane and Thomas 1961; Donaldson and Fagerlund 1968; Idler, Ronald and Schmidt 1959; Goswami and Sundararaj 1971a, Fagerlund and

Donaldson 1969; Fagerlund 1967; Hane, Robertson and Wexler 1966; Fuller et al 1974; Scott 1963; Ball/1976; Fuller, Scott and Fraser 1974; Fuller, Scott and Fraser 1976). In many cases, however, these observations are open to doubt, for three main reasons, i.e.;

(i) The criteria for adrenocortical activity may be subjective, depending on histological changes (which are not easy to quantify) in the adrenocortical tissue.

(ii) The species studied may be one in which other physiological phenomena occur which are difficult to dissociate from reproduction (e.g. the Pacific salmon studied by Robertson and Wexler 1959 undergo extreme changes in osmotic needs during their spawning migration; and they are senescent at this time, dying after spawning has taken place).

(iii) It is now well-established that the level of circulating adrenocortical hormones in the blood is affected by the treatment to which the fish are subjected. Maintenance of specimens in aquaria raises the hormone level, and even the method by which the fish are caught and killed can result in a ten-fold increase in hormone concentration in Coregonus lavaretus (Fuller, Scott and Fraser 1976). So rapid is the rise in hormone concentration in the latter species that the authors consider that a delay of only a few minutes between catching and killing the fish would result in unacceptably elevated hormone levels (Scott, D.B.C. personal communication).

The particular aims of this project are therefore to use objective criteria to study the changes in adrenocortical cells activity in relation to the reproductive cycle of a teleost species which, firstly, has no extraneous metabolic requirements which might obscure adreno-cortical-reproductive interractions, and secondly, which can be caught

and killed with the minimal chance of any artifact changes in the tissues concerned.

2. Materials and Methods.

(a) The species.

The species chosen for this project was the European Minnow, Phoxinus phoxinus L. It is a member of the family Cyprinidae of the Order Ostariophysi (Greenwood et al 1966). It occurs in most freshwaters of Europe, and is widespread in Britain, except for the Highlands of Scotland (Maitland 1972). Minnows are abundant in the middle reaches of rivers, in shallow lakes, and in the littoral regions of deep lakes, where wave action is moderate and the substrate stony. They are less common where aquatic vegetation is dense and the substrate is organic, and in such habitats ~~it is~~ they are replaced by the sticklebacks (Gasterosteus spp.). They are likewise replaced by Brown trout (Salmo trutta .) and Loach (Nemacheilus barbatula) in the uppermost reaches of fast-flowing waters. In certain areas (e.g. round St. Andrews) minnows have disappeared from many waters, possibly as a result of agricultural pollution.

Its abundance and accessibility make the minnow a suitable species for the present study. In addition, its life cycle and in particular its reproductive cycle are relatively simple. The minnow is a strictly freshwater species, spending most of the year in the still waters of ponds and lakes or slow flowing rivers. From May to July the fish generally move upstream to spawn in fast-flowing shallow streams (as in the population studies in this project), though in the absence of suitable streams, they may spawn on stony substrates in still water. In any case, the migration is not far. A further advantage is that the general biology of the species is well documented (Yarrell 1836; Couch 1855;

Day 1880; Regan 1911; Mottram 1922; Bullough 1939, 1940; Frost 1943; Scott 1963; Vladykov 1927-28), and so is its reproductive biology (Mottram 1922; Bullough 1939-40; Frost 1943; Scott 1963) and other related physiological factors e.g. the thyroid cycle (Barrington and Matty 1953) and the pituitary (Barrington and Matty 1955). It is known that the minnow spawns annually for at least four years in succession, and there is no evidence of senescence or change in reproductive potential with age.

(b) The location.

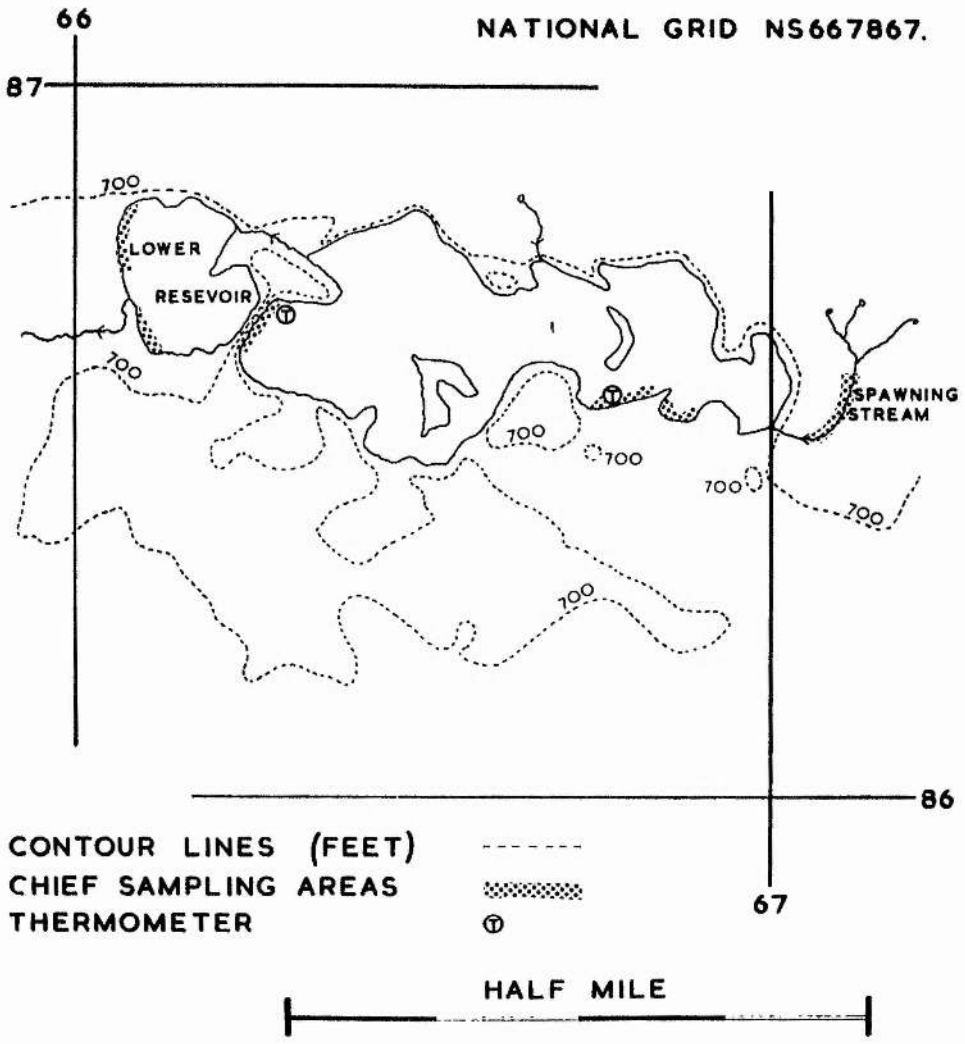
The population chosen for study was the one which inhabits the Walton reservoir in the Gargunnock Hills, Scotland (56° 3' 15" N; 4° 8' 30" W) (Fig. 11). A study of the ecology of this population has been made (Maitland 1972) and of its reproductive cycle, though of females only (Scott 1963). Since such a widely distributed species as the minnow is bound to have local variations in its biology because of differences in latitude, elevation, etc., it is valuable to have accurate background information on the specific population under investigation.

The reservoir is 1.5 Km long with a mean width of 0.4 Km and a maximum depth of 4m. The inflow is from two main streams (in the larger of which the minnows spawn) and seepage from many springs on the hillside above. The reservoir is contained by an artificial dam at its western end, and drains first into a smaller reservoir about 10m lower and thence via the River Endrick, Loch Lomond, and the River Clyde, to the sea. The reservoir contains, besides minnows, brown trout (Salmo trutta L.), eels (Anguilla anguilla L.), three-spined sticklebacks (Gasterosteus aculeatus L.) but no migratory salmonids, as they cannot ascend waterfalls on the Endrick. The entire body of water, including the spawning stream, is easily accessible and so fish should be easy to collect in statistically valid numbers and under controlled conditions. Unfortunately, the size

Fig. 11 Map of Walton Reservoir, showing locatities at which most minnows were cought. Minnows were also collected from the lower reservoir immediately to the west.

WALTON RESERVOIR

56° 3' 15"N, 4° 8' 30"W.
NATIONAL GRID NS667867.



of the minnow population has declined since Scott's study (1963), and it proved difficult, in the present study, to collect sufficient fish. The reason for this decline in numbers has not been investigated, but the Walton Reservoir is intensively managed as a game fishery for brown trout (Salmo trutta) and anglers' records show that the mean weight of individual trout has increased over the past ten years. The mean size of minnows also seems to have increased (Fig. 12). It may be that the same factors are affecting the minnows as the trout, or that the larger trout eliminate by predation, many of the smaller minnows.

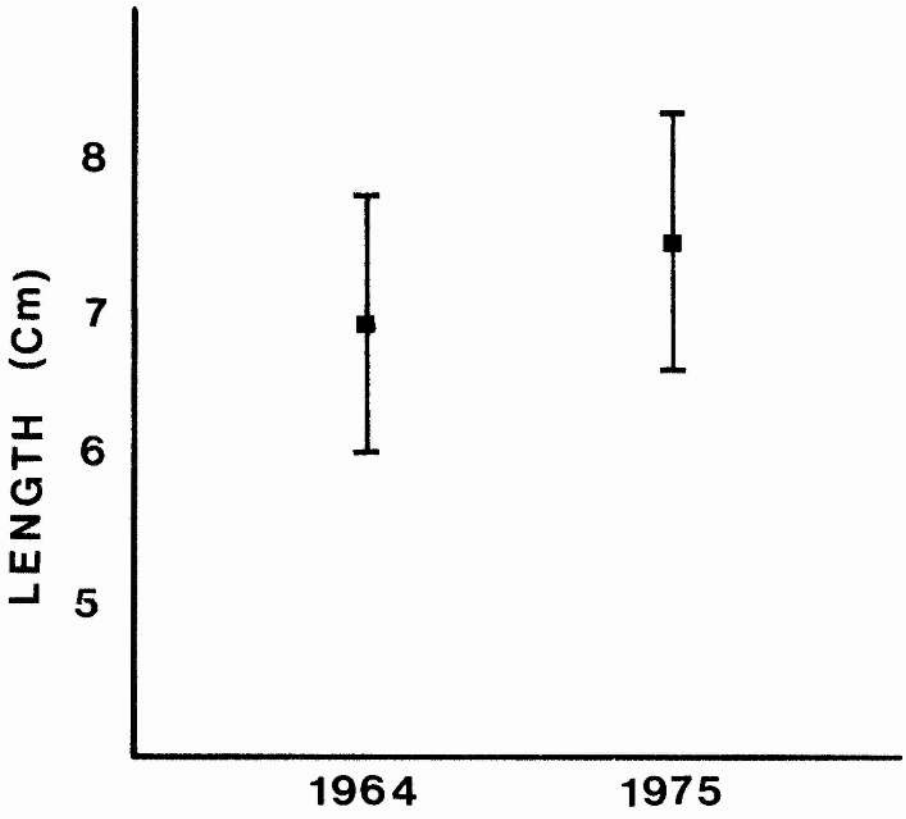
I am most grateful to the Walton Reservoir Angling Society for their permission to collect minnows from the reservoir.

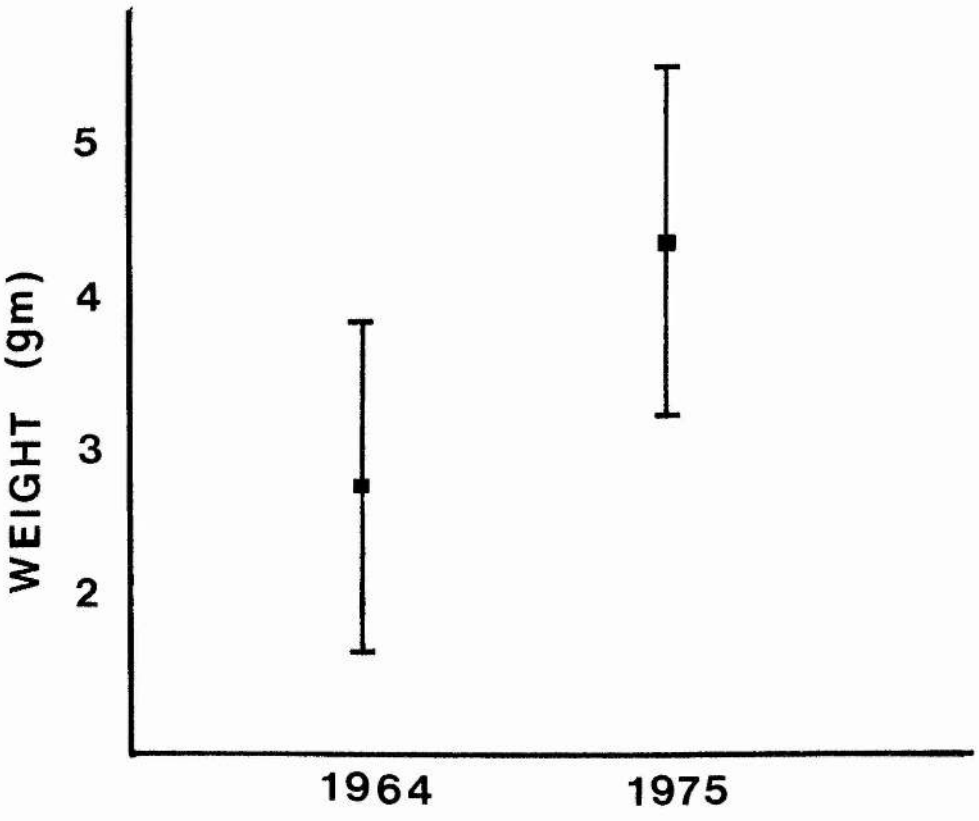
(c) Sampling and Maintenance.

All fish used in this study were caught by electrofishing round the shores of the Walton reservoir in depths of from 0 - 1.5m. The electrofishing device used a rotary transformer with an input of 18v from three 6v 12 a-h lead-acid accumulators. The output of 450v was pulsed by means of a mechanical make-and-break which was variable from 20 - 200 pulses per second. 40 - 50 pulses per second proved most effective for minnows in the size range 5-10cm. The current on/current off ratio per pulse was also variable, and the optimum was about 50/50. The two electrodes were mounted 0.5m apart on a single hand-held pole. With this equipment the fish, which are usually to be found hiding amongst large stones, are first attracted towards the negative electrode from a range of up to 1-2m, and they are then stunned as soon as they swim to within 10-20 cm and can be collected in a hand-net.

Fish required alive were transferred to stainless steel containers 40cm x 27cm x 30cm., where they generally recovered within one minute. Occasionally fish failed to recover, probably as a result of contact with the electrodes. To determine the rate of acclimatization and whether

Fig. 12 Size of minnows caught in Walton Reservoir in May, June and July 1964 and 1975 compared, showing an increase in both length and weight in 1975.


LENGTH (Cm)
8
7
6
5
1964
1975


WEIGHT (gm)
5
4
3
2
1964
1975

delay in killing the fish resulted in histological and endocrine changes, as have been reported by Fuller, Scott and Frazer (1976), minnows were maintained in fibreglass tanks 2m x 2m x 1m deep at the Walton reservoir supplied with running water from the hillside. They were fed with commercial trout food (fishmeal). Samples were killed after one day, seven days, thirty days and three months. The sample of three hours were killed after three hours from catching.

Fish required for light microscopy were transferred either:

(i) to s solution of the anaesthetic MS222, 0.001% in reservoir water, for one-two minutes, after which the abdomen was opened and they were immersed in Bouin's fixative for twenty-four hours, or

(ii) directly, while still under electronarcosis, to Bouin's fixative.

Fish required for electron microscopy (Chapter IV) were killed either in MS222 or by decapitation, the abdomen opened and flooded with buffered glutaraldehyde fixative and the head-kidney was dissected out, fixed for one hour in glutaraldehyde and post-fixed in osmium tetroxide (see page 57). Samples were taken during two years:

(i) 1964 collected by D.B.C. Scott by electrofishing MS222 anaesthesia, fixed in Bouin and stored in 70% ehanol.

(ii) 1975-76 collected by the author. I am grateful for the occasional help of Mr. R. Dempsey and Mr. D. Roche during these collections.

Samples were taken at monthly or fortnightly intervals (sometimes more often if catches were poor), and the aim was twenty-five fish per sample. The actual numbers caught were:

Month	1964-65	1975-76
January	___	20
February	___	27
March	81	31
April	74	55
May	100	47
June	102	40
July	94	57
August	53	42
September	___	30
October	22	6 (♂)
November	25	23
December	23	20

Only fish of 60mm total length and over were taken, as specimens of this size in this habitat, are invariably adult.

(d) Assessment of the reproductive cycle.

An excellent background to the present study is available in a study of the reproductive cycle of female minnows in the Walton reservoir (Scott 1963) (Fig. 13). In the present study both males and females were examined. In the case of the males, the state of the reproductive cycle was assessed by the gonadosomatic ratio. In the case of the females, in addition to the gonadosomatic ratio, the proportions of the various oocyte stages present was measured, as this gives a finer distinction between the reproductive phases. An indication pf the ratio of body weight to length was obtained by calculating the condition factor and the somatic condition factor (see below) for each fish.

After fixation in Bouin for twenty-four hours, the minnows were dehydrated in 30% ethanol (3 hours); 50% ethanol (3 hours) and 70% ethanol (several changes). After two-four days each fish was measured to the nearest mm, the total length from snout to fully-extended caudal fin being taken. The gonads were then dissected out and after drying the fish superficially with tissues, the fish and its gonads were weighed to the nearest mg. The head-kidneys were next dissected out. Gonads and head-kidneys were embedded and sectioned. As previously described (pp 11,12) and particular care was taken to ensure that the histological procedures were identical in all cases, to avoid variations which might invalidate comparisons between individual fish (see p. below). The following criteria were used to assess the annual cycle of the fish:

(i) The condition factor of each fish was calculated as an index of "Fitness" (Scott 1963)

$$\frac{\text{total weight (g.)} \quad 10^2}{\text{total length}^3 \text{ (cm)}}$$

(ii) The somatic condition factor $\frac{\text{total weight - gonad weight (g.)}}{\text{total length}^3 \text{ (cm)}} 10^2$ as a percentage of each fish was also calculated. This excludes the weight of the gonad from the formula, and so gives an indication of changes in body reserves unobscured by the considerable variations in the weight of the gonad (Scott 1963).

(iii) The gonadosomatic ratio $\frac{\text{(gonad weight (g.))}}{\text{(total weight (g.)}} 10^3$ expressed as a percentage). This gives an indication of gonad development relative to body weight, and provides a reasonably sound assessment of the reproductive stage of the animal. However, a more accurate assessment in the case of females is provided by:

(iv) The proportions of the various stages of oocyte/spermatocyte development.

Sections of selected gonads (see below) were cut at a thickness of 6-8 µm, and every fifteenth section was stained in Masson Trichrome stain (p. 12), to a total of 6-8 sections per gonad. The numbers of oocytes at each of four stages of development were counted, viz:

(a) Primary oocytes (Pre-vitellogenic)

(b) Secondary oocytes (Vitellogenic)

(c) Ova (yolk-filled, mature oocytes)

(d) Atretic oocytes (undergoing resorption)

and the results expressed as the percentage of each stage present per ovary. In practice it was found that the reproductive cycle of males could be satisfactorily assessed by gonadosomatic ratio alone, and therefore the proportions of the spermatocyte stages were not accurately measured, but were estimated subjectively.

As this process is very time-consuming, not all fish in each sample were so examined. The fish selected for gonad examination were:

(a) The fish whose gonadosomatic ratio came nearest to the mean value for the sample.

(b) The fish with gonadosomatic ratio directly above (a)

(c) The fish with gonadosomatic ratio directly below (a).

(d) The fish with the highest gonadosomatic ratio.

(e) The fish with the lowest gonadosomatic ratio.

Five males and five females from each monthly or fortnightly sample were so examined in the 1964 collections; and ten males and ten females from each sample in the 1975-76 collection. These fish were the fish with gonadosomatic ratio came nearest to the mean value for the sample and from the fish with gonadosomatic ratio directly above or below the mean value of the gonadosomatic ratio.

(e) Assessment of adrenocortical and chromaffin tissue activity.

Ideally, measurement of circulating corticosteroids in the plasma would give a good idea of adrenocortical activity. Several such techniques are now available, including double isotope dilution (James, Townsend and Fraser 1967.; Fraser and James 1968; Arnold and James 1971;) competitive protein binding (Murphy and Patee 1964; Murphy 1967; Leyendecker, Wardlaw and Nocke 1972); radio immunoassay (Mayes, Furuyama and Nugent 1970; MiKhail, Wu, Ferrin and Vaude-Wiele 1970; Leyendecker, Wardlaw and Nocke 1072; Martin and Nugent 1973); gas-liquid chromatography (Fraser, Wilson and Holmes 1973; Fuller, Mason and Fraser 1976; Fuller 1974) and fluorimetry (Mattingly 1962). The small size of the minnow, however, rules out these methods because of the very small samples of blood available. To use a larger species would not necessarily solve this problem, because it has been shown (Fuller, Scott and Fraser 1976) that corticosteroid levels may rise rapidly as much as ten-fold in response to certain catching and killing techniques, so that there is a great advantage in dealing with a species which can be caught under controlled conditions with minimum stress. Fuller, Scott and Fraser for example, were forced to catch their coregonids by gill-netting, despite the fact that this method is known to elevate plasma cortisol. Phoxinus can in fact be caught by electrofishing throughout the year.

An alternative way of determining adrenocortical activity is to study histological changes in the adrenocortical tissue. Until the recent development of plasma steroid measuring techniques, indeed, this was the only method. Many histological criteria are unfortunately vague or subjective, however, and it was considered to be essential to use criteria which were objective, One such criterion, which has been repeatedly used by many workers is the measurement of nuclear size (diameter/area/volume) of adrenocortical cells (Stanworth 1953; Hanke, Bergerhoff and Chan 1967; Hanke and Chester Jones 1966; Robertson and Wexler 1959; Honma 1960).

It is known that there is no storage of hormones in the steroidogenic cells, so that intense synthetic activity would be expected when they are stimulated. In practice, in Phoxinus, nuclear volume may increase by two times. The exact cytological changes associated with this change in volume are unknown; in the final part of this thesis a study of the fine structure of adrenocortical cells is made, partly in an attempt to explain these changes.

The head-kidneys of the five males and five females which were selected for the gonads examination from the 1964-65 collections and the ten males and females of the 1975-76 collections, were serially sectioned at 4-5 µm. Great care was taken to ensure that all specimens underwent exactly the same procedures, so as to ensure that no artefact variations would be introduced into the measurements. Times in the embedding solutions were strictly adhered to, and 57° M.P. "Fibrowax" (A.R. Horwell) was used throughout. Every fifteenth and sixteenth sections was taken and from sixteen to twenty sections per fish were stained in Masson's trichrome and examined.

The nuclei of thirty to forty adrenocortical cells, and the nuclei of thirty to forty chromaffin cells were measured using a Watson Shearing Eyepiece(Casartelli 1965). Only nuclei which were clearly complete or nearly so, were measured, and the minimum diameter was measured in any cases where the outline of the nucleus was not circular.

As a preliminary exercise to locate and identify the adrenocortical and chromaffin tissue, the head-kidneys of eight fish were serially sectioned at 4-5 µm and all sections were stained for a basic study of the morphology of the head-kidney. Eight fish were additionally sectioned, with the head-kidney *in situ*, at 5-6 µm, and all sections stained.

3. Results

(a) Histology of the gonads.

The gonads in both sexes of Phoxinus phoxinus are paired elongated organs lying in the dorsal region of the body cavity, ventral to the swimbladder. The weight of the gonads varies cyclically during the year (Table 1).

The much higher mean values in 1975-76 compared with that in 1964 are associated with the fact that the mean size and weight of minnows also increased in that period, (Tables 2,3 Fig. 12).

As the histology of the gonads of Phoxinus has already been described (Bullough 1939; Scott 1963 (Females only)), only a brief description will be given here.

(i) The Ovary: (Plates 23,26). The ovaries of Phoxinus are solid structures, with no hollow core. In general five stages of oocyte development can be distinguished:

(i)	Oogonia	previtellogenic
(ii)	Primary oocytes	
(iii)	Secondary oocytes	vitellogenic
(iv)	Ova	
(v)	Atretic oocytes	resorptive

(i) Oogonia (Plate 23,26). The oogonium measures up to 8µm in diameter and stains deeply with Masson's Trichrome.

(ii) Primary oocytes. (Plate 23,26). Primary oocytes grow up to 250 µm in diameter. They have densely staining granular cytoplasm, and a large and conspicuous chromaphobic nucleus with peripheral acidophilic nucleoli. For the purposes of this study, counts of oocyte numbers did not distinguish between oogonia and primary oocytes; they were grouped together as a single group of "previtellogenic oocytes".

(iii) Secondary oocytes. (Plate 23,26). Secondary oocytes range from 250 µm to 500 µm in diameter. Yolk-precursor globules appear at the

periphery and spread gradually towards the centre. The cytoplasm loses its great affinity for stain, and the yolk-precursors take up the light green of Masson strongly. Inside the layers of the follicle a vitelline membrane, the oolemma (=zona radiata) is formed, strongly acidophitic and with marked radial striations.

(iv) Ova. (Plate 23,26) Ova grow to a maximum diameter of 1.4mm, and are recognised by the presence of red-staining yolk deposits. Yolk formation, unlike yolk-precursor formation, is centrifugal. There is no final fusion of the individual yolk globules before ovulation as described by Bower and Halliday (1961).

(v) Atretic oocytes. (Plate 23,26). Atretic oocytes are rare and do not occur in nature except during and after the spawning period. They are formed by the degeneration of unspawned ova and secondary oocytes which are being resorbed.

(ii) The Testis: (Plates 24,27)

The testes of Phoxinus phoxinus are paired elongated structures enclosed in a thin fibrous membrane. Dorsally the fibrous membrane is thickened, and contains the vas deferens, and the spermatic artery and vein. The seminiferous tubules are of varying sizes and shapes, with thin membranous walls. The seminiferous tubules radiate from the vas deferens, at right angles to the long axis of the testis. They end blindly at the periphery of the testis.

The spermatogenic elements of the testis lie in discrete cellular nests inside the seminiferous tubules. The cells in a single nest are all at the same spermatogenic stage (except that spermatogonia are generally present throughout). In general six stages of spermatocyte development can be distinguished:

(i) Primary spermatogonia

(ii) Secondary spermatogonia

(iii) Primary spermatocytes

(iv) Secondary spermatocytes

(v) Spermatids

(vi) Spermatozoa

(i) Primary spermatogonia (Plates 24,27) The primary spermatogonia are derived from the germ cells which are observed at all times of the year lying along the walls of the seminiferous tubules. The nuclei of the germ cells are measured 7.27 + 0.68 µm in diameter and the nucleoli 2.15 + 0.17 µm. The primary spermatogonia are smaller than the germ cells. Their nuclei are about 5.35 + 0.47 µm in diameter and the nucleoli about 1.73 + 0.15 µm. The nucleus has more chromatin than the nucleus of the germ cell.

(ii) Secondary spermatogonia: (Plates 24,27). Nuclear diameter is reduced to 4.24 + 0.37 µm and the cytoplasm is shrunk and stains more deeply. The nucleoli measure 1.3 + 0.09 µm in diameter. The chromatin can be recognised.

(iii) Primary spermatocytes. (Plates 24,27). The cytoplasm and nucleus are even smaller and more deeply staining. The nuclear diameter is reduced to 3.11 + 0.25 µm and the chromatin material is contracted to one side of the nucleus.

(iv) Secondary spermatocytes: (Plates 24,27). This is a transient stage in spermatogenesis. The nuclei are smaller (about 2.29 + 0.13 µm in diameter) and stain more deeply than all the earlier stages. Their granular chromatin distinguished them from the nuclei of spermatids.

(v) Spermatids: (Plates 24,27). Spermatids are produced by the second maturation division. The cell is spherical, stain solidly with Masson's trichrome and measure 1.88 + 0.05 µm in diameter. Spermatids are the final stage of development which takes place within the wall of the seminiferous tubules.

(vi) Spermatozoa: (Plates 24,27). Spermatozoa are found in the lumen of seminiferous tubules. The diameter of the nucleus (or head) is 1.58 + 0.07 µm. They are the smallest cells in spermatogenesis and the most intensely-staining.

(b) The annual reproductive cycle

(i) General.

The spawning period of Phoxinus phoxinus in the Walton Reservoir is from May to July. In early May small numbers of minnows migrate upstream (Fig. 11), but the peak of the spawning season is in June. During the whole spawning period, male minnows in full nuptial coloration are abundant in the upper reaches of the spawning stream, suggesting that there is a large population resident there throughout the period. Females are less common, suggesting that this sex stays on the upper reaches only for long enough to spawn.

Nuptial colours and associated characters (as described by Vladykov 1927; Frost 1943; Scott 1963) first appear in late April, but are not general until May. They persist, decreasing in intensity, until late August or early September. The normal colour of adult minnows is dark brown on the back and sides down as far as the lateral lines and white with a silver sheen on the ventral surface (Plate 25). During the spawning period the dorsal surface darkens and becomes suffused with metalic green; the belly becomes bright red with milky white patches at the bases of the fins, and white tubercles develop on the dorsal surface of the head. Both sexes develop this coloration, but it is much more intense in the male (Plate 25). The typical red belly of the nuptial livery sometimes reappears in fish caught in winter; no other feature of the nuptial coloration is seen at this time.

After spawning, during August and September, minnows become difficult to catch, and it is presumed that they move to the inaccessible deep

waters of the reservoir. Many fish caught during this time are infected with Saprolegnia and have open wounds in the skin. From October until the following spring season minnows are found under heaps of stones round the edge of the reservoir in water from 0-2m deep. According to Scott (1963) the minnows remain in darkness, under the stones during the daylight hours, but emerge into open water after dark. When the water temperature rises to 8-9°C (generally in March) the fish begin to swim in open water during the daylight hours also. This change in behaviour is accompanied by a sudden increase in the bulk of the gonads. Shortly thereafter the fish migrate upstream, and spawning takes place.

(ii) Females: [The annual reproductive cycle of female minnows in the Walton Reservoir is shown in Figs. 15,17 (1975-76); Figs. 14,16 (1964) and for comparison, the results of Scott (1959-61) have been redrawn in Fig. 13 (Scott 1963)]

The gonadosomatic ratio (Figs. 14,15) shows a regularly recurring cycle, with the lowest value in August in all years studied. There is a gradual increase in gonadosomatic ratio during the autumn months, followed by a period of inactivity during the winter. In March the gonadosomatic ratio begins again to increase, but in late spring there is a sudden rise to the highest values for the year, in May and June, the spawning period. [Scott (1963) ascribes this rapid phase of ovarian growth to photostimulation induced by a temperature-mediated change in the minnows' behaviour].

Corresponding changes are seen in the distribution of the various oocyte stages in the ovary (Figs. 14,15). Previtellogenic oocytes are abundant throughout the year, seldom accounting for less than 40% of the oocytes present in a section. This percentage, moreover, is clearly an underestimate because it is based on a two-dimensional count, and takes no account of the difference in volume between previtellogenic and later oocytes. The highest percentage of previtellogenic oocytes occurs immediately after spawning, in August. At this time the ovary contains

Fig. 13 Reproductive cycles of female Phoxinus phoxinus in Walton Reservoir from December 1959 to July 1961, showing minimum and maximum temperature, hours from sunrise to sunset, condition factor and somatic condition factor, gonadosomatic ratio ±1 standard deviation, and percentage oocyte stages. Redrawn from Scott 1963.

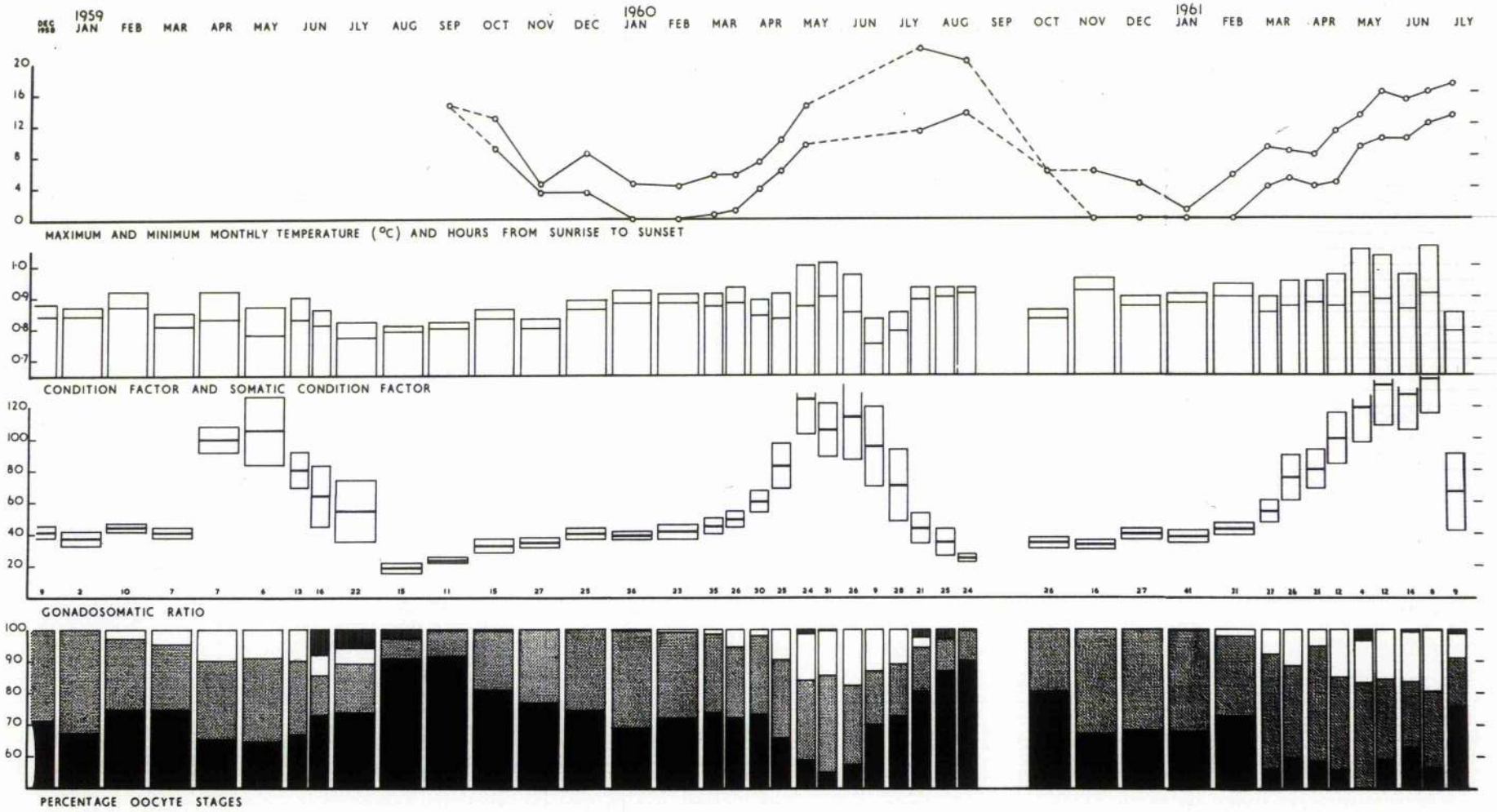

DEC 1958
1959
JAN FEB MAR APR MAY JUN JLY AUG SEP OCT NOV DEC
1960
JAN FEB MAR APR MAY JUN JLY AUG SEP OCT NOV DEC
1961
JAN FEB MAR APR MAY JUN JLY
20
16
12
8
4
0
MAXIMUM AND MINIMUM MONTHLY TEMPERATURE (°C) AND HOURS FROM SUNRISE TO SUNSET
1·0
0·9
0·8
0·7
CONDITION FACTOR AND SOMATIC CONDITION FACTOR
120
100
80
60
40
20
9 2 10 7 7 6 13 16 22 15 11 15 27 25 36 23 35 26 30 25 24 31 26 9 28 21 25 24 26 16 27 41 31 27 26 31 12 4 12 16 8 9
GONADOSOMATIC RATIO
100
90
80
70
60
PERCENTAGE OOCYTE STAGES

Fig. 14 Gonadosomatic ratio of females Phoxinus phoxinus during 1964 ±1 standard deviation.

Percentage oocyte stages of female Phoxinus phoxinus during 1964.

Black = primary oocytes

Coarse stippling = secondary oocytes

Fine stippling = ova

White = Atretic oocytes.

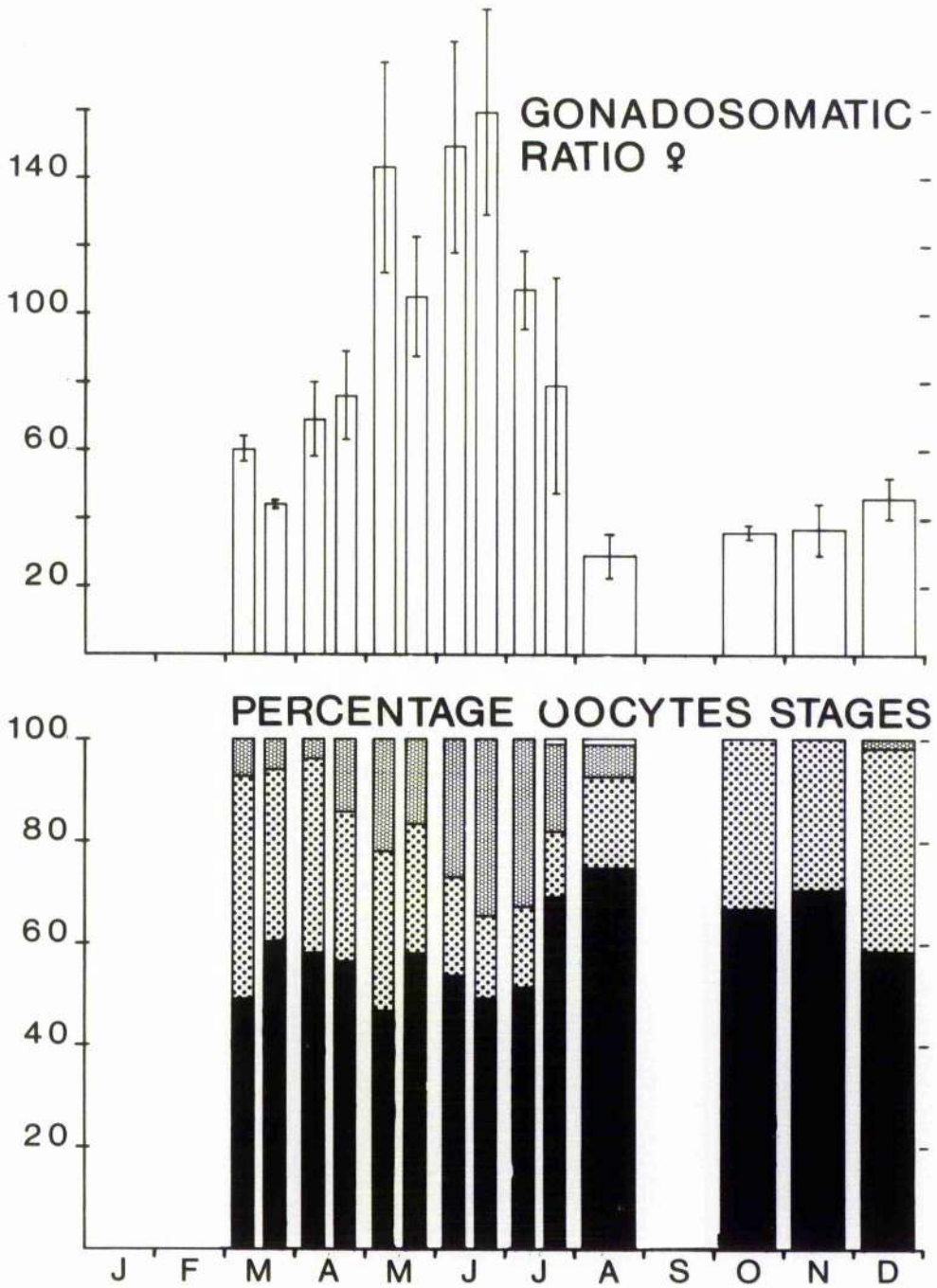

GONADOSOMATIC RATIO ♀
140
100
60
20
PERCENTAGE OOCYTES STAGES
100
80
60
40
20
J
F
M
A
M
J
J
A
S
O
N
D

Fig. 15 Gonadosomatic ratio of females Phoxinus phoxinus during 1975–76 ±1 standard deviation.

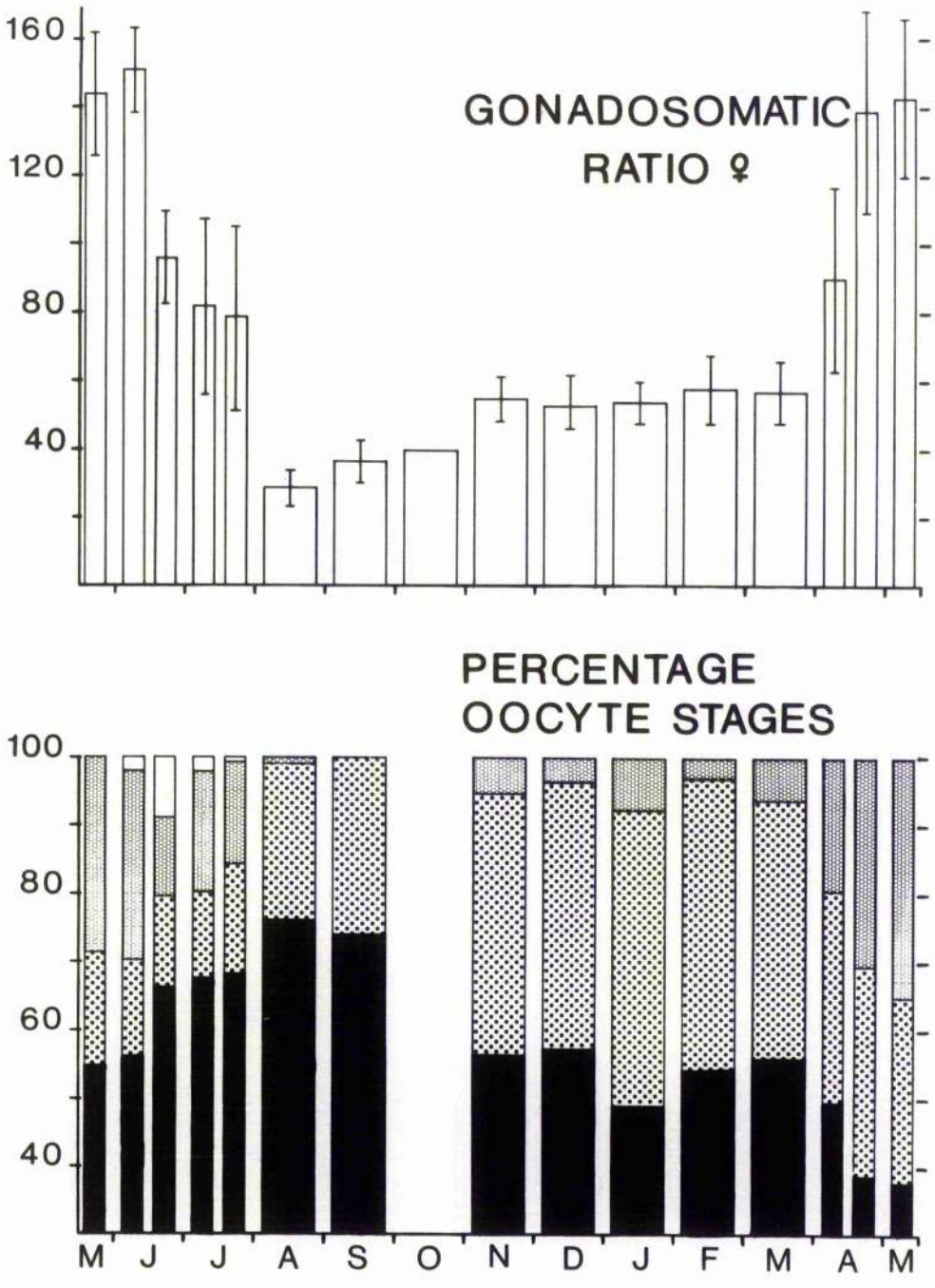
Percentage oocyte stages of females Phoxinus phoxinus during 1975–76.

Black = primary oocytes

Coarse stippling = secondary oocytes

Fine stippling = ova

White = Atretic oocytes.


GONADOSOMATIC
RATIO ♀
160
120
80
40
PERCENTAGE
OOCYTE STAGES
100
80
60
40
M
J
J
A
S
O
N
D
J
F
M
A
M

only previtellogenic oocytes, later (vitellogenic) oocytes which are being resorbed (Atretic oocytes) and the empty follicles from which ripe ova have been ovulated (Plates. 26).

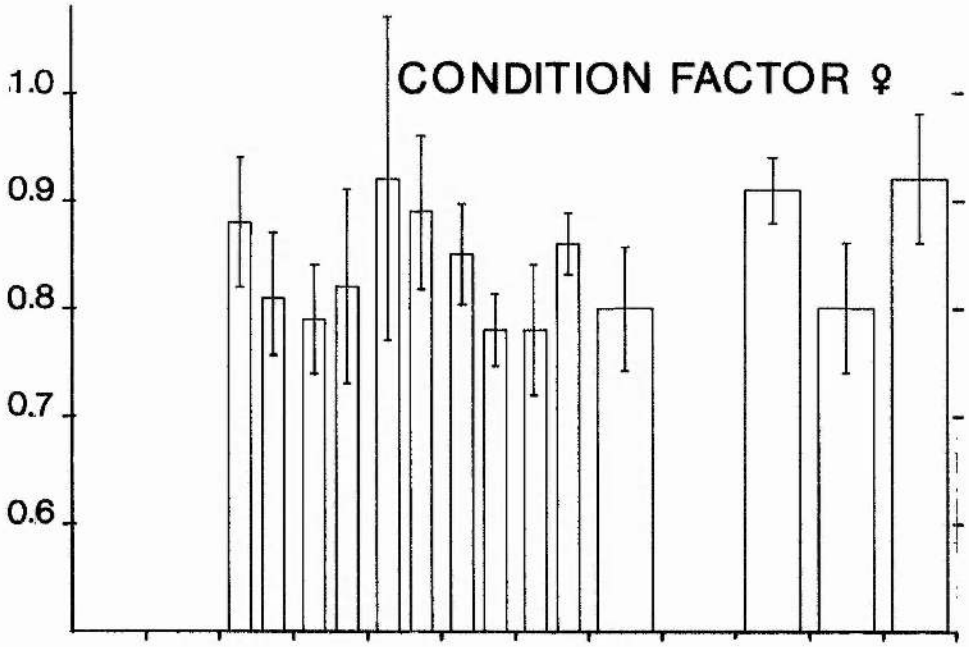
Atretic oocytes occur chiefly during the spawning period, late May at the earliest, and they reach their maximum percentage after spawning late June in 1975 and late July in 1964 (Figs. 14,15). Atretic oocytes occur occasionally at other times of the year, but in low numbers. [Scott (1963) has suggested that oocytes may become atretic when fish are under stress; this may account for their presence outside the spawning season].

Secondary oocytes also appear in the figures (Figs. 14,15) throughout the year, but this is due to the fact that the figures represent the mean values of several fish, where spawning periods have not coincided exactly. In individual fish, after spawning, any remaining secondary oocytes become atretic and are resorbed, so that the ovary contains only previtellogenic oocytes. Secondary oocytes increase in number in autumn (so that their development cannot be dependent on increasing daylength) and again in early spring (Plate 26). Later in spring, however, their numbers stabilise (Fig. 14) or even fall (Fig. 15) as ova develop. Ova appear in very small numbers in autumn 1964, and more abundantly, in 40% - 50% of all fish in the sample in 1975-76, though in neither case in sufficient numbers as to be apparent in the figures (Fig. 15). In 1959-61, ova were rare in autumn, developing only in very few fish (Scott 1963). Ova develop most rapidly in late spring, immediately prior to spawning (Plate 26).

The condition factor and somatic condition factor show less clear cyclical changes (Fig. 16,17). The difference between these two measurements reflects the cyclical changes in gonad weight. The somatic condition factor, which ignores gonad weight, gives a good indication of the state of the fish's body tissue reserves. In 1964 the lowest value

Fig. 16 Condition factor of female Phoxinus phoxinus during 1964, ±1 standard deviation.

Somatic condition factor of female Phoxinus phoxinus during 1964, ±1 standard deviation.


CONDITION FACTOR ♀
1.0
0.9
0.8
0.7
0.6

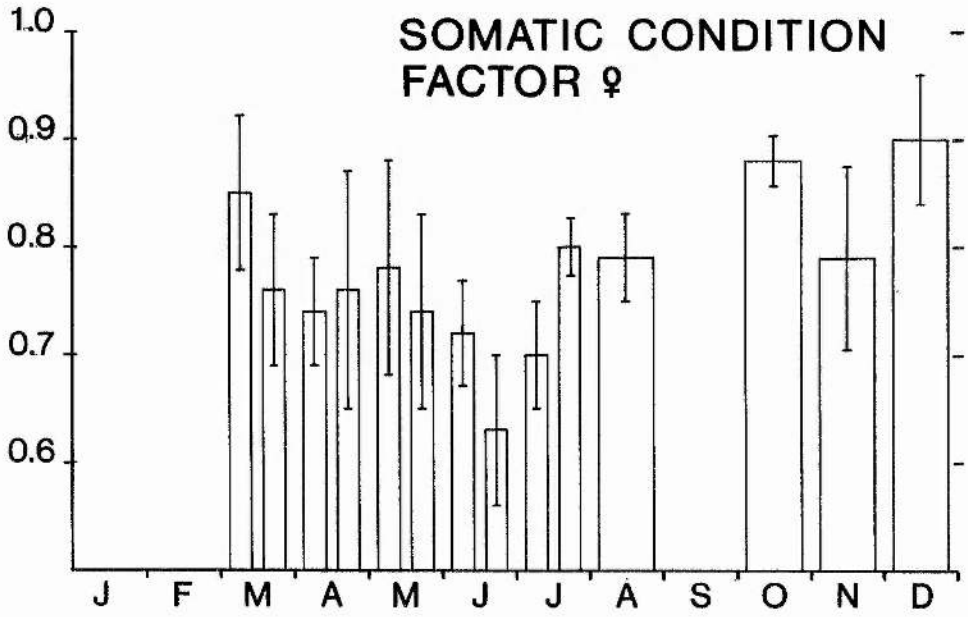
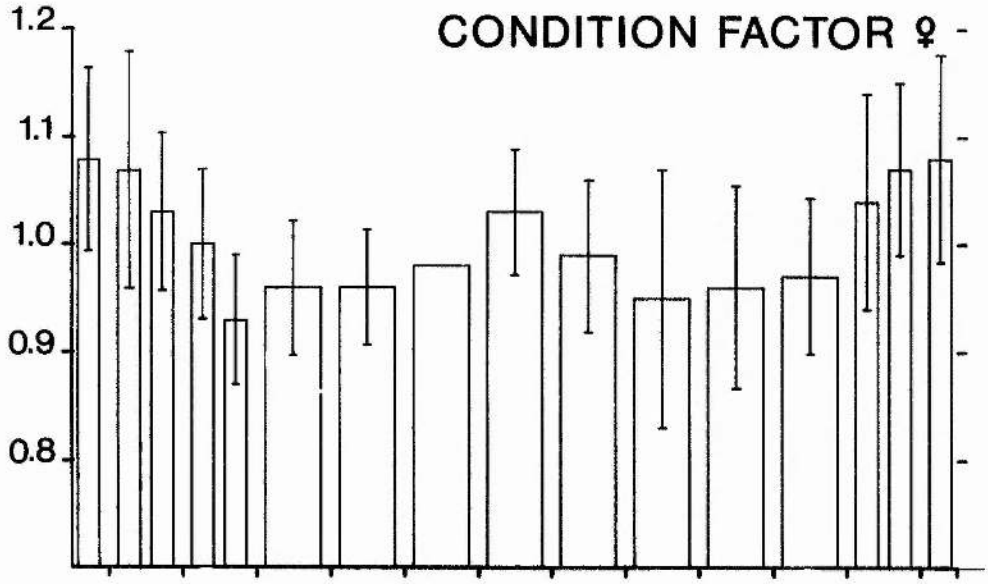
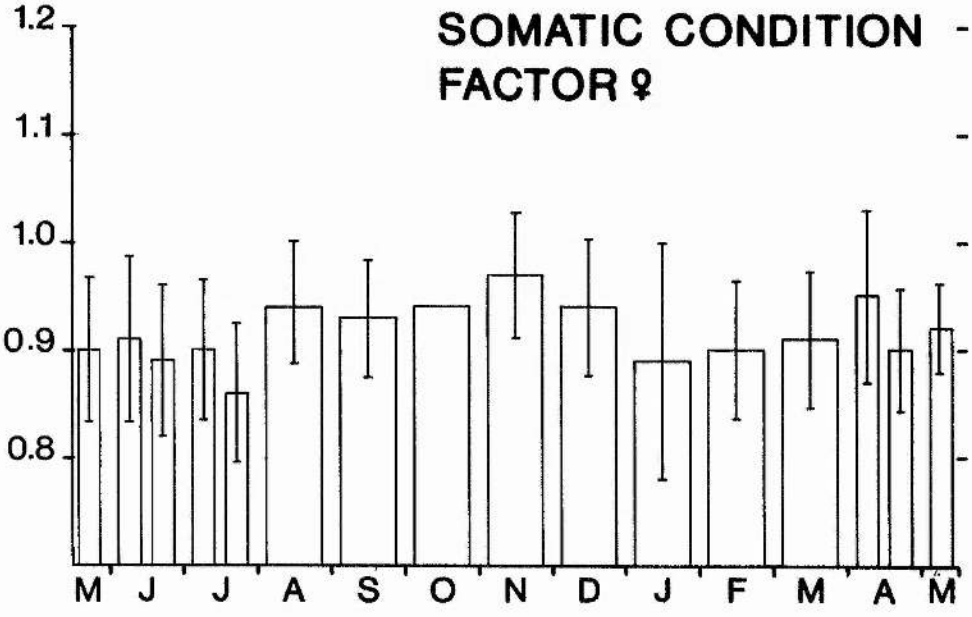

SOMATIC CONDITION FACTOR ♀
1.0
0.9
0.8
0.7
0.6
J
F
M
A
M
J
J
A
S
O
N
D

Fig. 17 Condition factor of female Phoxinus phoxinus during 1975-76, ±1 standard deviation.

Somatic condition factor of female Phoxinus phoxinus during 1975-76, ±1 standard deviation.


CONDITION FACTOR ♀
1.2
1.1
1.0
0.9
0.8


SOMATIC CONDITION FACTOR ♀
1.2
1.1
1.0
0.9
0.8
M J J A S O N D J F M A M

of somatic condition factor (0.63 + 0.06) occurred in June and in 1975 (0.86 + 0.06) in July. In 1959-61 (0.75) in June or July. Low condition factors are generally associated with the end of the spawning period (Scott 1974; Scott and Fuller 1976; Fuller, Scott and Fraser 1976).

(iii) Males [The annual reproductive cycle of male minnows in the Walton Reservoir is shown in Figs. 20,22 (1975-76); and Figs. 18,21 (1964). No comparable data for 1959-61 are available]

The gonadosomatic ratio (Figs. 18,20) shows a cycle not unlike that of females, with a minimum value in August. Slight gonad growth in autumn, a relatively quiescent period in winter, resumed growth in early spring, and accelerated growth immediately prior to spawning.

In the weeks following spawning, the testis passes through a resting stage which consists mainly of primary spermatogonia and secondary spermatogonia (Plate 27). A very small percentage of primary spermatocytes may be present. Spermatogonial division continues throughout the summer and autumn. Primary spermatocytes appear about September, but by October many primary spermatocytes have appeared, and the size of the testis increases accordingly. (Plate 27).

Very little change occurs during the winter, from October until early March, but spermatogonia continue to divide. Secondary spermatocytes appear in February at the earliest. In April and May, however, spermatogenic activity increases markedly, and in addition to the production of more primary and secondary spermatocytes, both spermatids and spermatozoa appear in the seminiferous tubules (Plate 27). In late May the seminiferous tubules as well as the sperm ducts are full of spermatozoa (Plate 27). In the mature testis at this stage, spermatogonia are present in considerable numbers, but they are rarely seen in division. Spermatids and earlier stages are scarce.

In July, after spawning, the testis shrinks as the spermatozoa are released. At this stage primary spermatogonia and residual spermatozoa comprise the content of the tubules (Plate 27).

Fig. 18 Gonadosomatic ratio of male Phoxinus phoxinus during 1964, ±1 standard deviation.

Fig. 19 Effect of killing female Phoxinus phoxinus by fixative or anaesthetic on:

i) condition factor and somatic condition factor.

ii) Gonadosomatic ratio.

iii) Nuclear diameter of chromaffin (upper) and adrenocortical (lower) cells.

(B = Bouin's fixative; MS = MS222)

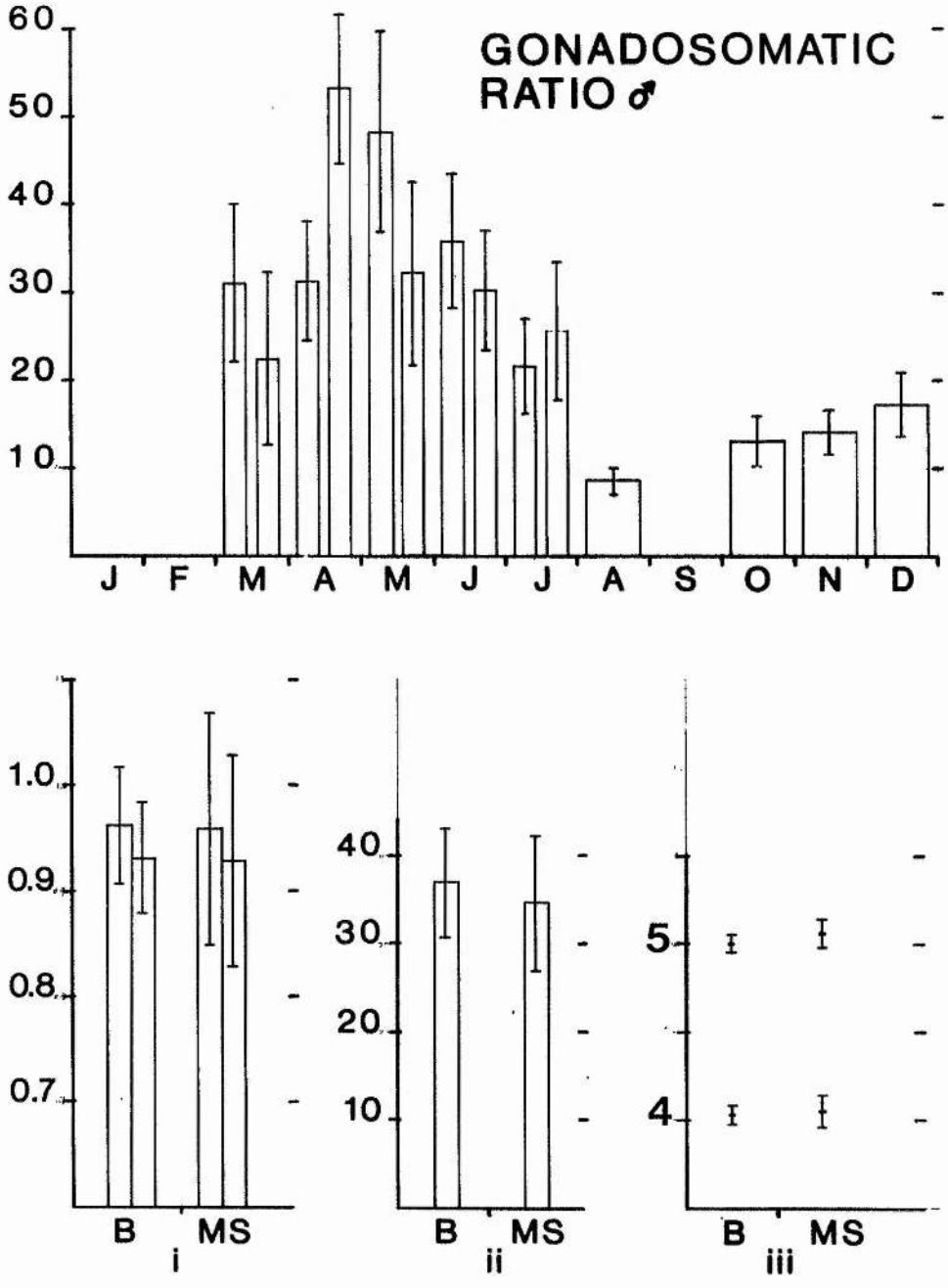

GONADOSOMATIC
RATIO ♂
60
50
40
30
20
10
J F M A M J J A S O N D
1.0
0.9
0.8
0.7
B MS
i
40
30
20
10
B MS
ii
5
4
B MS
iii

Fig. 20 Gonadosomatic ratio of male Phoxinus phoxinus during 1975-76, ±1 standard deviation.

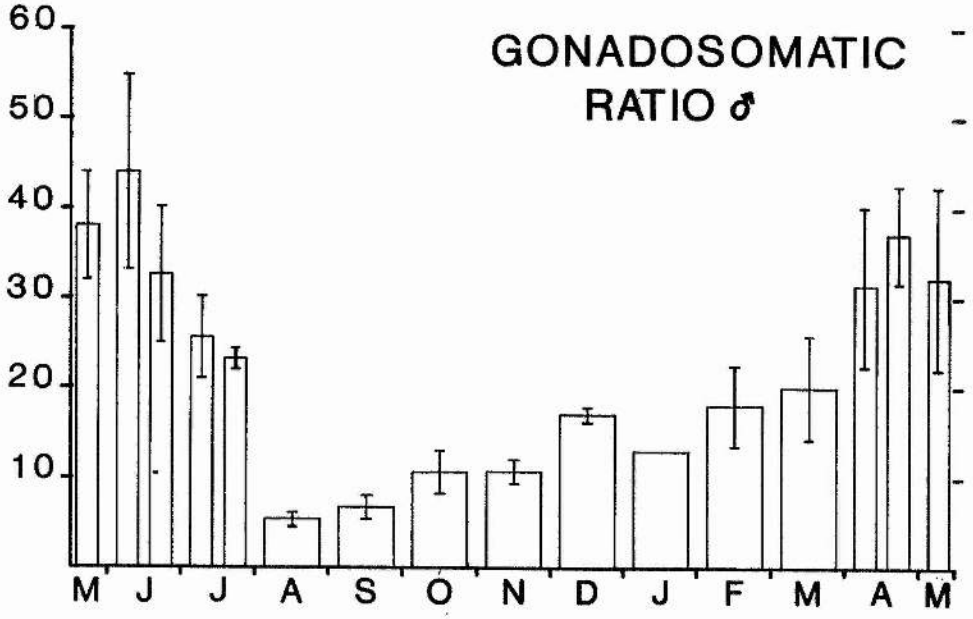
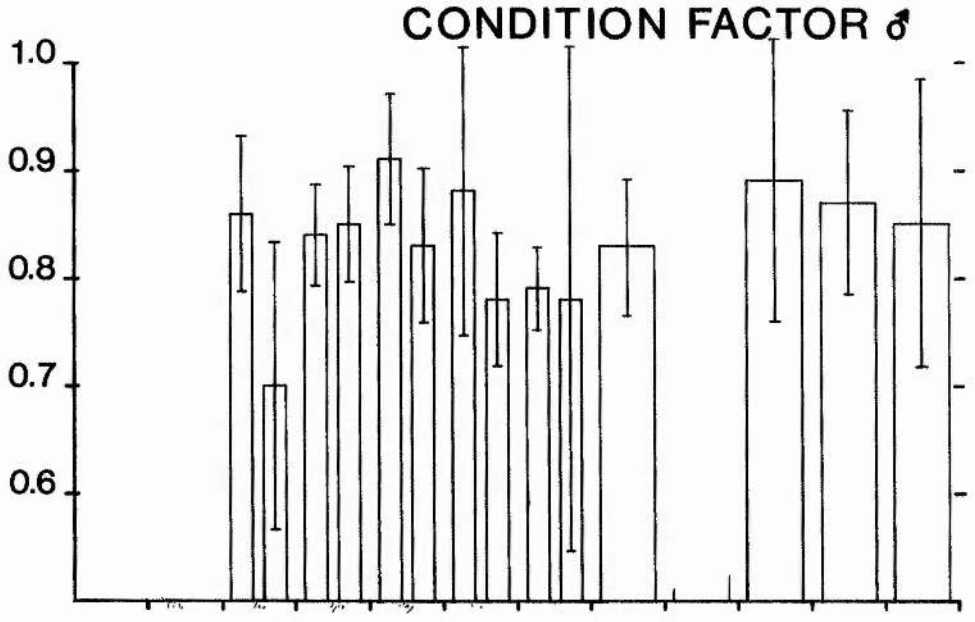

GONADOSOMATIC
RATIO ♂
60
50
40
30
20
10
M
J
J
A
S
O
N
D
J
F
M
A
M

Fig. 21 Condition factor of male Phoxinus phoxinus during 1964, ±1 standard deviation.

Somatic condition factor of male Phoxinus phoxinus during 1964, ±1 standard deviation.


CONDITION FACTOR ♂
1.0
0.9
0.8
0.7
0.6

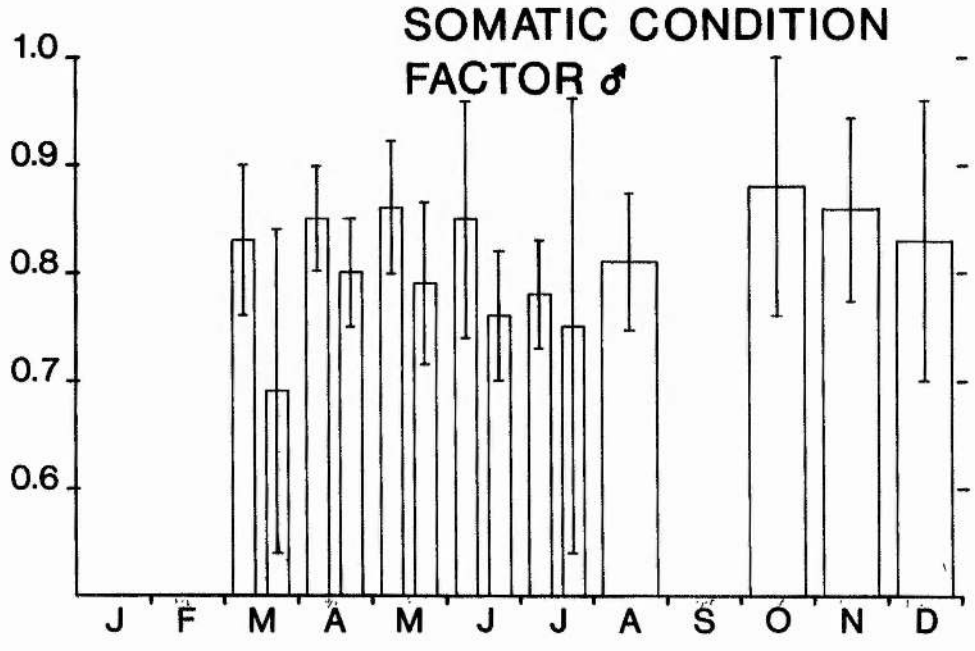
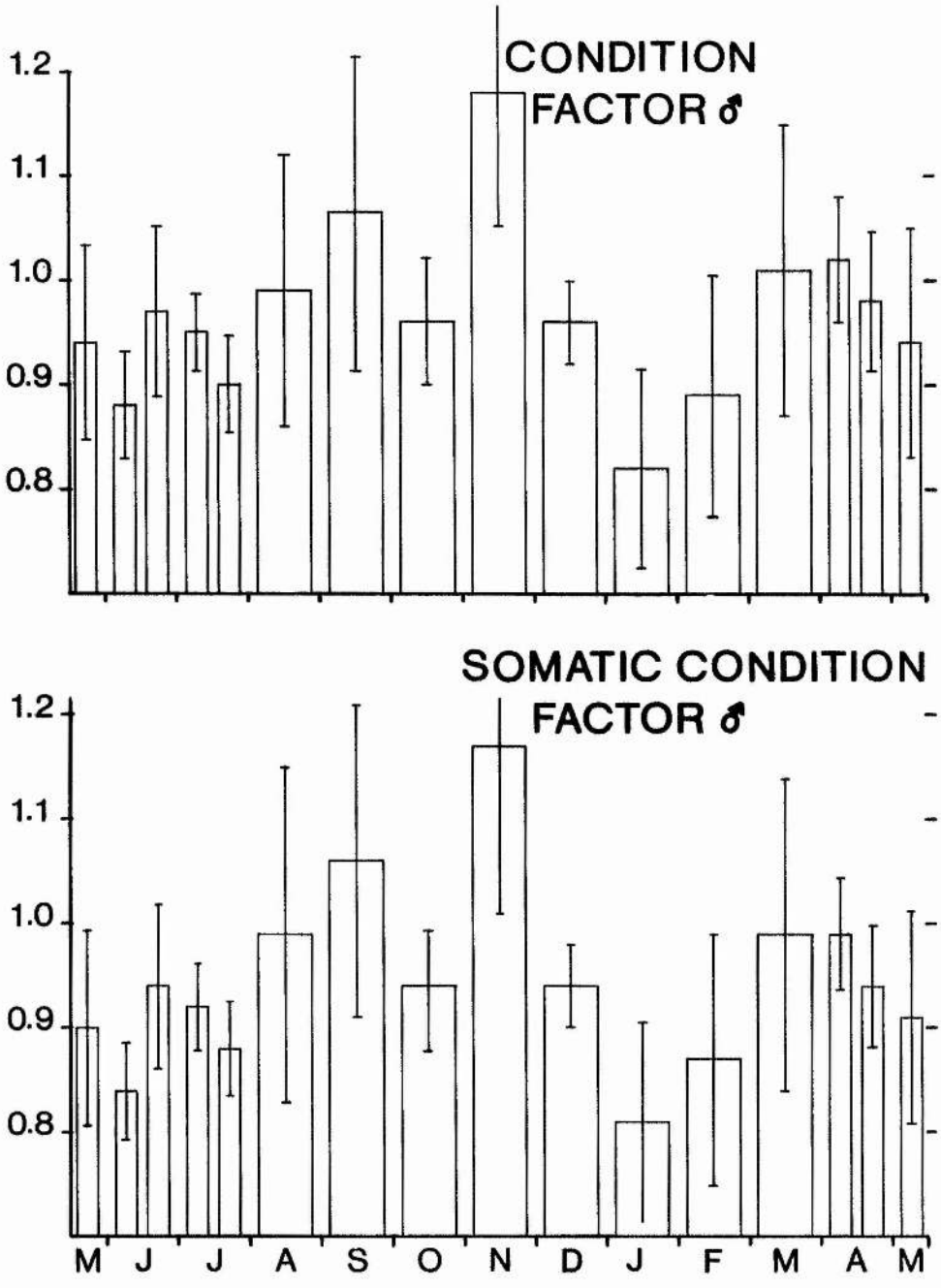

SOMATIC CONDITION FACTOR ♂
1.0
0.9
0.8
0.7
0.6
J F M A M J J A S O N D

Fig. 22 Condition factor of male Phoxinus phoxinus during 1975-76, ±1 standard deviation.

Somatic condition factor of male Phoxinus phoxinus during 1975-76, ±1 standard deviation.


CONDITION
FACTOR ♂
1.2
1.1
1.0
0.9
0.8
SOMATIC CONDITION
FACTOR ♂
1.2
1.1
1.0
0.9
0.8
M J J A S O N D J F M A M

The somatic condition factors of male minnows are less clearly cyclical than in the case of females. There is no evidence of a signigicant fall in somatic condition factor after (or during) spawning time, as there is in females. (Figs. 21,22).

(c) The Morphology of the head-kidney

(i) General morphology (Fig. 23)

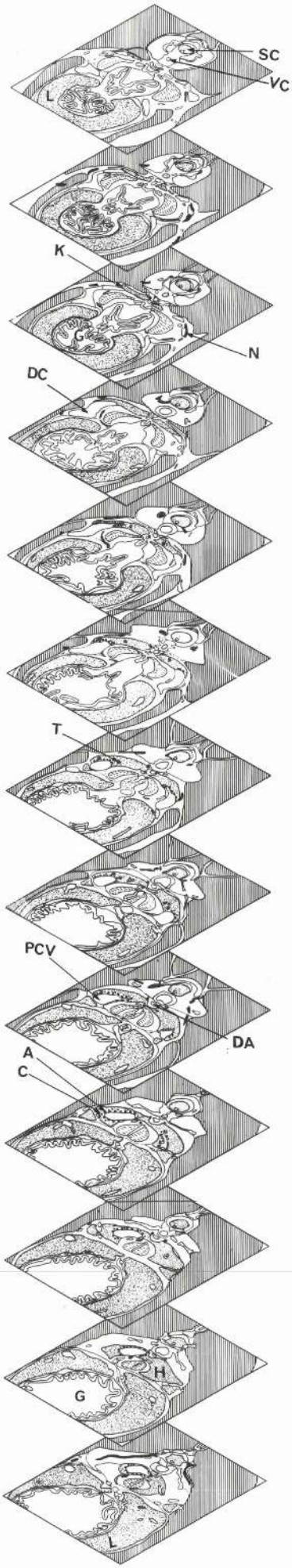
The head-kidney of Phoxinus phoxinus is a discrete structure, separate from and anterior to the trunk-kidney, to which it is connected only by a fine strand of haemopoietic tissue on the right side. The head-kidney is bilobed, and the two dorso-ventrally flattened lateral lobes are connected to each other by two narrow bridges of haemopoietic tissue, one towards the anterior end, one towards the posterior end, of the head-kidney. Through the central space between the lobes and these bridges, passes the gastromesenteric artery, running from the dorsal aorta (immediately dorsal to the head-kidney)to the organs of the gut.

The trunk-kidney is drained by two posterior cardinal veins, but (as in many fish) one of these is more important than the other, and deals with most of the drainage. In the case of Phoxinus the right posterior cardinal is by far the larger vein, and several transverse vessels transport blood from the left lobe of the trunk-kidney to the right lobe. Only the anteriormost part of the left trunk-kidney lobe drains via a small left posterior cardinal vein.

Both right (major) and left (minor) posterior cardinal veins pass into their corresponding head-kidney lobes, near the midline. They then pass through the head-kidney, swinging outwards, and emerge from the antero-lateral region of the two head-kidney lobes as the ducts of Cuvier,which travel ventrally into the sinus venosus of the heart (Plate 28).

The dorsal aorta lies near the midline immediately dorsal to the head-kidney, and oxygenated blood passes to it via small arterioles.

Fig. 23 Isomatric drawings of serial transverse sections through specimen of Phoxinus phoxinus, 60 µm apart, anterior uppermost. Right side of fish on left side of drawing. Each section is 7 mm long × 5 mm wide. (see text pages 50–51.
(Legend as for figure 5.)


SC
VC
L
K
G
N
DC
T
PCV
DA
A
C
H
G
L

As already mentioned, the gastromesenteric artery passes between the head-kidney lobes on its way to the gut (Plate 28).

The innervation of the region has not been investigated in detail, but segmental sympathetic nerves can be seen in sections, and conspicuous sympathetic ganglia and nerve trunks are associated with the gastromesenteric artery (Plate 28). Major branches run to the gut, and minor branches run to the head-kidney lobes, though it has not proved possible (except by electron microscopy, see pages 55ff) to trace their endings. Parasympathetic nerves, derived from the cranial nerves, are also visible in the region. Occasionally some nerves or ganglia were observed in association with the posterior cardinal veins.

(ii) Histology

The head-kidney is composed of haemopoietic tissue, a mass of lymphoid cells permeated by sinusoids and erythrocytes, with sparse connective tissue. There are one to three renal tubules.

As the two posterior cardinal veins travel through the head-kidney, they are surrounded by adrenocortical and chromaffin cells. The adrenocortical cells form a layer two to six cells in thickness, lying close to the endothelium of the vein. Adrenocortical tissue also surrounds the main tributary veins for a short distance from their fusion with the main cardinal veins. This arrangement corresponds with Nandi's Type I (see page 5). The adrenocortical cells are rounded, ovoid and polygonal in shape (between 8-10 μm in diameter) with spherical nuclei. The diameter of the nuclei ranges between 4.1 and 4.6 μm in both sexes.

The chromaffin cells are less numerous than the adrenocortical cells, but are most abundant towards the posterior part of the head-kidney. They are generally polymorphic cells, between 10.4 - 15.2 μm in length, with large irregular outlined nucleus, sometimes rounded, measuring between 5 - 5.7 μm in diameter. They are scattered amongst the adrenocortical cells or sometimes separately from them, (Nandi's Type V).

It is sometimes difficult to distinguish the two types of cells. However electron microscope studies (Page 55) confirm that the identification is correct.

(d) Variations in adrenocortical and chromaffin activity:

(i) Variations in response to techniques used,

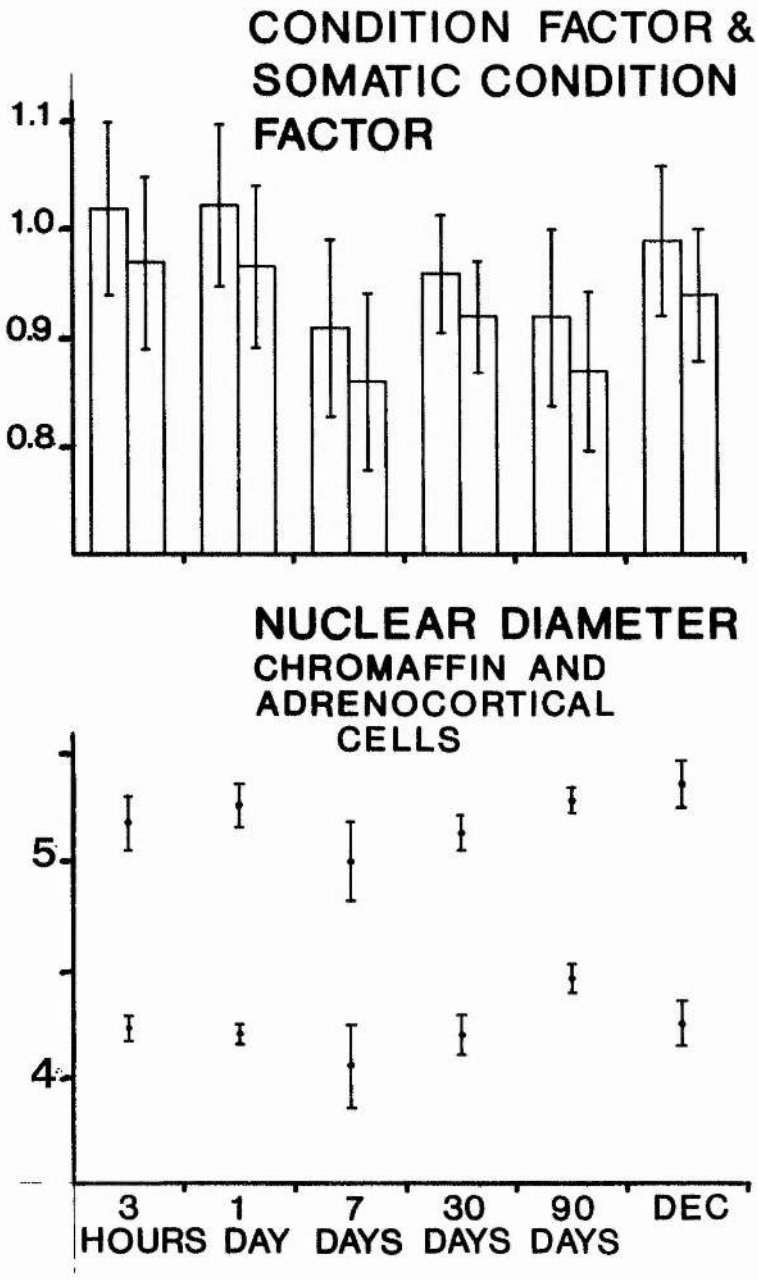
It has been shown (see page 33) that adrenocortical activity may be affected by capture and maintenance techniques. Two killing methods were therefore compared (Fig. 19) using fifty-eight mature female minnows caught by electrofishing in September. There was no significant difference in gonadosomatic ratio, condition factor, somatic condition factor, or nuclear diameters of adrenocortical and chromaffin cells. Only twenty female minnows of the fifty-eight, whose gonadosomatic ratios were closest to the mean value of the sample, were used for measuring the nuclear diameter of adrenocortical and chromaffin cells (Table 9).

To determine whether maintenance in captivity affects adrenocortical activity, sixty-five females were kept in aquaria (see page 37) for three months, and samples of ten fish were killed at three hours, one day, and seven days, of thirteen fish at thirty days and of twenty-two fish at ninety days after capture in December 1975 (Fig. 24). The condition factor and somatic condition factor fell in one week, but thenremained at only slightly below December level for the remainder of the period in captivity. There was a corresponding, but not statistically significant change in the nuclear diameter of adrenocortical and chromaffin cells (Table10). A tentative conclusion would be that after an initial period of stress, the fish become acclimatised to their new environment.

(ii) Seasonal variations:

Adrenocortical cells: The mean nuclear diameter ranges from 3.9 + 0.1 μm in the female and 3.9 + 0.1 μm in the males in August 1975

Fig. 24 Condition factor and somatic condition factor, of 65 female Phoxinus phoxinus caught by electro-fishing in December 1975 and maintained in aquaria for three hours, one day, seven days, thirty days and ninety days; and nuclear diameter of adreno-cortical and chromaffin cells of ten fish of each group.


CONDITION FACTOR &
SOMATIC CONDITION
FACTOR
1.1
1.0
0.9
0.8
NUCLEAR DIAMETER
CHROMAFFIN AND
ADRENOCORTICAL
CELLS
5
4
3
HOURS
1
DAY
7
DAYS
30
DAYS
90
DAYS
DEC

to 4.6 ± 0.12 µm in the female and 4.6 ± 0.06 µm in male in May 1975 and from 4.0 ± 0.1 µm in female and 4.2 ± 0.1 µm in male in August 1964 to 4.6 ± 0.1 µm in female and 4.6 ± 0.1 µm in male in May 1964. In general there is no significant difference between the nuclear diameters of males and females and the results of 1975-76 and 1964. The increase in diameter of about 0.7 µm from the minimum represents an increase in nuclear volume (calculated as a sphere) of about two times (Figs.25,26)

After spawning, in late July - August, the adrenocortical cells form a narrow collar, only one to two cells in thickness around the veins. The nuclei are small in both sexes, 1964 = males 4.2 ± 0.1 µm, females 4.1 ± 0.1 µm; 1975 = males 3.9 ± 0.1 µm, females 3.9 ± 0.1 µm. The cytoplasm is densely stained, (Plate 30).

There is a gradual increase in nuclear diameter during the autumn months in both 1964 and 1975-6, but whereas in 1964 little change took place during the winter months, in 1975-76 the increase in nuclear diameter continued without interruption (Figs. 25,26), from 4.1 ± 0.1 µm in September 1975 to 4.3 ± 0.1 µm in February 1976 in females, and from 4.1 ± 0.1µm in September 1975 to 4.4 ± 0.1µm in February 1976 in males. During this period there is a slight increase in adrenocortical cell size and in the number of layers of cells round the veins (Plate 30).

Nuclear diameter increases more rapidly during spring 1975 = from 4.3 ± 0.1 µm to 4.5 ± 0.1 µm in females, and from 4.4 ± 0.1 µm to 4.5 ± 0.1 µm in males and reeches its maximum at spawning time in late May and early June. Hyperplasia of the adrenocortical tissue, and an increase in the number of cell-layers are conspicuous (Plate 30). During late June - July the volume of the adrenocortical tissue is reduced and the nuclear diameter decreases rapidly from its maximum to its minimum in both sexes.

Chromaffin cells= The mean diameter ranges from 5.22 ± 0.14 µm in females and 5.34 ± 0.2 µm in males in late July or August 1964 to

Fig. 25, Mean nuclear diameter (µm) ±1standard deviation of chromaffin (upper) and adrenocortical (lower) cells from March to December 1964.

A Females

B Males.

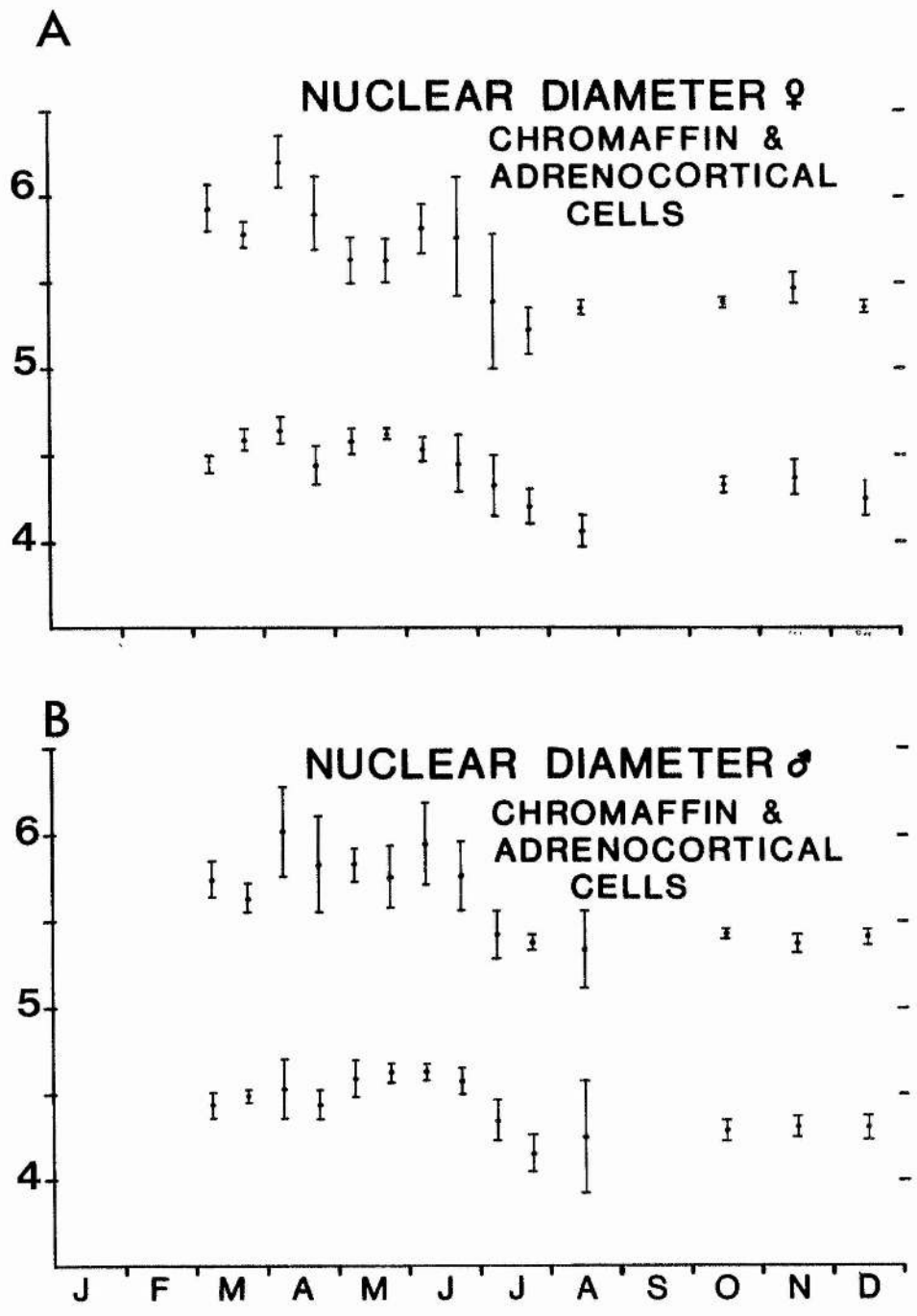
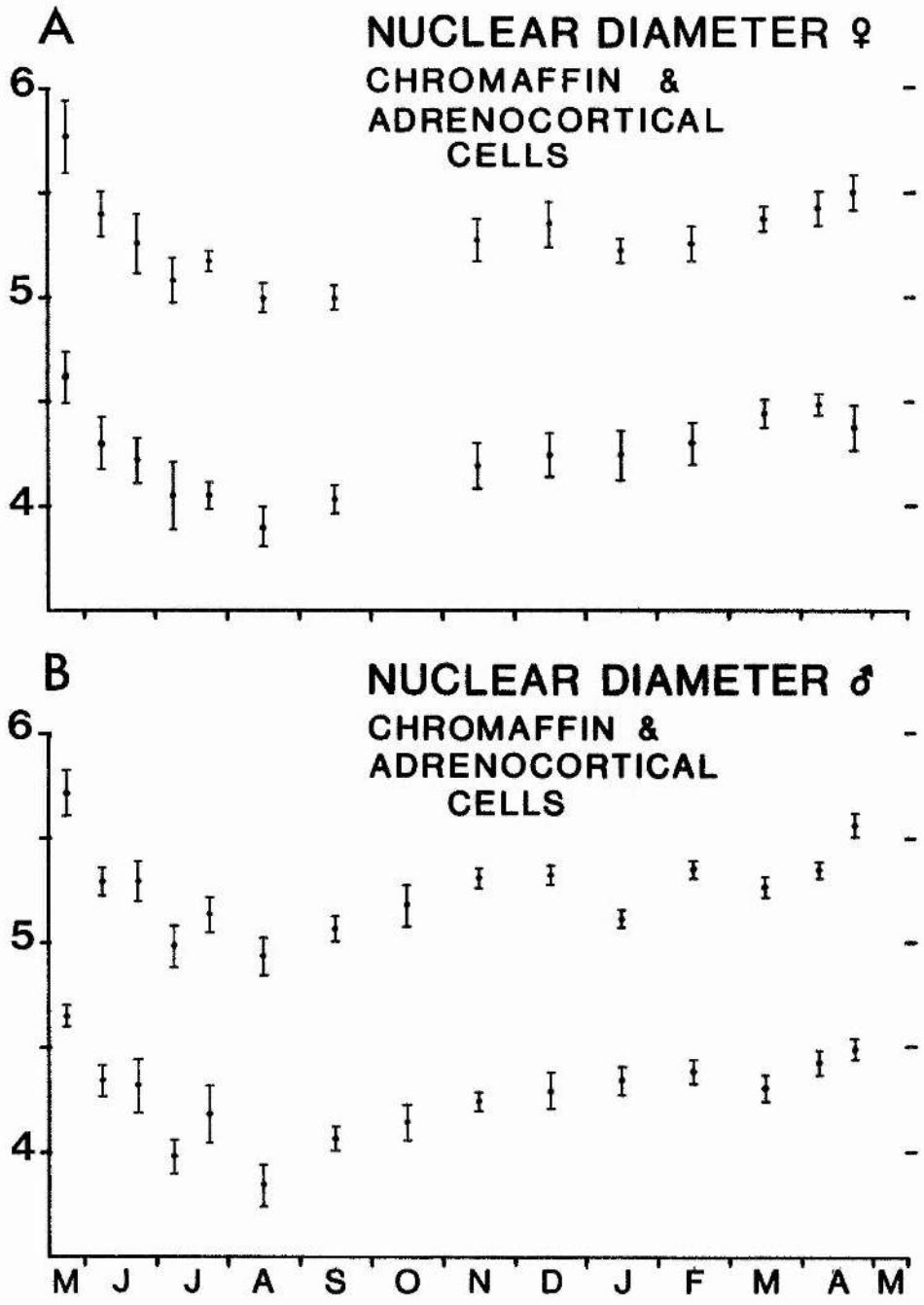

A
NUCLEAR DIAMETER ♀
CHROMAFFIN &
ADRENOCORTICAL
CELLS
6
5
4
B
NUCLEAR DIAMETER ♂
CHROMAFFIN &
ADRENOCORTICAL
CELLS
6
5
4
J
F
M
A
M
J
J
A
S
O
N
D

Fig. 26. Mean nuclear diameter (μm) ±1standard deviation of chromaffin (upper) and adrenocortical (lower) cells from May 1975 to April 1976.

A Females.

B Males.


A
NUCLEAR DIAMETER ♀
CHROMAFFIN &
ADRENOCORTICAL
CELLS
6
5
4
B
NUCLEAR DIAMETER ♂
CHROMAFFIN &
ADRENOCORTICAL
CELLS
6
5
4
M J J A S O N D J F M A M

6.14 ± 0.15 µm in females and 5.83 ± 0.09 µm in males in April or May 1964, and from 5.0 ± 0.07 µm in females and 4.94 ± 0.09 µm in males in August 1975 to 5.76 ± 0.17 µm in female and 5.71 ± 0.1 µm in males in May 1975. In general the chromaffin cells, like adrenocortical cells do not show any significant difference between their nuclear diameters in males and females. The difference between the maximum and the minimum values of diameter (about 0.79 µm) represents the change in the nuclear volume which takes place through a year, (Fig.25,26). The chromaffin cells reach their smallest size of the year immediately after the spawning period. A gradual increase in size then occurred during autumn months in 1975-76, but in 1964 little change took place. In January 1976 the nuclear diameter drop a little from 5.37 ± 0.11 µm in females and 5.32 ± 0.04 µm in males to 5.22 ± 0.05 µm in females and 5.14 ± 0.03 µm in males. This reduction is probably due to reduction in the fishes activity because of the low temperature. After January until late April or early May the nuclear diameter of chromaffin cells increases rapidly from 5.22 ± 0.06 µm in females and 5.14 ± 0.03 µm in males in 1976 to 5.61 ± 0.09 µm in females and 5.59 ± 0.08 µm in males.

During the spawning period the nuclear diameter decreases very rapidly from 5.76 ± 0.17 µm in females and 5.71 ± 0.1 µm in males in 1975 to 5.0 ± 0.07 µm in females and 4.94 ± 0.09 µm in males in 1975. The same phenomenon took place in 1964.

CHAPTER IV

Fine structure of the Head-Kidney of Teleost fish.

A. Introduction

Although the endocrine head-kidney tissues of fish have been the subject of study for some time (see pages 1,3,32), most of the research effort seems to have been directed more towards physiological and bio-chemical aspects rather than towards a detailed analysis of structure. In comparison to the extensive literature on the fine structure of the corresponding endocrine tissues in mammals, only very few published descriptions of the fine structure of the adrenocortical and chromaffin tissues of bony fish exist. (Yamamoto and Onozato 1965; Ogawa, 1967).

What information there is, however, suggests a high degree of uniformity of structure of these endocrine tissues in the various vertebrate classes. The adrenocortical cells have mitochondria with tubulo-vesicular internal structure, as opposed to the more usual cristae. These tubulo-vesicular cristae are characteristic of all steroidogenic tissue such as the ovarian follicle, corpus luteum, interstitial cells of testis and the placenta, (Sheridan 1963, Belt and Pease, 1956). Similarly the fine structure of chromaffin cells is characterised by the presence of abundant chromaffin granules scattered throughout the cytoplasm (Coupland, 1965; Zelander, 1964).

In those classes in which the adrenocortical tissues are distributed in discrete zones with different functions (e.g. the amniotes, and at least some sharks), fine structural differences in the cells of each zone have been described. With each zone, too, changes in fine structure associated with degree of activity have been described by certain workers. (Lever 1956; Nishikawa, Murone and Sato 1963; Taylor, Honn and Chavin 1975).

In the bony fish, where such zoning is not apparent (at the light microscope level, at any rate), it is more difficult to decide whether differences in fine structure of adrenocortical cells are due to differences in activity level, or to differences in the basic function of the cells.

Two distinct types of chromaffin cells have been described in many vertebrates, (Lever, 1955; Coupland 1965; Yates, Wood and Duncan 1962; Piezzi 1967; Unsicker 1973), secreting adrenalin and noradrenalin respectively. The existence of types has been confirmed by histochemical methods (Tramezzani, Chiocchio and Wassermann 1964), as well as by comparison of fine structure. As in the case of the adrenocortical cells, it is difficult, on the basis of fine structure alone, to decide whether two discrete cell types, or one type at different levels of activity, are involved. No comparable studies on the chromaffin tissues of teleosts have been made.

The aim of this part of the project is to examine the fine structure of the head-kidney tissues, in particular the endocrine tissues; and to identify the changes which are observable at different levels of activity. Such changes, as observed at the light microscope level, were used in a previous section as an index of cells activity (Chapter III, pages 52-54), and it would clearly be of value to know in detail the nature of the changes which were used for this purpose.

B. Materials and Methods =

1. The fish Two species were studied;

(a) The Rainbow Trout, Salmo gairdneri irideus, chiefly because of the ease with which the endocrine cells could be located in small pieces of head-kidney and:

(b) The Minnow, Phoxinus phoxinus, because of the useful basic information on head-kidney structure and function described in Chapter III pages 50-54 .

The trout, about 20 cm. in length, were purchased from a commercial fish-farm, and maintained in an aquarium with running water until required for use. They were then killed by immersion in a lethal concentration of MS222 (Sandoz).

The minnows were caught by electrofishing in the Walton Reservoir (see pages35,36). All were adult females from 7.5 to 8 cm. in length. Four fish were killed immediately, while still under electronarcosis in one of two ways:

(a) Immersion in MS222, (b) Decapitation.

Four were brought back to St. Andrews and maintained in an aquarium 56 x 30 x 30 cm. in the laboratory, where they were subjected to disturbance for twenty-four hours. They were then killed in either MS222 or by decapitation.

2. Fixation and Embedding.

Immediately after death the fish were opened by a median ventral incision. In the case of the trout (which the dissection of the kidney is relatively easy) the head-kidney region was dissected out and a small piece was cut from each of three parts - right and left anterior lobes, and the posterior lobe. In the case of the minnows (in which the discrete, small, head-kidney is difficult to dissect in fresh specimens) the abdominal coelom was flooded with fixative which served to harden the tissues. The head-kidney was then dissscted out under gluteraldehyde. Fixation etc. followed the following scheme=

(1) Fix in 2.5% gluteraldehyde buffered to pH7.2 for one hour
[Phosphate buffer = 8.0ml. of 0.0066M Na_2HPO_4
2.0ml. of 0.0066M $Na\ H_2\ PO_4$
Mix one part of 25% gluteraldehyde with nine parts buffer to make 2.5% gluteraldehyde, pH 7.25 - 7.45].

(2) Wash in phosphate buffer, three changes of two minutes each.

(3) Post-fix in 1% osmium tetroxide, pH 7.4, thirty minutes.
[2% osmium tetroxide 5ml.
Veronal acetate buffer 2ml. (Palade, 1952).

0.1N HCl 2ml.
Water 0.5ml. /

(4) Wash in distilled water, three changes of two minutes.

(5) Dehydrate (i) 70% Ethanol thirty minutes
(ii) 95% " one hour
(iii) 100% " one - twenty four hours

(6) Embed (i) Soak in propylene oxide 1,2 epoxy propane), two changes of thirty minutes

(ii) Soak in propylene oxide /araldite mixture (2/1) one hour

/ Araldite = { Araldite resin CY212 27ml. } { Hardener HY964 23ml. } { Accelerator DY064 1.0ml. }

(iii) Add an equal volume of araldite and leave it for one hour at room temperature.

(iv) Soak in pure araldite, four hours at room temperature.

(v) Transfer specimens to bottom of dry gelatin capsule and fill with fresh araldite, incubate at 37°C for twelve hours, 45°C for twelve hours, and 60°C for twelve hours.

(7) Section: The araldite blocks were trimmed to a pyramidical shape with the specimen at apex, and sections cut using glass knives on a L.K.B. Ultratome III. As a first step thick sections (1µm.) were cut. stained with methylene blue, and examined under the light microscope to locate adrenocortical and chromaffin cells. The block was then further trimmed down to these limits, and thin sections (700-900 A) were cut. Silver and grey sections were mounted on hexagonal type grids 3.05mm. diameter with supporting films (Smethurst Highlight, Ltd.).

(8) Stain: The sections were stained in uranyl acetate (saturated solution in 50% ethanol, centrifuged before use) for one hour, then washed three times within two minutes in distilled water, dried and stained with lead citrate (pH 12.0) for ten minutes (Reynolds, 1963).

(g) Examination and Photography.

The stained sections were examined on a Philips Electron Microscope E.M. 301 HRG, operated at 60 Kv with a 50 µm objective aperture. The material was photographed on Ilford E.M.4 plates at various magnifications. The plates were developed for two and half minutes at 20°C in Ilford PQ universal developer, and fixed in "Amfix".

Results

The descriptions which follow apply essentially to Phoxinus phoxinus. Significant differences in Salmo gairdnerii irideus are mentioned where appropriate at the end of each paragraph.

1. Haemopoietic tissue. In electron microscope sections, the haemopoietic tissue is clearly delimited from the endocrine tissues in the vein wall. (Plates 31, 32). White blood cells (5.6-7.9 µm in diameter) are abundant in the haemopoietic tissue. Most of these cells in Phoxinus phoxinus contain many electron-opaque ellipsoid granules. Others, however, contain few or no granules (Plates 31,32). In sinusoids within the haemopoietic tissue are elongated erythrocytes (8.9um - 9.5µm in length) with uniform osmiophilic cytoplasm and regular ovoid nuclei (Plates 31,36). Collagen fibres and fibroblasts are also found (Plate 31) and many nerve fibres (Plate 32), some of which innervate the endocrine cells, and which will be described in that context (see page 63,64 below).

In Salmo gairdnerii irideus haemopoietic tissue is essentially similar (Plates 33,34), except that cells with the ellipsoid granules seen in Phoxinus phoxinus are absent and the cells contain either no granules, or a few small circular granules. Very few cells occur containing groups of dense, large, circular granules (Plate 33).

2. Endocrine tissues. As previously described on the basis of light microscope studies (pages 51,52), the adrenocortical and chromaffin cells of Phoxinus phoxinus surround the posterior cardinal veins and their main tributaries in the haemopoietic tissues of the head-kidney. The adrenocortical cells are generally proximal to the lumen of the vein, the chromaffin cells forming an irregular layer distally (Plates 31,32, 36,37). Electron microscope preparations show that the endocrine tissues are separated from the haemopoietic tissue by a discrete layer of tissue (Plates 31,32,48). Lymphocytes, though abundant in the haemopoietic tissue, never occur inside the membrane bounded endocrine region.

Within the endocrine zone, the cells are relatively closely-packed, though some intercellular spaces occur, containing granular material and what appeared to be microvilli protruding from the walls of adrenocortical cells (Plates 38,48). These spaces may be continuous with the periendothelial space behind the vein lining, and with the spaces in the membranous boundary between the haemopoietic and endocrine tissues.

In Salmo gairdnerii irideus the adrenocortical and chromaffin tissues occur separately; the adrenocortical tissue anteriorly and the chromaffin tissue posteriorly, so that only one type of endocrine cells appears in each plate (Plates 33,34,35).

3. The adrenocortical cells of Phoxinus (Plates 31,32,36,37) are variable in shape, being circular, oval, or columnar in section. They are bounded by a typical smoothly-contoured double cell-membrane 0.02um in width. They form a layer or layers next to the wall of the vein, and are generally closely-packed, though the cell surface contiguous with intercellular spaces may be raised into microvilli of irregular form (Plate 38).

The nuclei are generally circular in section, rarely oval, and are usually centrally situated within the cell. Within the nuclear

membrane is a zone of osmiophilic granules lined up parallel to the inner membrane and only interrupted at the pores where the membranes are contiguous. The same type of granules is also dispersed throughout the nucleus. One or two nucleoli, of high electron density, are usually visible in each nuclear section (Plates 32,34,39).

The mitochondria are very numerous, of large size (up to 0.9µm) and with the tubulo-vescular internal structure which is typical of steroidogenic cells (Belt and Pease 1956). In some cells the mitochondria are more or less circular in section, and only weakly osmiophilic, so that their internal structure can be clearly seen (Plate 40). This type of mitchondrion may occur in such numbers as apparently to fill the cell. In other cells, however, in the same fish, the mitochondria are elongated and intensely osmiophilic and tend to be smaller than the former type (Plate 40). A complete range of types between the two extremes occurs, so that it is likely that the morphological differences reflect different levels of activity, rather than representing two distinct cells types having different functions.

In some cases mitochondria were observed in which the cristae appeared to be breaking down, leaving a clear lumen. Open form mitochondria were also present, their lumen opening either into the cytoplasm or, more usually, into many-layered circinate structures which are presumably derived from the mitochondria. Such structures appeared to be further developments of the 'light' type of mitochondria (Plates 41,42).

There is an extensive smooth encoplasmic reticulum, and groups of ribosomes can also be seen in association with short lengths of endoplasmic reticulum. Free ribosomes are abundant (Plates 39,40). Microbodies occurred in cells with light mitochondria (Plate 42). These are electron-dense structures, smaller than mitochondria, but with similar internal structure.

The cytoplasmic matrix ranges from weakly osmiophilic to intensely osmiophilic. Large vacuoles are often abundant in cells with a light matrix, and much rarer in those with a dark matrix, but exceptions are common. The different types of adrenocortical cells lie mingled together in an apparently random manner (Plates 32,35,37).

Golgi complexes are apparent in some adrenocortical cells close to the nucleus (Plate 39). They consist of flattened sacs with vesicles clustering at their ends.

In Salmo (Plates 33,43) the adrenocortical cells are similar though the range of types is more extensive. The pale stage mitochondria of Salmo are less uniform in shape than those of Phoxinus which are almost all circular to oval.

4. The Chromaffin cells are generally situated away from the vein wall, close to the haemopoietic tissue, but as described in light microscope studies (pp. 51), the distribution of chromaffin tissue is erratic, so that some sections may contain no chromaffin cells, while in others they form the bulk of the endocrine tissue. Chromaffin cells at some distance from the vein have elongated processes running towards it between the adrenocortical cells (Plates 32,37,40). (The situation in Salmo is quite different, see below). Chromaffin cells are characterised by the presence in their cytoplasm of a great many chromaffin vesicles. These vesicles are rounded or oval, from 0.2μm to 0.3μm in diameter, and they contain varying amounts of intensely osmiophilic material. In extreme cases the vesicles are empty or filled to capacity with osmiophilic material (Plates 32,35,36,44). A complete range occurs, randomly arranged within a section.

The chromaffin cells are generally polyhedral or elongated, often with a narrow cellular projection towards the vein, which may appear as a small isolated patch when cut transversely (Plates 32,37). They are bounded by a double layered plasma membrane. The cell surface is smooth

and generally without microvilli except on surfaces closely related to blood sinuses (in the periendothelial spaces) (Plates 38,45).

The nucleus is large and tends to be irregular in outline, often not centrally placed in the cell. Nucleoli are conspicuous (Plates 31, 32,40,45).

The mitochondria are few in number and variable in shape - round, oval or rod-shaped in section, with smooth contours. They have typical cristae, and are concentrated in the middle of the cell (Plates 44,45).

Many ribosomes occur, often in groups or associated with short endoplasmic membranes forming rough endoplasmic reticulum.

The golgi apparatus is in the form of smooth surfaced membranes, vacuoles and small vesicles in a paranuclear position (Plates 44,45).

In Salmo, (Plates 46,47), the chromaffin cells are situated in the posterior region of the head-kidney. They lie in the vein wall, unlike the chromaffin cells of Phoxinus, which are generally separated by adrenocortical cells from the vein wall. The basic cell structure is similar in both species but the range of stages of activity appears more extensive in Salmo than in Phoxinus.

5. The innervation of the head-kidney of Phoxinus phoxinus is derived from sympathetic fibres as described in light microscope studies (p. 51). Nerve axons and ganglion cells appear on most sections of head-kidney tissue (Plate 28). In electron microscope sections, nerve axons are conspicuous (Plate 32), and nerve junctions with chromaffin cells are commonly seen (Plates 48,49,50). The nerve endings often protrude into the chromaffin cell. The degree of interdigitation between nerve ending and cell varies. Electron density is high in the area of contiguity, which corresponds to the pre-and post-synaptic thickening associated with nerve synapses, and sometimes the nerve synapse occurs on an invagination of the plasma membranes into the nerve ending. Most nerve endings contain many small light coloured synaptic vesicles and

fewer larger, dark-cored ones (Plates 48,49,50). This description accords with the cholinergic synapses described by Unsicker (1973b), who found this to be by far the most abundant type of synapse in bird adrenals. Synapses of Unisicker's adrenergic type with two types of large, dark vesicles, were not found, but axons with marked varicosities, associated with this type of synapse, did occur (Plate 54).

Chromaffin cells with more than one apparent synapse (with a single nerve) were found, but there was no difference between the degree or type of innervation in chromaffin cells of differing chromaffin vesicle content (Plate 48). No synapses on adrenocortical cells were found.

In Salmo details of innervation are the same (Plates 51,52,53).

6. Effects of Killing and Maintenance Techniques.

As corticosteroid hormones are not stored, but are synthesised as demand arises, it would be reasonable to expect the fine structure of adrenocortical cells to show morphological changes associated with changes in secretion rate. It has been shown (p. 33) that many forms of stress, even different catching techniques, elicit a rapid rise in plasma corticosteroid level, and consequently it is difficult to be certain whether one is examining a resting or an active cell. To determine the fine structure differences between active and inactive cells, four Phoxinus were caught by electrofishing in the Walton Reservoir, and of these, two were killed by MS222 anaesthesia and two by decapitation while still under electronarcosis. These methods are least likely to stimulate corticosteroid secretion (p. 33). To provide "stressed" fish for comparison, four Phoxinus were transported to the laboratory and kept for twenty-four hours in a 56 cm x 30 cm x 30 cm aquarium, and were there disturbed with a net for ten minutes, before being killed by the same methods.

All specimens of Salmo used were aquarium-maintained fish which had been bred and reared under similar conditions.

In Phoxinus, unstressed fish contained a high percentage of adrenocortical cells with numerous large, pale, circular or sub-circular mitochondria (Plate 32). Stressed fish contained a higher proportion of cells with fewer mitochondria of irregular shape, often elongated and densely osmiophilic (Plates 32,40). A complete range of intermediate types occurred.

The percentage of adrenocortical cells containing few dark mitochondria and adrenocortical cells which contain many pale mitochondria in unstressed and stressed Phoxinus.

Mitochondria	Unstressed	Stressed
Pale	69%	34%
Dark	31%	66%

There was no visible difference between fish killed by MS222 anaesthesia or decapitation.

In general, but not invariably, the large, pale mitochondria were associated with relatively dense cytoplasm lacking vacuoles (Plates 32,40). The dark, irregular mitochondria were likewise often associated with pale cytoplasm with many vacuoles, some very large. The association of a particular type of mitochondria with a particular type of cytoplasm or vascuoles was, however, not consistent (Plate 40).

In Salmo also a full range of cell types was present. Mitochondria of the pale large form often occurred in very large numbers in some cells (Plate 55). The dark, irregular forms of mitochondria were always sparser within a cell, and often (but not always) associated with pale cytoplasm with numerous large vacuoles (Plate 55).

In this species mitochondria apparently degenerate, the cristae progressively breaking down until only a few tubulo-vesicular fragments remain (Plate 55), and finally merely a vacuole bounded by the mitochondrial membrane (Plates 33,41,55). A similar process occurs in Phoxinus, where

the number of mitochondria decreases from the light to the dark form. In *Salmo* (but not in *Phoxinus*,) multi-layered circinate structures occur, apparently derived from (or associated with) mitochondria (Plate 55).

CHAPTER V

DISCUSSION

1. Morphology of the head-kidney of teleost fish.

Early studies of the head-kidney of teleost fish (pp. 1,3) recognised it as a part of the renal system having no renal functions, often completely separate from the functional trunk kidney situated posteriorly. Because the head-kidney was so distinct from the trunk-kidney, it was regarded as being homologous with the embryonic pronephros, which is the functional renal organ of larval fish (and of adult lampreys). However, as more species were investigated, it became clear that the head-kidney and trunk-kidney were not always clearly distinct, and that a complete range of morphological types exist, from the extreme case of a discrete head-kidney without renal elements to the other extreme in which renal elements extend to the anteriormost end of the kidney, and the head-kidney, (if such a term can be used in this case) is recognisable only because of the presence of endocrine cells and lymphocytes amongst the glomeruli and tubules (e.g. Xiphophorus; Brachydanio pp. 16,17). As a result, dispute arose about the exact homology of the head-kidney, as to whether it was solely derived from the pronephros, or whether it also contained a mesonephric component. This dispute seems never to have been settled, because more interest now is directed towards understanding the functions of the head-kidney tissues. The terms "Head-kidney" and "Trunk-kidney" are consequently now generally used, without implying their embryonic origins. Even these simple terms can cause confusion, however, as in the case of those species referred to above in which

the kidney contains functional tissue throughout its length.

Detailed study of the distribution of the endocrine tissues of the head-kidney began somewhat later (pp. 1,3), and attempts were made to set up categories of different types, e.g. by Nandi (1962) and Oguri and Hibiya (1957). Again, as more species are investigated, it is becoming apparent that there is a complete unbroken range of types, and such elaborate systems of classification are not really useful. Each species is better described in its own right.

Indications that the different families of teleosts might each have a specific type of head-kidney morphology (see, e.g. Nandi 1962), have also not been confirmed by studies on more species. In species studied in the present work, for example, amongst the family cyprinidae two have discrete head-kidneys separate from the trunk-kidney and containing no renal elements (Phoxinus, p. 50 Rasbora, p. 13) while one has renal elements extending throughout the kidney and the head-kidney is represented only by the endocrine tissues and some lymphocytes scattered amongst the renal elements near the anterior end, (Brachydanio, p. 16). Likewise, similar morphological types occur in different families. For example, the arrangement in Brachydanio (Cyprinidae) is closely resembled by that in Xiphophorus (Poeciliidae) p. 17 , and by Aphanius (Cyprinodontidae) p. 18 .

Amongst the Red Sea fishes, also, dissimilar types of morphology occurred in closely-related species: in Acanthopagrus (p. 20) the chromaffin tissue lies inside the vein wall, whereas in Scarus (p. 21) the adrenocortical and chromaffin tissues lie together. Both species belong to the Sparidae, and similar tyoes in diverse families: In Chanos (chanidae) p. 19 the arrangement is similar to that in Thalassoma lunare and T. amblycephalus (Labridae), in Acanthopagrus (sparidae) p. 20 is similar to that of Lethrinus (Lethrinidae) pp. 26 ,27.

Some uniformity of type in related species does seem to exist.

For example, in the Salmonidae (Salmo pp. 14,15; Coregonus pp. 15,16), the chromaffin tissue lies separately from the adrenocortical tissue, and posterior to it. Similar distribution has been described in other salmonids (Giacomini 1922; Nandi 1962). It is interesting to speculate why the two endocrine tissues a re separate in this family, especially as it has been shown that enzymes in the adrenocortical tissue are involved in the synthesis of adrenalin in the chromaffin tissue (Chester Jones 1976).

In most species the staining properties of chromaffin cells in Masson's trichrome are very different from those of adrenocortical cells, and the two are easy to distinguish. In some species, however, the differences between these two endocrine cell-types is less marked, and in those species in which chromaffin cells are relatively rare, they may easily go unnoticed. So, for example, Nandi (1965) found no chromaffin cells in Brachydanio, though the present study (p. 16) shows that they do in fact occur. There does appear to be a great variation in the relative abundance of chromaffin and adrenocortical tissues in different species - chromaffin is particularly rare in Herklotsichthys (Clupeidae, p. 22), in Hepsetia (Atherinidae, pp. 27,28), and Rasbora (Cyprinidae p . 13) as well as in Brachydanio. Such variations in abundance might not, of course, be a permanent feature of the species, but due to seasonal variation or sampling technique.

Even when the staining properties of the two endocrine cell-types are not markedly different, they can be readily distinguished by a comparison of cellular detail. For example, the nuclear size of chromaffin cells is significantly larger than that of adrenocortical cells (pp. 53-54). Consequently it is not necessary to use histochemical methods to identify the cells, such as the 3β - ol steroid dehydrogenase technique (Wattenberg 1958; Chieffi and Botte 1963)

or the reaction of potassium dichromate with the chromaffin cells, from which they derive their name (Hillarp and Hökfelt 1955). Studies of the fine structure of the cell-types of the head-kidney, using the electron microscope confirmed that the identification made by light microscopy in the present work was correct (pp.13-54).

2. Physiological aspects of the head-kidney.

The object of the second part of the project was to determine the relationship between adrenocortical activity and the reproductive cycle, a relationship which has been claimed to exist by various workers (V. pp.32,33).

The reproductive cycle was determined primarily by measuring gonad weight relative to body weight in fixed specimens. Fixation (and partial dehydration, V. p. 37) prior to weighing,obviously introduces errors, but this method makes it possible to ensure histological material which is free from post-mortem artefacts. Histological assessment of gonad state, in either sex, provides a much more accurate criterion by which to judge the stage of the reproductive cycle (Scott 1963).

The state of activity of the adrenocortical tissue was determined by measuring the nuclear diameter of the adrenocortical cells. This (or similar) techniques have been used by many workers and are generally accepted as valid. Recent investigations (Scott, unpublished) in which the nuclear diameter of adrenocortical cells and the concentration of cortisol in the plasma of the same fish were measured, suggest that there is a correlation between the two, though with a correlation coefficient of only about 0.6. As an index of adrenocortical activity in fish which are too small to provide enough blood for direct steroid hormone measurement, nuclear diameter is an acceptable criterion.

The method used to catch and kill the fish, and the maintenance of fish in aquaria after catching but before killing, may have a considerable effect on circulating cortisol level (V. p. 33) and therefore presumably on nuclear diameter of the adrenocortical cells. In the present study, electrofishing followed by killing in either MS222 anaesthetic or in Bouin's fixative while still under electronarcosis were used. Although no measurements of cortisol in the plasma of fish caught by this

method are available, Scott (1963) suggests on the basis of survival rate and their resumption of normal gametogenesis, that this is a relatively stress-free method of catching Phoxinus, and therefore likely to have minimal effect on adrenocortical cytology. Fish were maintained in large, open-air aquarium tanks for periods up to three months, and as expected showed an initial stress reaction followed by a degree of adaptation. Aquarium maintainance, however, clearly has an effect on adrenocortical cytology (a fact revealed even more clearly in the electron microscope studies, V. pp.64,65), and allowance must be made for stress-induced changes in both adrenocortical and chromaffin cytology when using such fish.

While nuclear diameter is a valid, though inexact, criterion of adrenocortical activity, it is less certain that this criterion can be applied to chromaffin cells. Because adrenocortical cells do not store their steroid hormones, but synthesise them as required, it is reasonable to expect that the cells (and therefore the nuclei) might show marked variations in size. Chromaffin cells, however, store catecholamine hormones, and no investigations have shown a correlation between nuclear diameter and noradrenalin or adrenalin concentration in the plasma. However, a definite cycle of chromaffin cell nuclear diameter is apparent (V. pp.53,54).

The reproductive cycle and the adrenocortical cycle recur uniformly from year to year, with only minor variations. In 1975 an exceptionally high percentage of ova appeared in autumn, apparently in response to high summer and autumn temperatures. The adrenocortical cells had minimum nuclear diameter during and after spawning, suggesting maximum activity at this time. This agrees with the findings of Fuller, Scott and Fraser (1976) that cortisol level is high at this time.

3. The Fine Structure of the Tissues of the Head-kidney.

There exist remarkably few electron microscope studies of the head-kidney tissues of teleost fish. The earliest on goldfish (Carassius auratus L.) was made by Yamamoto and Onozato (1965), and a more detailed study, on the same species, by Ogawa (1967). Apart from mammals, indeed, very few fine structural studies are available on any vertebrate group.

(Agnatha – Hardisty and Baines 1971;
Elasmobranches – Taylor, Honn and Chavin 1975;
Amphibians – Burgos 1959; Piezzi 1965,1966,1967;
Reptiles – Sheridan 1963; Conte; 1977.
Birds – Unsicker 1973 a.b.c.d.e.)

Because of this scarcity of studies on non-mammalian vertebrates, it is inevitable that when interpreting the present study on teleost fish, comparisons must be made with what is known in the mammalian system, even though the arrangement of the tissues in these two groups is very different. So far as we know, there is no zonation of the adrenocortical tissue of teleosts as there is in mammals. Such comparisons between widely diverse groups of animals must obviously be made with caution, but they do provide a basis for interpretation of new observations.

In the present investigation, no attempt has been made to study the non-endocrine tissues of the head-kidney in any detail. Typical nucleated erythrocytes are seen, collagenous connective tissue, fibroblasts, and various types of white blood cells. In Phoxinus (p. 59) the majority of the white cells contain many dark lenticular granules, similar to those of eosinophilic granulocytes of mammals (Bessis,1964). Others lack granulation. In Salmo (p. 59) the former type is absent, and four other types occur; a) with many small spherical granules;

b) without granules; c) with many intensely dark large granules; d) similar cells to c) but lacking granules. The differences between the two species may not be taxonomic, but be due to the season, or to the physiological states of the fish, but this was not investigated.

The adrenocortical cells are typical of the steroidogenic cells of all vertebrates in having a tubulo-vesicular internal structure in the mitochondria (Belt and Pease 1956), and a well-developed smooth endoplasmic reticulum. In general their fine structure corresponds with that of goldfish (Yamamoto and Onozato 1965; Ogawa 1967), but it has proved possible to observe a greater range of structural types than described by these workers (pp.60,61,62). Because these types form a continuous uninterrupted series, it is concluded that they represent different stages in the activity of a single cell-type. This conclusion necessarily implies that a single cell-type produces all the cortico-steroid hormones in teleosts (or at least in the two species studied). In contrast to the situation in mammals, where the adrenal cortex is divisible into zones secreting different hormones (Chester Jones 1976), however, there is evidence that in mammals, the zones are not rigidly divided, and that cells in different phases of activity may occur in successive zones (Nishikawa, Murone and Sato 1963).

The most consistent criterion for assessing the activity stage of an adrenocortical cell in Phoxinus or Salmo is the structure of its mitochondria. Some are small (0.4µm), irregularly-shaped and often elongated, electron-opaque structures, whose internal structure is difficult to make out because of their dark matrix. This type of mitochondrion generally occurs sparsely within a cell, and will be referred to as Type I. A second type is large (0.9µm), circular in section, and with a pale matrix so that the tubulo-vesicular internal structure is easily discerned. This type of mitochondrion generally

occurs in very large numbers within a cell, so that in extreme cases the entire cell is packed with them, and will be referred to as Type II. A third type is similar in most respects, except that the internal structure is clearly breaking down, so that only a few tubulo-vesicular remnants are visible in its extreme form, all the internal structure of the mitochondrion is lost, and all that remains is the double outer membrane. This type of mitochondrion occurs, like Type II, in large numbers in a cell, but this is not always apparent in extreme cases (mostly seen in Salmo pp. 62) when all that remains is a number of empty membrane-bounded vacuoles. Such mitochondria will be referred to as Type III. A complete series of intermediate forms is, of course, found.

The cytoplasm of the adrenocortical cells may appear either dark or light, depending on the number of ribosomes, amount of granulation, extent of the smooth endoplasmic reticulum, number of vacuoles or exhausted Type III mitochondria, etc. There is very little correlation between the darkness of the cytoplasm and the type of mitochondrion, though a subjective impression is that cells with Type I mitochondria tend to have light cytoplasm, and those with Type II mitochondria darker cytoplasm. In the case of Type III mitochondria, especially in the final stages of disintegration, the cell gives an overall impression of lightness, but this impression may be largely due to the vacuolar remains of exhausted mitochondria. The form of the mitochondria is clearly likely to be the most objective way of assessing the activity of an adrenocortical cell, but cell "darkness" or "lightness" have been used by Ogawa (1967) and are considered here for that reason.

It is clearly necessary to determine which are the active and which are the inactive stages. This is not a straightforward problem, as all stages are present in any given section, only the relative proportions of the stages differing in different fish. Moreover, it

is well established that catching, handling, and aquarium maintenance can elicit considerable changes in corticosteroid levels in the blood of teleosts, and unless the conditions to which the specimens have been subjected before tissue fixation are known, it is impossible to decide whether the cells were fixed while active or inactive. Neither Yamamoto and Onozato (1965) nor Ogawa (1967) make this clear. Ogawa (1967)hypophysectomised goldfish, whose adrenocortical cells would presumably be inactivated, but his description of the consequent fine structure changes (" .....the appearance of a highly electron-opaque cell-type") is not clear; he might be referring either to the mitochondria or to the cytoplasm, or to the cell generally.

In Phoxinus, caught by electrofishing and killed immediately, so that corticosteroid secretion is likely to be low, all cell-types were present, but the most abundant (69%) were those with Type II mitochondria. Cells with Type I mitochondria comprised only 31% of the total. In Phoxinus killed after twenty-four hours in aquaria, the proportions were reversed: cells with Type II mitochondria 34%; cells with Type I mitochondria 66%. At first sight these figures suggest that cells with Type II mitochondria are inactive, and that Type I mitochondria develop with increasing activity. Two arguments contradict this interpretation:

(a) Often (but not always) the cytoplasm of cells with Type II mitochondria is relatively light, whereas that of cells with light mitochondria is often dark, rich in ribosomes, and with a conspicuous endoplasmic reticulum and golgi complex, suggesting activity.

(b) In Salmo, the most common adrenocortical cell-type contains many very closely-packed Type II mitochondria, despite the fact that these specimens had been aquarium-maintained for a much longer period than Phoxinus. In addition, many Salmo

Fig. 27. Nurse Shark adrenocortical gland divided into three zones. The schematic cells illustrated represent a composite of general cellular characteristics of each zone (Taylor, Honn and Chavin 1975).

Fig. 28. Changes of mitochondria in the adrenal cortex of adult rat (Nishikawa, Murone and Sato (1962).

Fig. 27

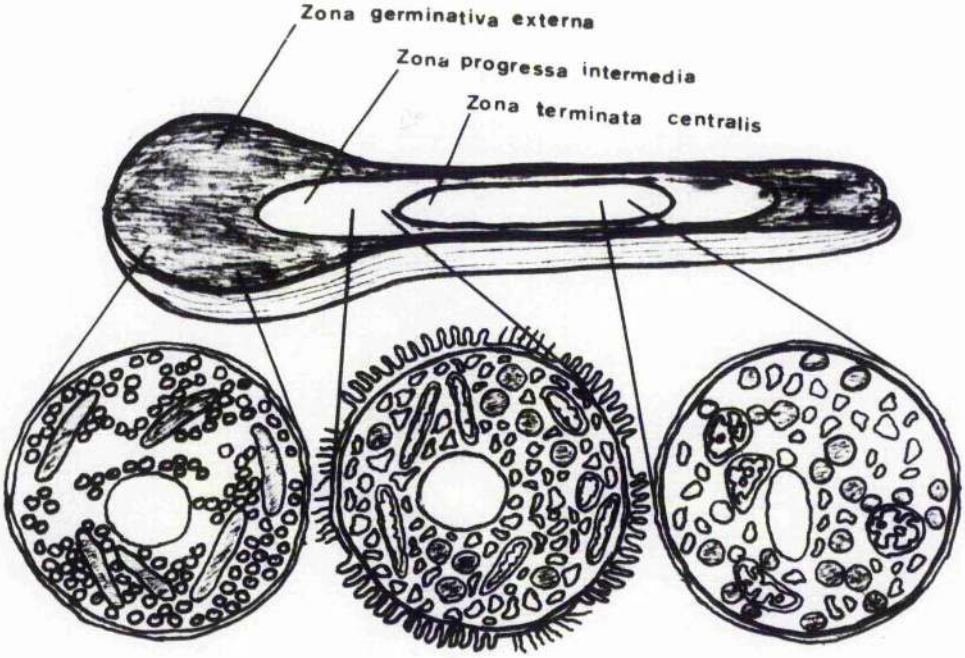
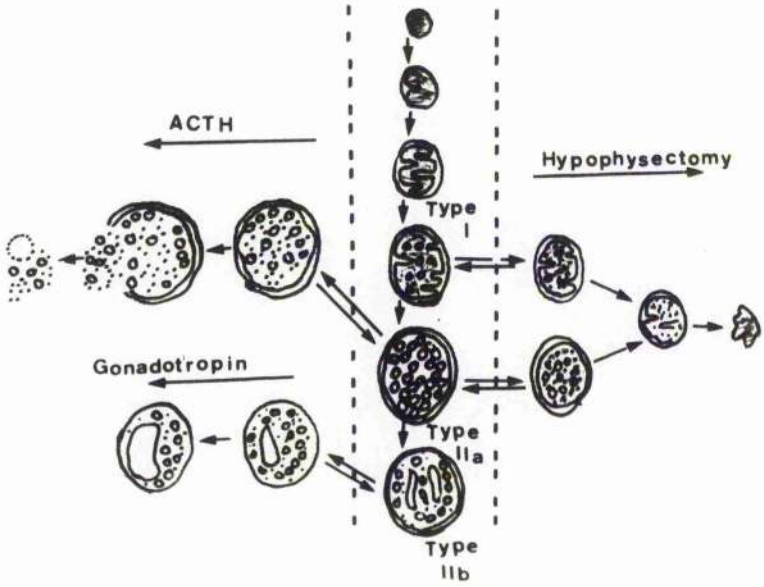

Zona germinativa externa
Zona progressa intermedia
Zona terminata centralis

Fig. 28


ACTH
Hypophysectomy
Type I
Gonadotropin
Type IIa
Type IIb

cells contained Type III mitochondria whose internal structure was clearly breaking down, or which had completely disappeared. Such cells also occurred in Phoxinus, but much less commonly.

The interpretation which best fits these observations is that the cells with Type I mitochondria are at the beginning of their cycle of activity, and when the fish are first exposed to stressful conditions, the number of cells with Type I mitochondria increase in the head-kidney. Increasing secretion of hormones is associated with the change of the small, dark Type I mitochondria into the large, pale Type II. The number of mitochondria per cell increases correspondingly, and dark cytoplasm may be associated with this phase of activity. Eventually the mitochondrial structure breaks down, leaving cells which, because of the many residual vacuoles, look pale. Whether such cells repeat the cycle or not is uncertain. In nurse sharks, (Ginglymostoma cirratum Bonnaterre) Taylor, Honn and Chavin (1975) have reported that cell death follows the release of the corticosteroid hormones. In these animals, the progressively maturing adrenocortical cells apparently migrate through zones in the adrenocortical mass of tissue (Fig. 27). Taylor, Honn and Chavin (1975) describe also the fine structural changes in these adrenocortical cells. In the outermost zone, the zona germintiva externa the cells have elongated to spherical mitochondria with short lamellar cristae projecting into a dense matrix; numerous spherical vesicles; no smooth endoplasmic reticulum; no lipid droplets; no cilia or microvilli; cell death does not occur. In the middle zone, zona progressa intermedia the cells have elongated to spherical mitochondria with dilated cristae; numerous irregularly shaped vesicles; smooth endoplasmic reticulum; liquid droplets; cilia or microvilli; there is a limited amount of cell death. In the innermost zone, the zona terminata centralis, the cells have elongated to irregularly shaped mitochondria with vesicular cristae; numerous irregularly shaped vesicles;

smooth endoplasmic reticulum; lipid droplets; no cilia or microvilli; there is extensive cell death.

Although there is no evidence of such zonation of the adrenocortical cells in teleosts, the series of fine structural changes described in the nurse shark accords well with the present author's observations in Phoxinus and Salmo.

Allowing for the complication of zonation in the mammalian adrenal cortex, the above interpretation also accords with the observations in rats described by Nishikawa, Murone and Sato (1963) (Fig. 28). They describe "Type I" ("an immature type") mitochondria which are restricted to the zona glomerulosa, and which correspond closely with Type I mitochondria as described in the present work. Nishikawa et al's "Type I - intermediate mitochondria" develop into their "Type IIa", chiefly in the outer layers of the zona fasciculata. Under the influence of ACTH, "Type IIa" mitochondria develop into "open form" mitochondria. Hypophysectomy has no effect on "Type I", but does cause degenerative changes in "intermediate" and "Type IIa" mitochondria, which are transformed to small mitochondria with a dark matrix.

Nishikawa, Murone and Sato (1963) point out that classifying adrenocortical cells into "light" or "dark" categories is not a sufficient indication of their state of activity, as "dark" cells may contain either the degenerate mitochondria resulting from hypophysectomy - presumably inactive - or "Type II" mitochondria - presumably active. Hence Ogawa's description (see above) of an "electron opaque cell-type" after hypophysectomy presumably refers to inactive cells. His subsequent conclusion that "light cells have a great capacity for steroid biosynthesis" is probably therefore an oversimplification, as in both Phoxinus and Salmo Type II mitochondria occur in cells with either light or dark cytoplasm. Increasing "lightness" of the cell is probably due to the accumulation

of vacuoles as the stage of exhaustion approaches, in Type III. Probably therefore, maximum activity is occurring in cells with Type II mitochondria and dark cytoplasm,

The microbodies seen in cells with light mitochondria (p. 61) may be the early stages in the development of new mitochondria. Such development of microbodies into mitochondria has been previously described by Nishikawa, Murone and Sato (1963), and by Belt (1958).

In birds, Unsicker (1973d) has drawn attention to differences in the mitochondrial structure in different zones of the avian "interrenal gland". He also describes " ..... great numbers of concentrically arranged fenestrated double membranes (whorls) which have been known to exist in several steroid producing glands....". These whorls he interprets as being specialisations of the smooth endoplasmic reticulum, and ".....the result of certain kinds of stress".

Although various cell-types in an animal give the classical chromaffin reaction with potassium dichromate, the term "chromaffin cell" has become an accepted name for the endocrine cells secreting noradrenalin and adrenalin which are often (but not always) associated with the adrenocortical cells. Chromaffin cells are readily identifiable by their fine structures,and are typified by the presence of a great many chromaffin vesicles more or less filled with material of high electron density (Unsicker (1973a). With only minor differences, the fine structure of chromaffin cells seems uniform in all vertebrates so far studied. There is, as in the case of the adrenocortical tissue a considerable volume of work on mammalian chromaffin tissue (reviewed by Unsicker, 1973a), but less information is available concerning non-mammalian vertebrates (Coupland 1971). Studies on avian chromaffin cells exist (Unsicker 1973a), a few on amphibia (Piezzi, 1965,1966,1967; /Burgos 1959, but apparently none on fish.

In the present study the fine structure of the chromaffin cells of Phoxinus and Salmo has been described (pp. 62,63). In most respects they resemble the chromaffin cells of other vertebrates. However, no cilia were observed in the teleost cells whereas cilia are described as being of regular occurrence in the chromaffin cells of birds, a single cilium occurs in the majority, if not all, chromaffin cell (Unsicker 1973a), and of sporadic occurrence in amphibia (Piezzi 1967). In all the other vertebrate groups it has proved possible to distinguish two distinct types of chromaffin cells, those producing adrenalin, and those producing noradrenalin. In birds (Unsicker 1973a), the two types can be distinguished on the bäsis of the structure of the chromaffin vesicles. In noradrenalin-secreting cells the contents are homogeneous and electron-dense, whereas in adrenalin-secreting cells the vesicular contents show a granular sub-structure of less electron density. Adrenalin-secreting cells are the more common. Non-adrenalin-secreting cells have "cavities and light spots in the hyaloplasm; adrenalin-secreting cells have a hyaloplasm of uniform density.

In reptiles also two different types of chromaffin cells have been described (Wassermann, and Tramezzani 1961,1963; Benedeczky, Puppi, Tigyi and Lissak 1964).

In Bufo arenarum (Piezzi 1967) the two types of chromaffin cells are mixed together - noradrenalin - secreting cells are elongated with densely osmiophillic chromaffin granules. They have a moderate number of spherical mitochondria and empty vesicles. Adrenalin-secreting cells are spherical or polygonal, with elongated mitochondria having an electron-opaque matrix. The chromaffin granules are less abundant, and vary in their osmiophilia.

It is not possible, without making histochemical tests, to state definitely whether the chromaffin cells in Phoxinus and Salmo are nor-adrenalin or adrenalin - secreting. The chromaffin granules have a granular sub-structure but the cytoplasm is not uniform, so that

comparison with the avian situation is not possible. The amount of electron-opaque/core contained in the chromaffin vesicles is very variable (depending presumably on the rate of secretion) so that it is not possible to use this criterion of Piezzi (1967), though the elongate shape of the mitochondria suggests, by analogy with Bufo, that the cells secrete adrenalin.

The variation in the content of the chromaffin vesicles is clearly associated with the activity of the cell, but no attempt has been made in the present study to establish an exact relationship, because of the difficulties in obtaining specimens in an unshocked condition. Cells of all degrees of vesicular filling, from empty to completely filled with electron-dense material occurred in all sections in an uninterrupted series, suggesting that a single cell-type is involved.

Synaptic contacts with nerves were very commonly observed, and occurred equally on chromaffin cells of all types confirming that only a single cell-type is present. Adrenergic and cholinergic synapses have been described on chromaffin cells in amphibia and in birds. In Bufo (Piezzi 1965,1967) adrenergic synapses are characterised by synaptic vesicles 500 - 600A in diameter. The cholinergic synapses by synaptic vesicles of various sizes (700A - 1300A). In birds (Unsicker 1973b,c) of the efferent fibres are cholinergic, the synapses typically containing two populations of dense-cored vesicles. The terminals of adrenergic fibres lack synaptic membrane specialisations.

In Phoxinus and Salmo, by analogy, all the synapses observed were cholinergic, but occasional axons with varicosities were observed, so that adrenergic synapse, may be present, and the presence of rare adrenergic synapses cannot be eliminated.

SUMMARY

This is a study of the structure and functions of the endocrine tissues in the head-kidney of the teleost fish, the homologous tissues to the mammalian adrenal cortex (=adrenocortical tissue) and adrenal medulla (=chromaffin tissue). The study is divided into three main sections:

1. The first section comprises a study of the general morphology, at the anatomical and histological level, of the different types of head-kidney which occur in teleost fish. The range of types is illustrated by studies on twenty-four species, some of which have been previously investigated, and including in particular sixteen marine species from the Red Sea coast of Saudi Arabia collected by the author. No previous studies of the head-kidneys of middle-eastern teleosts have been made, despite their economic importance. From these twenty-four species, and 129 previously studied by Nandi (1962), there is clearly a considerable range in the anatomical configuration of the head-kidneys and the trunk-kidneys = The head-kidney may be separate from the trunk-kidney, completely fused with it, or intermediate in form. Haemopoietic tissue may occupy most of the head-kidney and functional renal elements may be absent, or renal elements may predominate. A complete range of intermediate forms exist.

Melanophore-macrophage complexes occur in the haemopoietic tissue. The distribution of the endocrine tissues is also very variable from species to species. Both the adrenocortical and chromaffin tissues are in some way associated with the main veins draining the head-kidney into the heart. They may exist as sheaths around major veins (either the posterior cardinals, or the ducts of Cuvier), as diffuse masses of

tissue permeated by minor sinusoidal branches of the veins, or as discrete masses of tissue embedded in the haemopoietic tissue. The adrenocortical and chromaffin tissues may lie intermingled together, or they may form separate layers around the vessels, sometimes with the chromaffin closer to the vein lumen, sometimes with the adrenocortical in this position. The adrenocortical tissue and chromaffin tissue may lie separately with the chromaffin tissue posterior. There is little evidence for the association of a particular morphological type with a particular taxonomic group of teleosts, so that each species is best investigated in its own right.

2. The second section of this thesis comprises a detailed study of the morphology of the head-kidney of one particular species, Phoxinus phoxinus (Linnaeus), and a two year study of seasonal variations in the activity of its adrenocortical and chromaffin tissue. Samples of fish were collected from a population in the Walton Reservoir, Scotland, at monthly intervals. The activity of the adrenocortical tissue was assessed by measuring nuclear diameter of the adrenocortical cells, a criterion already widely used for this purpose. The activity of the chromaffin cells was similarly assessed, though the methodology is less well established in this case. The effects were compared of electrofishing followed by anaesthesia and immersion in Bouin's fixative while still under electronarcosis. Both proved to be relatively stress-free methods. The reproductive cycle of both males and females sampled were assessed by measuring the gonadosomatic ratio, and by counting the proportions of different oocyte stages and observing the different spermatocyte stages in gonad sections. The cycle was found to vary a little from year to year, dependent on weather conditions. Nuclear diameter of both adrenocortical and chromaffin cells showed a seasonal variation which was closely correlated with the reproductive cycle; minimum mean nuclear diameter in both cases occurring at the end of

spawinig period. A high degree of adrenocortical activity accompanying spawning has been described independently in other teleosts.

3. The third section of this thesis comprises a study of the fine structure of the endocrine tissues of the head-kidney of Phoxinus phoxinus and Salmo gairdnerii. Electron micrographs were prepared using fish caught under stress-free conditions in the Walton Reservoir, and from aquarium-maintained fish. The adrenocortical cells in both species are characterised by having a great many conspicuous mitochondria with tubulo-vesicular internal structure. The nucleus is circular in section, and centrally situated in the cell. There is an extensive smooth endoplasmic reticulum and numerous ribosomes. Microvilli occur on cell surfaces in contact with veins. There is a wide range in the structure of the adrenocortical cells of individual fish; mitochondria range from small, elongated structure, with a dark matrix to large, circular structures in which the internal structures eventually breaks down. The cytoplasm as a whole tends to be pale in cells with small dense mitochondria, and dense in cells with large, paler mitochondria. Pale highly vacuolated cytoplasm is associated with cells in which the mitochondria are breaking down; the vacuoles are probably associated with the degenerating mitochondria. In Phoxinus maintained in aquaria for twenty-four hours before killing, the proportion of adrenocortical cells with small mitochondria with dark matrices, as cpmpared to fish caught by stress-free methods and fixed immediately. In Salmo, which had been maintained in aquaria for longer periods, the proportion of cells showing mitochondrial degeneration and cytoplasmic vacuolation is higher. It is concluded that small, dark matrix mitochondria are typical of early stages of adrenal activity; dense cytoplasm and an increased number of large, circular mitochondria are typical of maximum activity; and mitochondrial degeneration and vacuolation of

the cytoplasm is typical of exhaustion. Chromaffin cells, not hitherto described in teleost fish, are of the type found in other vertebrates, with many chromaffin vesicles containing varying amounts of granular inclusion. Synaptic contacts occur commonly, apparently all of cholinergic type. In Salmo the chromaffin and adrenocortical cells lie separately, but in Phoxinus the adrenocortical cells form a sheath round the posterior cardinal veins and their main tributaries, and the chromaffin cells lie beyond them, against the haemopoietic tissue. These chromaffin cells communicate with the vein by elongated projections running amongst the adrenocortical cells.

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Table 1. Mean weight of gonads during 1964 and 1975–6
(Mean of all fish in each sample)

Month 1964	mean gonad weight		Month 1975–76	mean gonad weight	
	♀	♂		♀	♂
March	0.17	0.05			
April	0.22	0.07			
May	0.40	0.10	May 75	0.89	0.15
June	0.42	0.10	June	0.51	0.13
July	0.26	0.05	July	0.36	0.08
August	0.12	0.04	August	0.2	0.03
Sept.			Sept.	0.22	0.03
Oct.	0.1	0.03	Oct.		0.05
Nov.	0.11	0.03	Nov.	0.35	0.06
Dec.	0.19	0.04	Dec.	0.37	0.09
			Jan.76	0.37	0.08
			Feb.	0.37	0.08
			March	0.46	0.12
			April	0.96	0.18

Table 2. Mean total weight of fish during 1964 and 1975–76.
(Mean of all fish in each monthly sample)

Month 1964	mean total weight		Month 1975–76	mean total weight	
	♀	♂		♀	♂
March	3.2	2.1			
April	3.48	2.6			
May	3.23	2.44	May 75	4.24	3.43
June	2.55	2.4	June	4.34	3.27
July	2.8	2.09	July	4.4	3.34
August	2.87	2.3	August	6.1	3.78
Sept.			Sept.	5.9	4.72
Oct.	2.7	2.24	Oct.		4.8
Nov.	2.61	2.5	Nov.	6.0	5.6
Dec.	3.86	2.5	Dec.	6.5	5.7
			Jan.76	5.9	4.4
			Feb.	5.89	4.7
			March	5.9	6.03
			April	6.5	6.1

Table 3. Mean length of fish during 1964 and 1975–76
(Mean of all fish in each mohthly sample)

Month 1964	Mean full length ♀	Mean full length ♂	Month 1975–76	Mean full length ♀	Mean full length ♂
March	7.19	5.38			
April	7.3	6.66			
May	7.13	6.52	May 75	7.2	7.14
June	6.64	6.39	June	7.47	6.99
July	6.94	6.36	July	7.6	7.1
August	7.02	7.3	August	8.61	7.4
Sept.			Sept.	8.4	7.6
Oct.	6.7	6.28	Oct.		7.9
Nov.	6.8	6.6	Nov.	8.5	7.9
Dec.	7.4	6.8	Dec.	8.64	8.35
			Jan.76	8.4	7.2
			Feb.	8.41	7.34
			March	8.6	8.43
			April	8.7	8.5

Table 4. Mean gonadosomatic ratio (g/s) ± standard deviation of fish during 1964 and 1975–76
(Mean of all fish in each fortnightly or monthly sample)

Month 1964	g/s Ratio ± S.D. ♀	g/s Ratio ± S.D. ♂	Month 1975–76	g/s Ratio ± S.D. ♀	g/s Ratio ± S.D. ♂
E. March	60.2±3.9	30.9±9.06			
L. March	44.8±0.9	22.5±9.86			
E. April	69.2±11.	31.27±6.79			
L. April	76.4±13.1	53.23±8.53			
E. May	143.9±31.2	48.27±11.5			
L. May	104.6±17.7	32.2±10.6	L. May75	144.67±17.9	38.5±6
E. June	149 ±30.9	35.8±7.6	E. June	151.2±12.5	44.7±10.9
L. June	159 ±43.2	30.2±6.7	L. June	96.3±13.7	32.78±7.6
E. July	107.9±13.9	21.7±5.4	E. July	82.3±25.7	25.8±4.6
L. July	79.4±31.7	25.6±7.8	L. July	79.3±27.1	23.3±1.1
August	29.4±6.7	8.6±1.5	August	29. ±4.8	5.4±0.8
Sept.			Sept.	37 ±6.3	6.7±1.3
Oct.	36.3±1.9	13.2±2.9	Oct.		10.8±2.4
Nov.	37.1±7.4	14 ±2.7	Nov.	55.9±6.7	10.8±1.2
Dec.	46.4±5.9	17.3±3.6	Dec.	53.4±9	10.99±0.7
			Jan. 76	54.8±6	13 ±0.2
			Feb.	58.2±10.2	18.2±4.45
			March	57.1±9.2	20.2±5.7
			E. April	90.7±26.98	31.5±8.9
			L. April	159.8±29.4	37.4±5.5
			E. May	143 ±22.8	32.3±4.2

Table 5. Mean condition factor (C,F,) ± 1 standard deviation of fish during 1964 and 1975-6 (Mean of all fish in each fortnightly or monthly sample)

Month 1964	C.F. ± S.D.		Month 1975-76	C.F. ± S.D.	
	♀	♂		♀	♂
E. March	0.88±0.06	0.86±0.07			
L. March	0.81±0.07	0.7 ±0.14			
E. April	0.79±0.05	0.84±0.05			
L. April	0.82±0.09	0.85±0.05			
E. May	0.92±0.15	0.91±0.06			
L. May	0.89±0.07	0.83±0.07	L. May 75	1.08±0.09	0.94±0.09
E. June	0.85±0.05	0.88±0.12	E. June	1.07±0.11	0.88±0.05
L. June	0.78±0.03	0.78±0.06	L. June	1.03±0.07	0.97±0.08
E. July	0.78±0.06	0.79±0.04	E. July	1.0 ±0.07	0.95±0.04
L. July	0.86±0.28	0.78±0.24	L. July	0.93±0.07	0.9 ±0.05
August	0.8 ±0.06	0.83±0.06	August	0.96±0.06	0.99±0.13
Sept.			Sept	0.96±0.05	1.07±0.15
Oct.	0.91±0.03	0.89±0.13	Oct.		0.96±0.06
Nov.	0.8 ±0.06	0.87±0.09	Nov.	1.03±0.06	1.18±0.16
Dec.	0.92±0.06	0.85±0.14	Dec.	0.99±0.07	0.96±0.04
			Jan. 76	0.95±0.12	0.82±0.09
			Feb.	0.95±0.09	0.89±0.12
			March	0.97±0.07	1.01±0.14
			E. April	1.04±0.1	1.02±0.06
			L. April	1.07±0.08	0.98±0.07
			E. May	1.08±0.1	0.94±0.11

Table 6. Mean somatic condition factor (S,C,F,) ± 1 standard deviation of fish during 1964 and 1975-76 (Mean of all fish in each fortnightly or monthly sample).

Month 1964	S.C.F. ± S.D.		Month 1975-76	S.C.F. ± S.D.	
	♀	♂		♀	♂
E. March	0.85±o.o7	0.83±0.07			
L. March	0.76±0.07	0.69±0.15			
E. April	0.74±0.05	0.85±0.05			
L. April	0.76±0.11	0.8 ±0.05			
E. May	0.78±0.1	0.86±0.06			
L. May	0.74±0.09	0.79±0.08	L. May 75	0.9 ±0.07	0.9 ±0.09
E. June	0.72±0.05	0.85±0.11	E. June	0.91±0.08	0.84±0.05
L. June	0.63±0.07	0.76±0.06	L. June	0.89±0.07	0.84±0.08
E. July	0.7 ±0.05	0.78±0.05	E. July	0.9 ±0.07	0.92±0.04
L. JUly	0.8 ±0.03	0;75±0.21	L. July	0.86±0.06	0.88±0.05
August	0.79±0.04	0.81±0.06	August	0.94±0.06	0.99±0.16
Sept.			Sept.	0.93±0.05	1.06±0.15
Oct.	0.88±0.024	0.88±0.12	Oct.		
Nov.	0.79±0.09	0.86±0.09	Nov.	0.97±0.06	1.17±0.16
Dec.	0.9 ±0.06	0.83±0.13	Dec.	0.94±0.06	0.94±0.04
			Jan. 76	0.89±0.11	0.81±0.1
			Feb.	0.9 ±0.08	0.87±0.12
			March	0.91±0.06	0.99±0.15
			E. April	0.95±0.08	0.99±0.05
			L. April	0.9 ±0.06	0.94±0.06
			E. May	0.92±0.04	0.91±0.11

Table 7. Mean nuclear diameter (N.D.) ± 1 standard deviation of adrenocortical (Ade.) cells of fish during 1964 and 1975-76. (Mean of ten - five females and ten-five males in each forthnight or monthly sample, 30 nuclei measured per fish).

Month 1964	Mean N.D. ± S.D. Adrenocortical ♀	♂	Month 1975-76	Mean N.D. ± S.D. Adrenocortical ♀	♂
E. March	4.45±0.05	4.44±0.07			
L. March	4.6 ±0.06	4.48±0.03			
E. April	4.64±0.07	4.53±0.17			
L. April	4.44±0.16	4.45±0.08			
E. May	4.58±0.08	4.59±0.1			
L. May	4.57±0.03	4.63±0.06	L. May 75	4.62±0.12	4.6 ±0.06
E. June	4.55±0.07	4.62±0.04	E. June	4.3 ±0.12	4.34±0.07
L. June	4.45±0.16	4.57±0.07	L. June	4.21±0.11	4.31±0.13
E. July	4.3 ±0.17	4.34±0.11	E. July	4.05±0.16	3.98±0.08
L. July	4.2 ±0.1	4.16±0.1	L. July	4.05±0.06	4.18±0.13
August	4.06±0.09	4.25±0.32	August	3.9 ±0.1	3.9 ±0.1
Sept.			Sept.	4.03±0.06	4.07±0.05
Oct.	4.32±0.04	4.29±0.06	Oct.		4.14±0.08
Nov.	4.35±0.1	4.3±0.05	Nov.	4.18±0.11	4.24±0.04
Dec.	4.2 ±0.1	4.3 ±0.07	Dec.	4.24±0.11	4.29±0.08
			Jan. 76	4.24±0.12	4.34±0.06
			Feb.	4.3 ±0.09	4.38±0.05
			March	4.44±0.07	4.32±0.07
			E. April	4.5 ±0.05	4.42±0.06
			L. April	4.39±0.11	4.49±0.05
			E. May	4.65±0.09	4.51±0.06

Table 8. Mean nuclear deameter (N.D.) ± 1 standard deviation of Chromaffin cells (Chr.) of fish during 1964 and 1975-76. (Mean of ten-five fish of each sex in each fortnightly or monthly sample, 30 nuclei measured per fish).

Month 1964	Mean N.D. ± S.D. Chromaffin ♀	♂	Month 1975-76	Mean N.D. ± S.D. Chromaffin ♀	♂
E. March	5.93±0.14	5.74±0.11			
L. March	5.8 ±0.07	5.63±0.09			
E. April	6.14±0.15	6.02±0.26			
L. April	5.89±0.22	5.83±0.27			
E. May	5.89±0.22	5.83±0.09			
L. May	5.63±0.13	5.76±0.13	L. May 75	5.76±0.17	5.71±0.1
E. June	5.66±0.14	5.95±0.24	E. June	5.4 ±0.1	5.29±0.07
L. June	5.76±0.35	5.75±0.2	L. June	5.26±0.14	5.29±0.09
E. July	5.39±0.23	5.42±0.13	E. July	5.08±0.1	4.98±0.1
L. July	5.22±0.14	5.38±0.04	L. July	5.17±0.05	5.13±0.09
August	5.3 ±0.04	5.34±0.2	August	5. ±0.07	4.94±0.09
Sept.			Sept.	5. ±0.05	5.07±0.05
Oct.	5.38±0.01	5.43±0.01	Oct.		5.17±0.1
Nov.	5.45±0.09	5.35±0.05	Nov.	5.28±0.1	5.31±0.04
Dec.	5.35±0.03	5.41±0.04	Dec.	5.37±0.11	5.32±0.04
			Jan. 76	5.22±0.06	5.14±0.03
			Feb.	5.26±0.08	5.3 ±0.04
			March	5.37±0.06	5.26±0.05
			E. April	5.43±0.08	5.34±0.04
			L. April	5.52±0.08	5.56±0.06
			E. May	5.61±0.09	5.59±0.08

Table 9. Mean nuclear diameter (N.D.) of adrenocortical cell (Adr.) and chromaffin cells (Chr.) of each fish in the sample. (Mean nuclear diameter was taken by measuring 30 nuclei per fish). ♀.

No. of fish	Killed in Bouin		Killed in MS222	
	N.D. Adr.	N.D. Chr.	N.D. Adr.	N.D. Chr.
1	4.1	5.0	4.0	5.0
2.	3.9	5.0	4.2	5.2
3.	4.1	4.9	4.0	5.0
4.	4.0	5.1	3.9	5.1
5.	4.1	5.1	4.0	5.0
6.	4.1	4.9	4.2	5.1
7.	3.9	5.0	4.1	5.0
8.	4.1	5.1	4.2	5.2
9.	4.1	5.0	4.0	5.1
10.	4.0	4.9	4.0	5.0
Mean S.D.	4.1 0.06	5.0 0.05	4.1 0.08	5.1 0.08

Table 10. Mean nuclear diameter (N.D.) ± 1 standard deviation of adrenocortical cell (Adr.) and chromaffin cells (Chr.) (Mean of all fish in each sample. 30 nuclei measured per fish). ♀.

Time in aquarium	Mean N.D. ± S.D. adrenocortical	Mean N.D. ± S.D. chromaffin
3 hours	4.22 ± 0.06	5.17 ± 0.12
24 hours	4.19 ± 0.04	5.25 ± 0.1
7 days	4.04 ± 0.19	4.99 ± 0.18
30 days	4.18 ± 0.09	5.13 ± 0.08
90 days	4.45 ± 0.07	5.28 ± 0.06

ENDOCRINOLOGY OF THE HEAD-KIDNEY TISSUES IN TELEOST FISH

Volume II (Plates)

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PLATES 1 – 30

ABBREVIATIONS

A = Adrenocortical cell

ACV = Anterior cardinal vein

AO = Atretic oocytes

C = Chromaffin cell

CA = Coeliacomesenteric artery

DA = Dorsal aorta

DC = Duct of cuvier

E = Erythrocytes

F = Follicle

FC = Follicular calyce

G = Gut

GC = Germ cell

GL = Glomerulus

H = Haemopoietic tissue

K = Head-kidney

KD = Kidney duct

L = Liver

M = Melanophore

N = Nerve

O = Oolemma

OG = Oogonium

OV = Ovium

PCV = Posterior cardinal vein

PG = Primary spermatogonium

PO = Primary oocytes

PS = Primary spermatocytes

S = Spermatozoa

SA = Sperm artery

SC = Spinal cord

SD = Sinusoid

SF = Siminiferus tubule

SG = Secondary spermatogonium

SM = Sperm duct

SO = Secondary oocytes

SS = Secondary spermatocytes

St = Spermatid

SV = Sinus venosus

T = Renal tubule

VB = Branch of posterior cardinal vein

VC = Vertebral column

VW = Vein wall

Y = Yolk

YP = Yolk precursors.

Plate 1. (A–D) Rasbora heteromorpha. Transverse sections, 6 µm, stained in Masson's trichrome.

(A) Section through whole fish, showing dorsal aorta and coeliacomesenteric artery, and left duct of Cuvier emerging from left lobe of head-kidney. The posterior cardinal vein branches extensively in the left lobe. Scale line corresponds to 300 µm.

(B) Section through right lobe of head-kidney, showing the median posterior cardinal veins forming the right duct of Cuvier, to the heart, without extensive branches as in the case of the left duct. Scale line corresponds to 200 µm.

(C) Section through head-kidney, showing both lobes of the head-kidney with median posterior cardinal vein and right duct of Cuvier. The coeliacomesenteric artery has branched off from the dorsal aorta and has passed through the kidney on its way to the gut. Scale line corresponds to 300 µm.

(D) Section posterior to the above, at the posterior end of the head-kidney. The right posterior cardinal vein lies to the left of the mid-line. The dorsal aorta lies in a groove in the angle between the kidney and the posterior cardinal vein On each side of the dorsal aorta is a branch of sympathetic nervous system. Dorsal to the dorsal aorta is the vertebral column. Scale line corresponds to 200 µm.

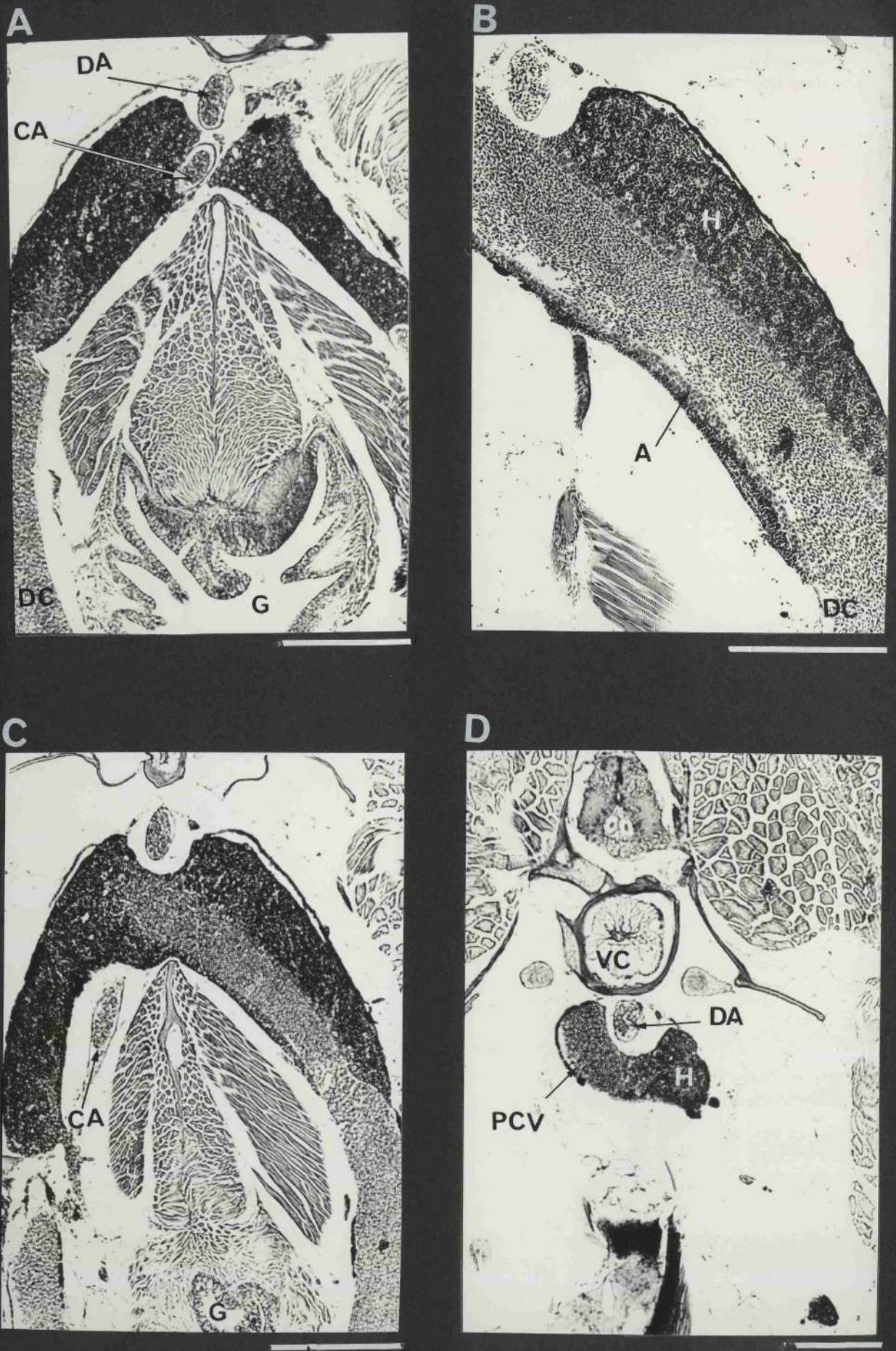
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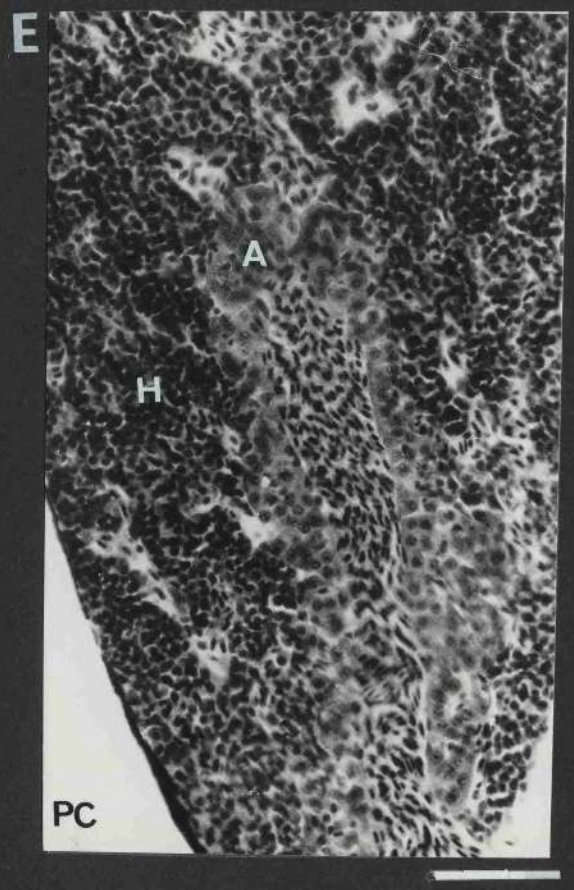
Plate 1 (E-F) Rasbora heteromorpha.

(E) Section of ventral part of left lobe of head-kidney, showing duct of Cuvier emerging. The vein is surrounded by several layers of adrenocortical cells, with very few chromaffin cells. The rest of the tissue is haemopoietic, and there are no functional renal elements present. Scale line corresponds to 50 µm.

(F) Section of right duct of Cuvier, showing, from left to right = erythrocytes; adrenocortical and chromaffin cells; haemopoietic tissue. Scale line corresponds to 25 µm.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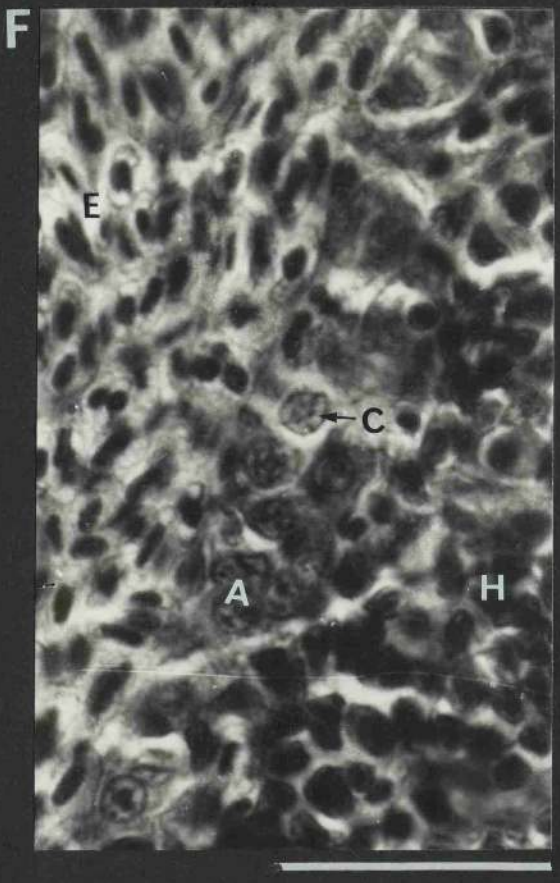
F
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Plate 2. *Scleropages formosus*. Longitudinal sections through head-kidney, 5 µm head.

(A) Islet of adrenocortical tissue in haemopoietic tissue containing melanophores. Scale line corresponds to 50 µm.

(B) Main branch of posterior cardinal vein in haemopoietic tissue. A few chromaffin cells lie in the walls of the vein. Scale line corresponds to 50 µm.

(C) As (B), showing individual chromaffin cells. Scale line corresponds to 25 µm.

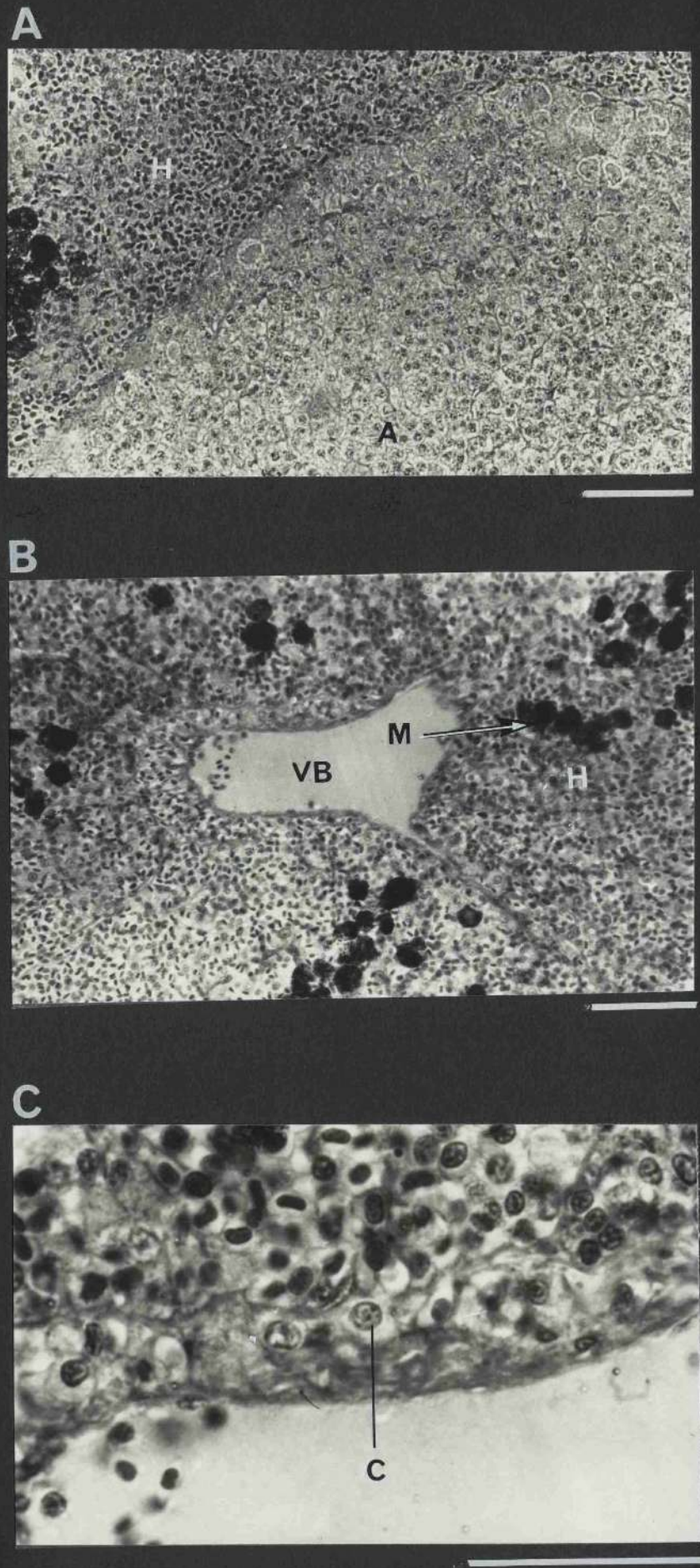
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Plate 3. *Salmo irideus* Longitudinal sections through the head-kidney, 6 µm, stained in Masson's trichrome.

(A) Adrenocortical tissue associated with the posterior cardinal vein, and surrounded by haemopoietic tissue. Scale line corresponds to 50 µm.

(B) The same cells as in (A). Scale line corresponds to 75 µm.

(C) Adrenocortical tissue, amongst haemopoietic tissue, not associated with a vein. Scale line corresponds to 75 µm.

(D) Chromaffin tissue in the wall of the posterior cardinal vein, in the posterior part of the head-kidney. Scale line corresponds to 50 µm.

(E) The same cells as in (D). Scale corresponds to 75 µm.

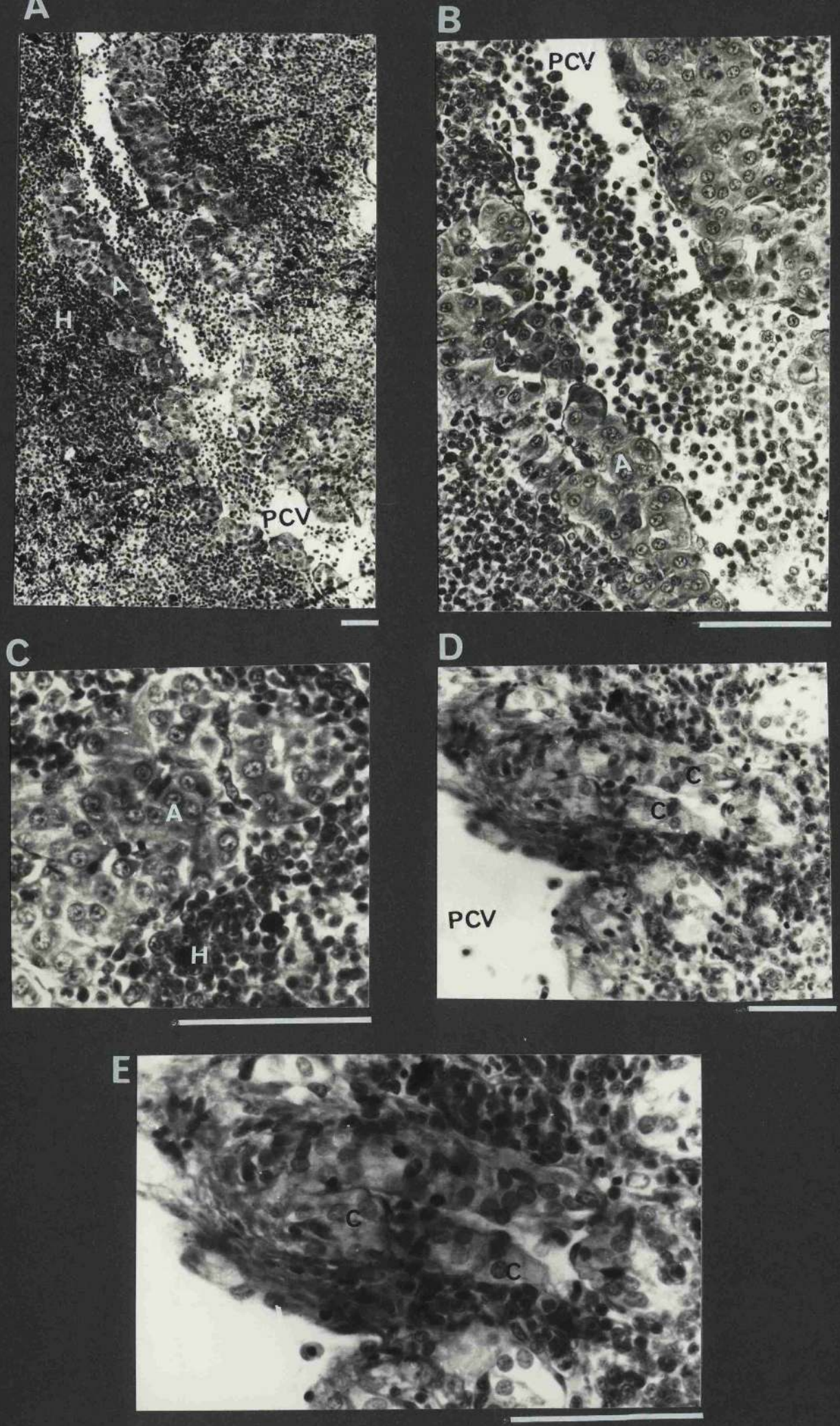
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Plate 4. *Coregonus lavaretus* Longitudinal sections through head-kidney, 6 µm, stained in Masson's trichrome.

(A) Section through right posterior cardinal vein, showing vein passing through haemopoietic tissue with little branching. Adrenocortical tissue is associated with the vein. Scale line corresponds to 200 µm.

(B) Section through left lobe of head-kidney, showing much-branched venous system with adrenocortical cells surrounding small veins. Melanophores occur in the haemopoietic tissue. Scale line corresponds to 50 µm.

(C) Section through right lobe of head-kidney, showing adrenocortical cells surrounding small branches of the main right posterior cardinal vein. Scale line corresponds to 200 µm.

(D) Section through right lobe near posterior end of head-kidney, showing groups of chromaffin cells in the wall of the right posterior cardinal vein. Scale line corresponds to 50 µm.

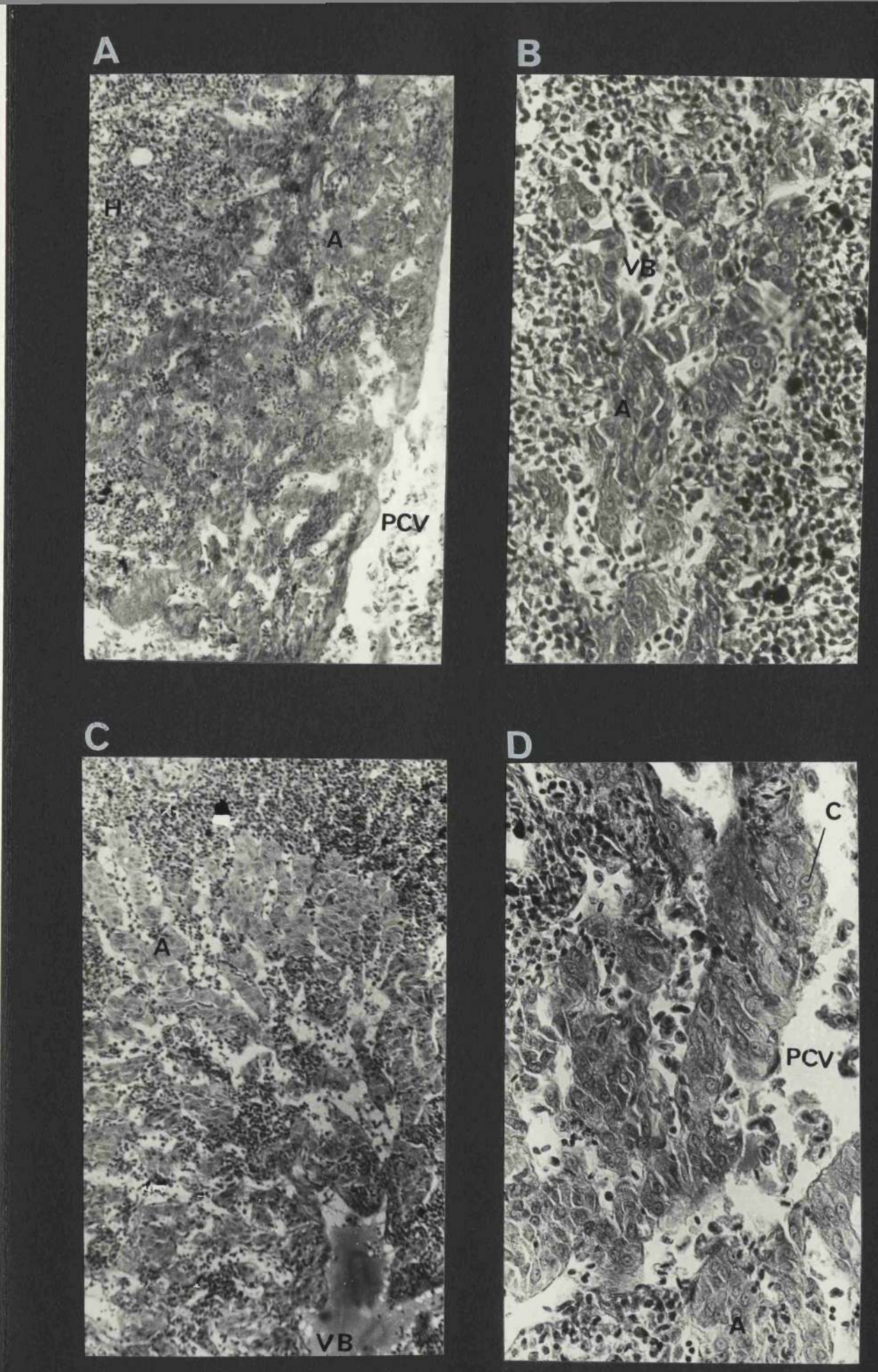
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Plate 5 (A–D) *Brachydanio rerio.* transverse sections through whole fish, 6 µm, stained in Masson's trichrome.

(A) Section at level of Ducts of Cuvier, showing bilobed kidney with renal elements extending to anterior end; dorsal aorta between kidney lobes branching; spinal nerves emerging from spinal cord . Scale line corresponds to 300 µm.

(B) Section slightly posterior to (A) showing right Duct of Cuvier (on the left of photograph) and smaller much-branched posterior cardinal vein in left lobe. Coeliacomesenteric artery lies in groove in ventral surface of the kidney. Scale line corresponds to 200 µm.

(C) Section slightly posterior to (B), showing right Duct of Cuvier (on left of photograph) surrounded by a narrow layer of adrenocortical tissue. In the left side of the kidney the posterior cardinal vein is broken up into many small vessels, some of which are also surrounded by adrenocortical tissue. Coeliacomesenteric artery runs enclosed in a channel in the kidney. Scale line corresponds to 200 µm.

(D) Section posterior to (C), showing single large posterior cardinal vein on the right side, with adrenocortical tissue lining the walls. The kidney forms a compact structure near the mid-line in its posterior region. Scale line corresponds to 200 µm.

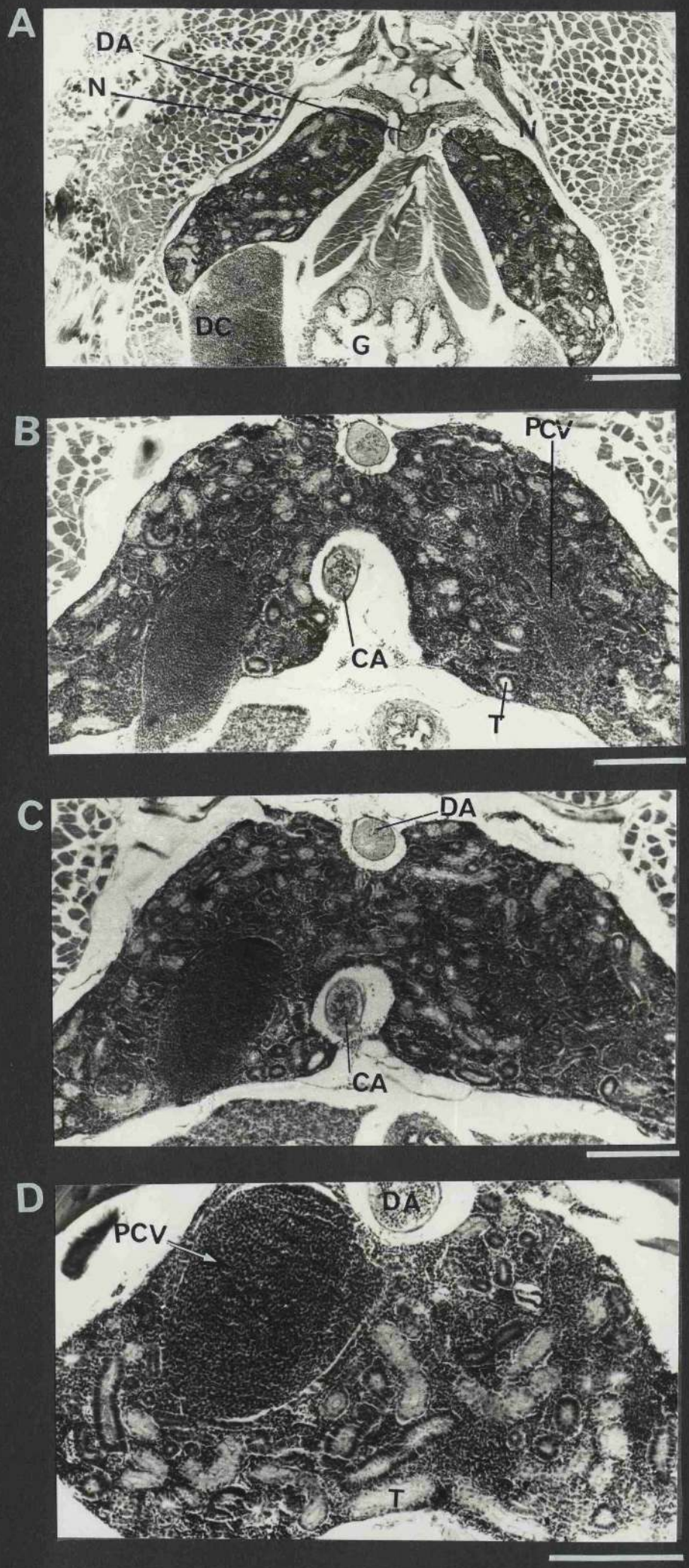
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Plate 5 (E-H) *Brachydanio rerio*, transverse sections through the anterior part of kidney, stained in Masson's trichrome.

(E) Section through the right lobe of the head-kidney, showing right Duct of Cuvier emerging from the kidney and surrounded by a layer of adrenocortical tissue, 1-3 cells thick. Renal elements are visible in the kidney tissue. Scale line corresponds to 50 µm.

(F) Section through left lobe of kidney showing left posterior cardinal vein branching extensively amongst the kidney tissues. Adrenocortical cells surround the main vein, and also extend for a short distance along smaller branches. Chromaffin cells are rare. Scale line corresponds to 50 µm.

(G) Section showing right Duct of Cuvier, surrounded by layers of adrenocortical cells, and branches in kidney tissue. Chromaffin cells are sparsely distributed. Scale line corresponds to 50 µm.

(H) Section of right Duct of Cuvier, showing adrenocortical cells and one chromaffin cells. Renal tubules are also present. Scale line corresponds to 25 µm.

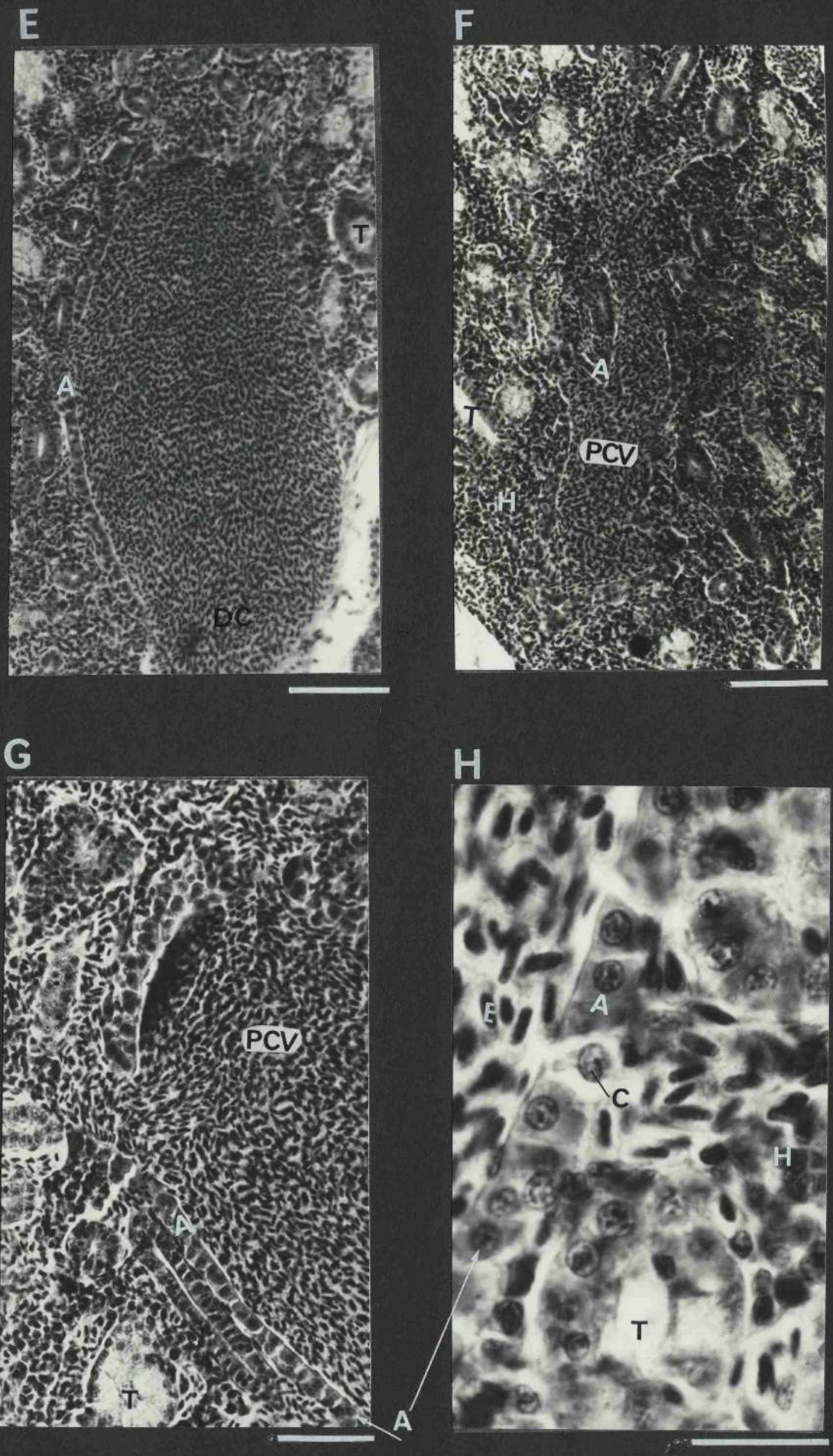
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Plate 6 (A–C) Xiphophorus helleri, transverse sections through whole fish, 6 µm, stained in Masson's trichrome.

(A) Section at level of Ducts of Cuvier. The right Duct of Cuvier (on the left of photograph) is emerging from the kidney. The left Duct of Cuvier extends from the kidney to the sinus venosus of the heart. Renal elements extend to the anterior end of the kidney, and the two lobes are separate. Scale line corresponds to 250 µm.

(B) Section more posteriorly, showing kidney lobes joined and a posterior cardinal vein in each lobe, the right bigger than the left. The coeliacomesenteric artery lies beside the gut. Scale line corresponds to 250 µm.

(C) Posterior cardinal vein in right lobe of kidney, with adrenocortical and chromaffin cells in wall. Scale line corresponds to 150 µm.

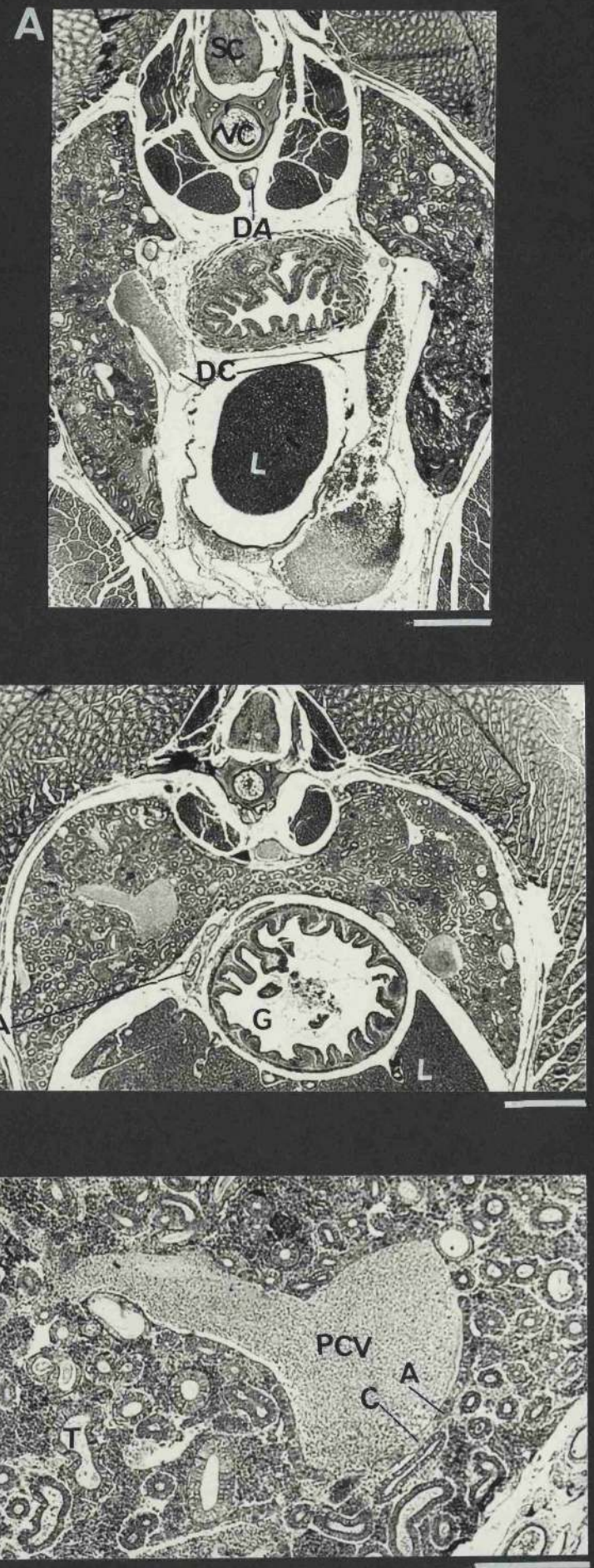
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Plate 6 (D–G) *Xiphophorus helleri* transverse sections through anterior end of kidney, 6 µm, stained in Masson's trichrome.

(D) Left Duct of Cuvier and its tributaries gathering from left kidney lobe. Adrenocortical and chromaffin cells line the walls of the vein. Scale line corresponds to 50 µm.

(E) As (D), but more anteriorly. A glomerulus is visible. Scale line corresponds to 50 µm.

(F) Right posterior cardinal vein, close to its junction with the Duct of Cuvier. Adrenocortical and chromaffin cells line the walls of the vein. Scale line corresponds to 50 µm.

(G) As (F), but more posteriorly. Scale line corresponds to 50 µm.

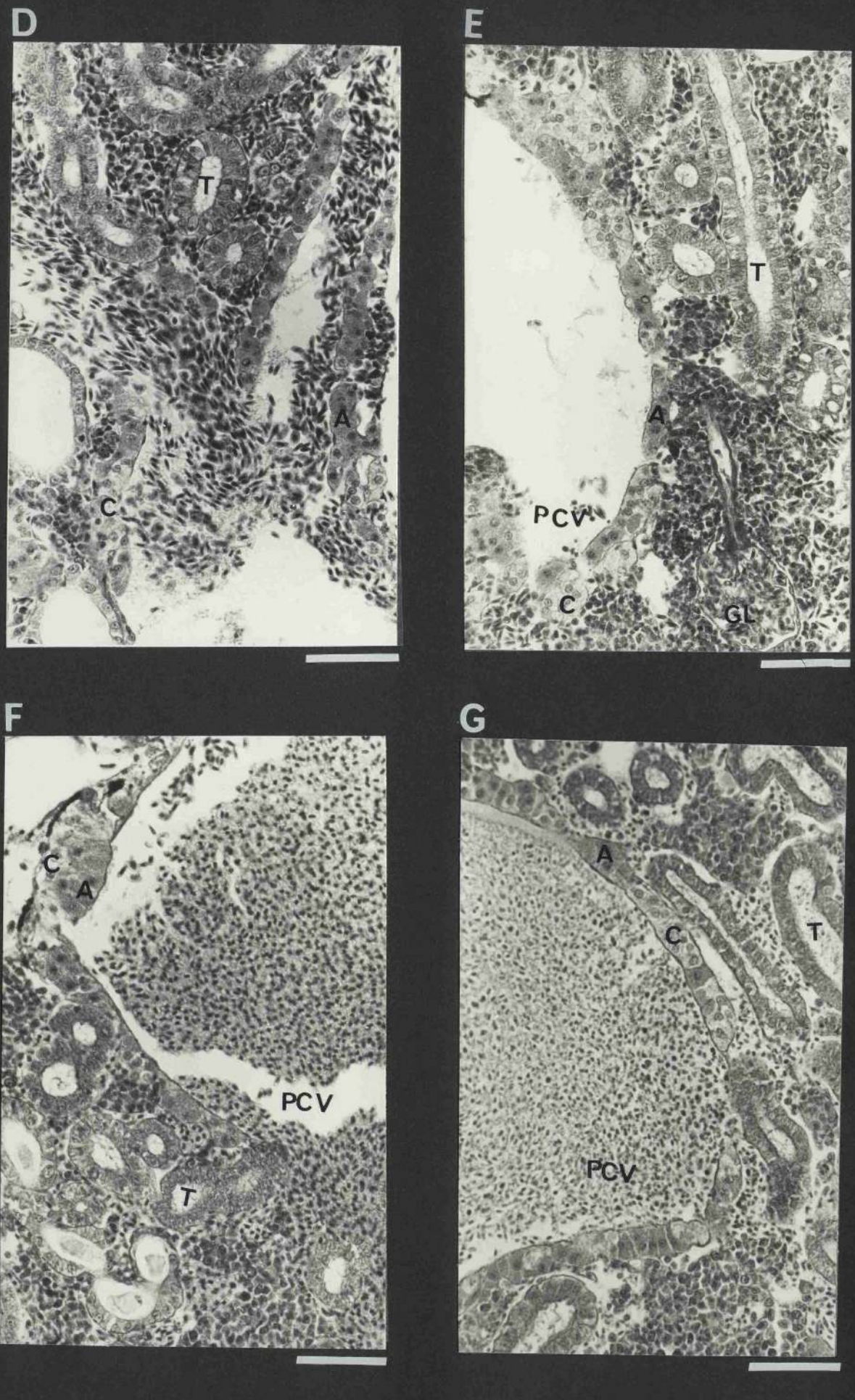
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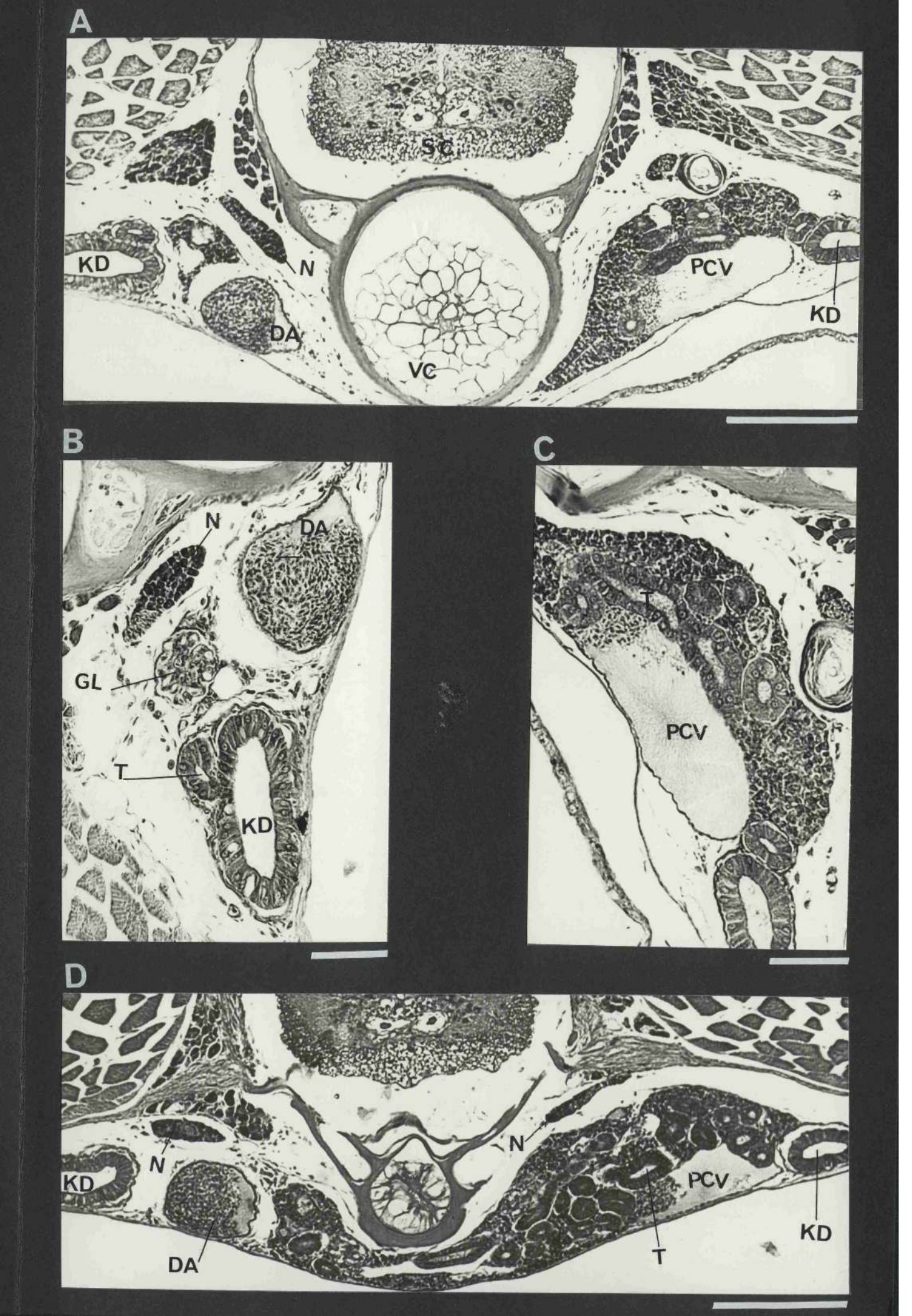
Plate 7 (A–D) Aphanius dispar. Transverse sections through whole fish, 6 µm, stained in Masson's trichrome.

(A) Section at region where the kidney is narrowest, between head-kidney and trunk-kidney. On the animals' left, (to the left in the photograph) kidney tissue is almost completely absent, and on the left only a narrow strand connect head and trunk kidneys. Scale line corresponds to 150 µm.

(B) Detail of left side of (A), showing kidney duct, one glomerulus and tubule, dorsal aorta and sympathetic nerves. Scale line corresponds to 50 µm.

(C) Detail of right side of (A) showing kidney duct, tubules, right posterior cardinal vein and sympathetic nerve. Scale line corresponds to 50 µm.

(D) Section slightly more posteriorly, showing trunk kidney developing on both sides of midline. The right posterior cardinal vein becomes progressively smaller posteriorly. Spinal nerve ganglia are evident. Scale line corresponds to 150 µm.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PCV
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DA
N
PCV
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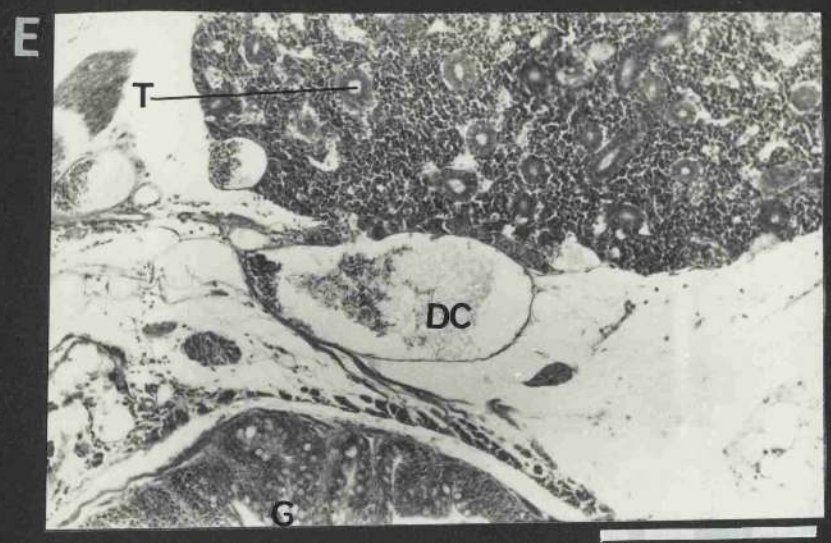
KD

Plate 7 (E–G) Aphanus dispar. Transverse sections through anterior region of kidney, 6 µm, stained in Masson's trichrome.

(E) Section at level of Duct of Cuvier. The Duct of Cuvier is emerging from the kidney, and adrenocortical and chromaffin cells are situated against its wall. Renal elements extend into the anterior region of the kidney. Scale line corresponds to 150 µm.

(F) Section through Duct of Cuvier showing adrenocortical and chromaffin cells in wall. Scale line corresponds to 50 µm.

(G) As for (F), but also showing sympathetic ganglia. Scale line corresponds to 50 µm.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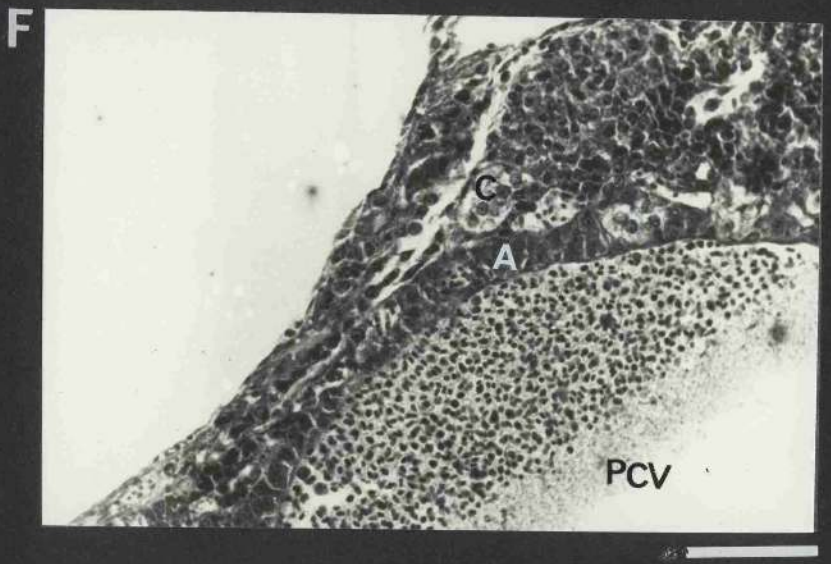
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Plate 8 Chanos chanos Transverse section through anterior region of kidney, 8 µm, stained in Masson's trichrome.

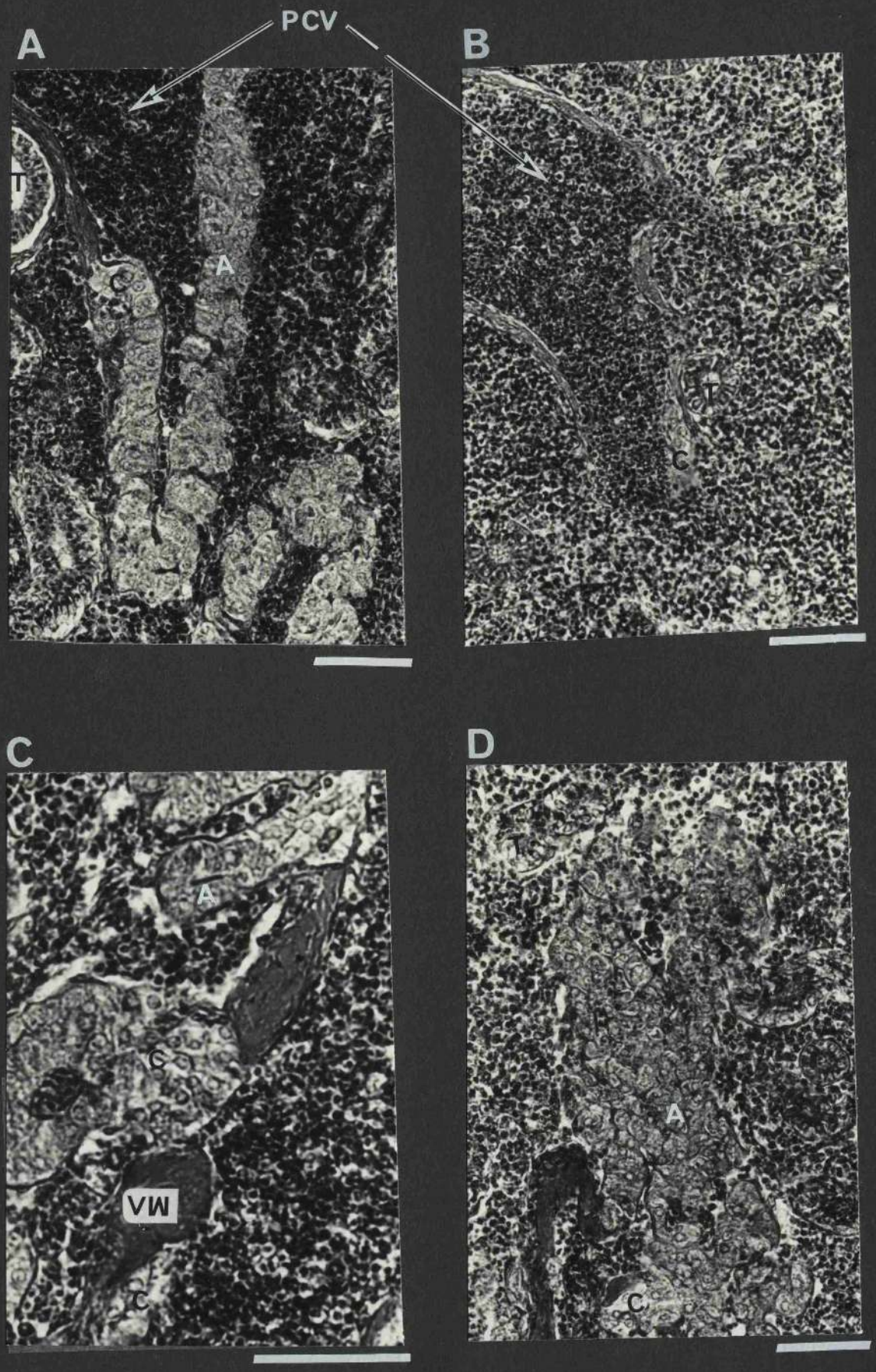
(A) Section showing posterior cardinal vein near anterior end of kidney, adrenocortical and chromaffin cells lining wall of vein. No renal tubules are visible in this section, but they do occur at this level. Scale line corresponds to 50 µm.

(B) Similar section to (A) but more posteriorly, showing chromaffin cells only, associated with branch of posterior cardinal vein in haemopoietic tissue. The main cardinal vein is at the bottom of the photograph. Renal tubules are visible in the haemopoietic tissue. Scale line corresponds to 150 µm.

(C) Section show posterior cardinal vein on left of photograph, showing groups of chromaffin cells embedded in thick wall of vein, groups of adrenocortical cells lie closer to the haemopoietic tissue and tubules. Scale line corresponds to 50 µm.

(D) Similar section to (C), but more posterior, showing adrenocortical cells extending into haemopoietic tissue and chromaffin cells in vein wall. Scale line corresponds to 50 µm.

[There was some delay in the fixation of the tissues of this specimen, and post-mortem changes have begun because of the high temperature.]


A
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VW
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C

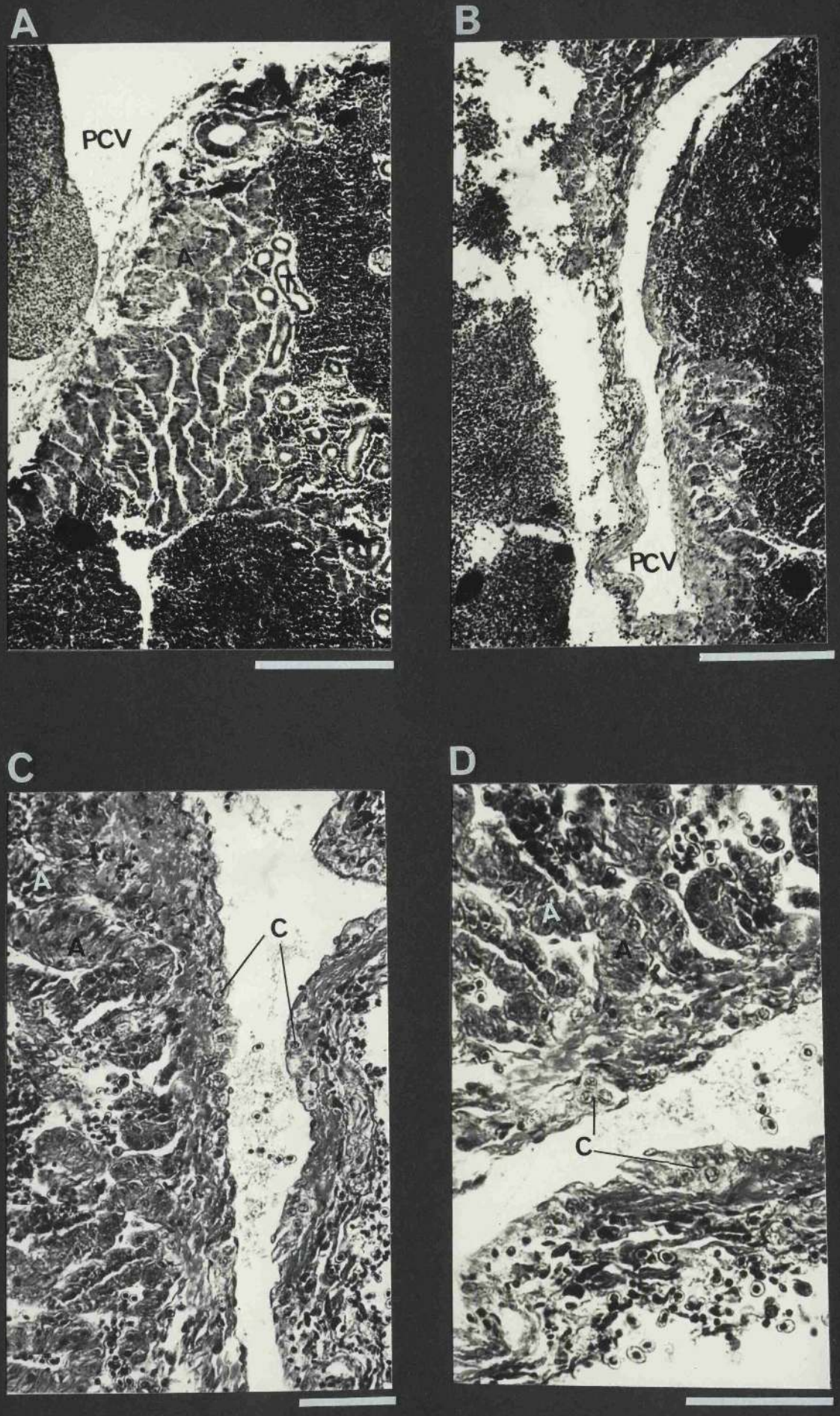
Plate 9. *Acanthopagrus bifasciatus*. Transverse sections through anterior region of kidney, 6 µm, stained in Masson's trichrome.

(A) Section through posterior cardinal vein, showing mass of adrenocortical cells surrounded by haemopoietic tissue and renal elements. Scale line corresponds to 200 µm.

(B) Section through empty posterior cardinal vein which has contracted with loss of fluid. Haemopoietic tissue with melanophores lies to the left and right of the vein. Adrenocortical cells lie behind the vein wall in the haemopoietic tissue; chromaffin cells lie in the vein wall itself. Scale line corresponds to 200 µm.

(C) The same section as (B), showing strands of adrenocortical cells permeated by small sinusoids, and chromaffin cells lying immediately behind the endothelium, protruding into the lumen of the vein. Scale line corresponds to 50 µm.

(D) The same section as (C). Scale line corresponds to 50 µm.


A
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C

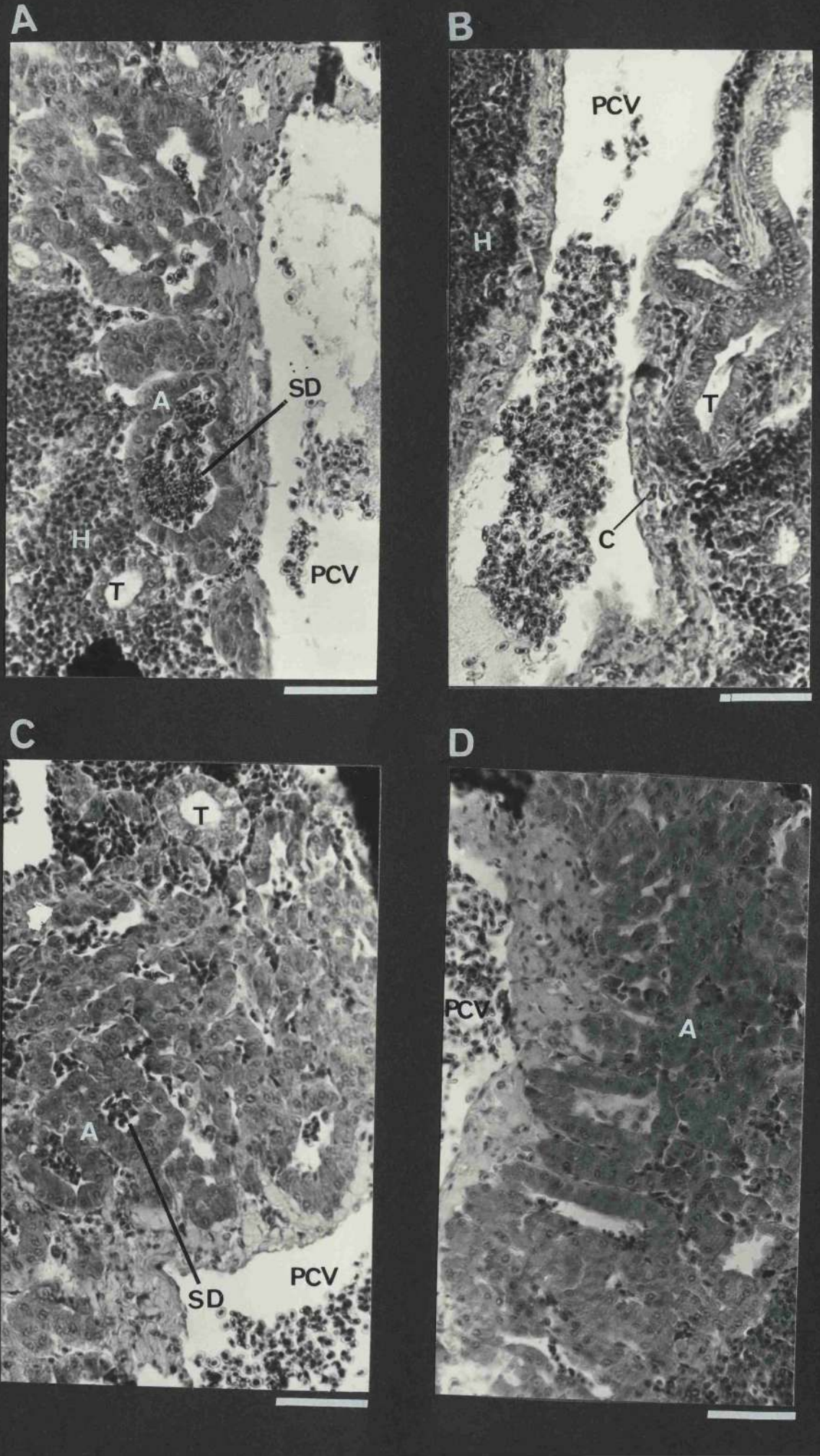
Plate 10. Crenidens crenidens. Transverse sections through anterior region of kidney, 6 µm, stained in Masson's trichrome.

(A) Section through posterior cardinal vein, near anterior end of kidney, showing adrenocortical cells behind vein wall, embedded in haemopoietic tissue with renal tubules and melanophores. The groups of adrenocortical cells are permeated by sinusoids. Scale line corresponds to 50 µm.

(B) Section through posterior cardinal vein, near posterior end of head-kidney, showing absence of adrenocortical cells, and presence of chromaffin cells in vein wall. Scale line corresponds to 50 µm.

(C) Section intermediate between (A) and (B), showing mass of adrenocortical cells, and a few chromaffin cells. Scale line corresponds to 50 µm.

(D) Similar section to (C). Scale line corresponds to 50 µm.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PCV
A

Plate 11. *Scarus* species. Transverse sections through anterior region of kidney, 6 µm, stained in Masson's trichrome.

(A) Section through posterior cardinal vein, showing vein wall lined by adrenocortical cells, with chromaffin cells behind them. The haemopoietic tissue contains many renal elements. Scale line corresponds to 200 µm.

(B) The same section, showing separation of adrenocortical and chromaffin cell-layers. Scale line corresponds to 50 µm.

(C) Similar section to (B) showing adrenocortical cells protruding into lumen of vein. Scale line corresponds to 50 µm.


A
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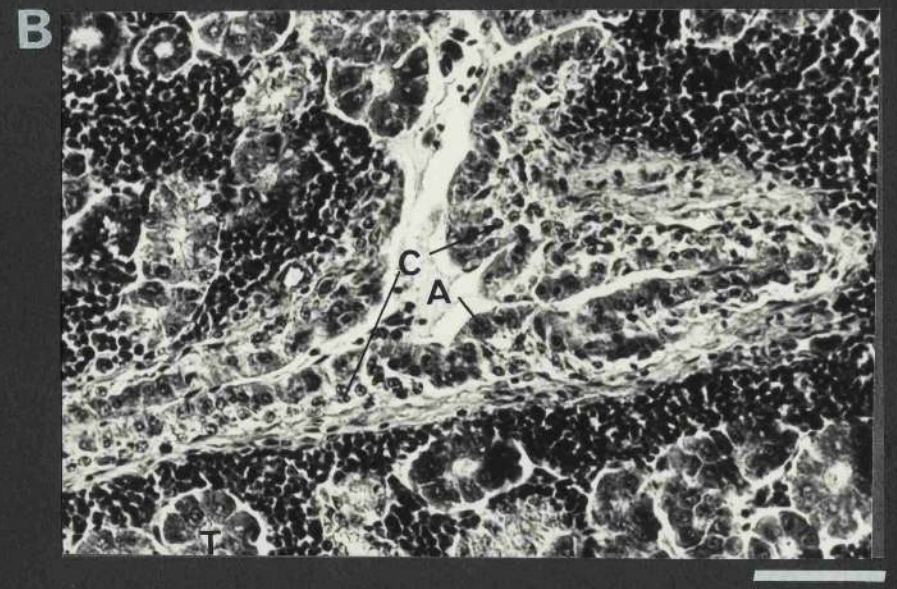
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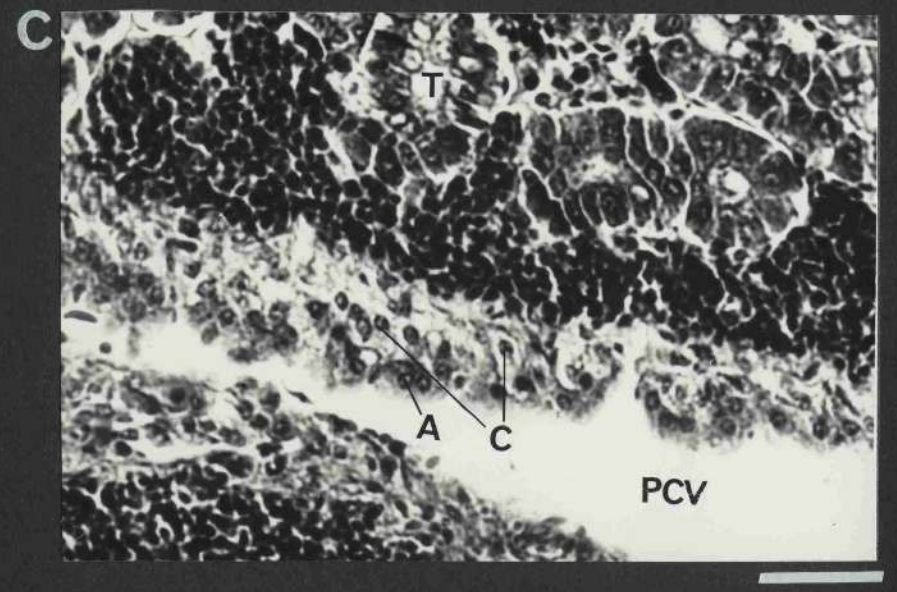
C
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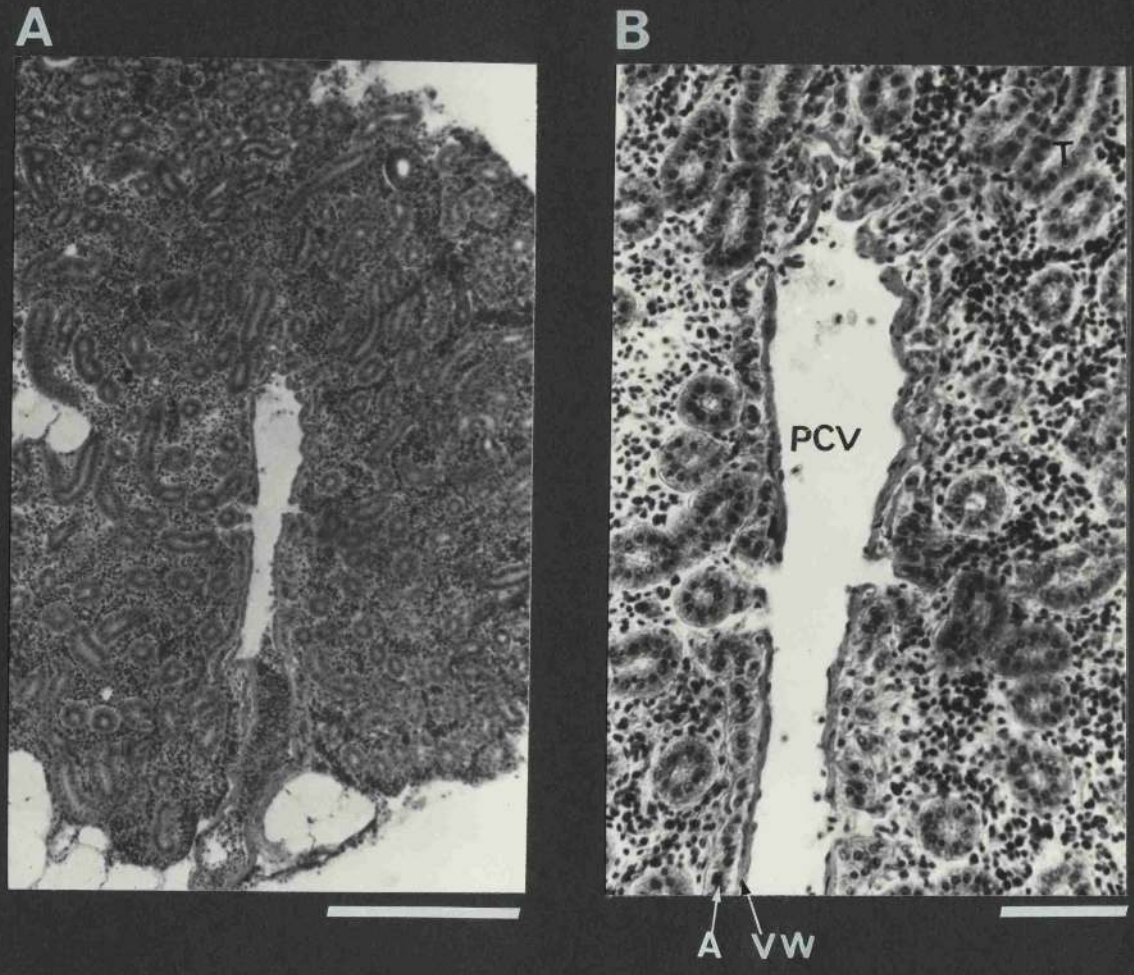
Plate 12. Herklotsichthys punctatus. Transverse sections through anterior region of kidney, 5 µm, stained in Masson's trichrome.

(A) Section through posterior cardinal vein, showing many renal elements. The wall of the vein is lined by a layer of adrenocortical cells. Scale line corresponds to 200 µm.

(B) The same section, showing that the adrenocortical cells form a layer 1–3 cell thick around the vein. Scale line corresponds to 50 µm.

(C) Similar section to (B). Scale line corresponds to 50 µm.

(D) Section more posterior, showing a few chromaffin cells in small vein walls and associated adrenocortical cells. Scale line corresponds to 50 µm.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A

Plate 13. (A–D). Thalassoma amblycephalus. Transverse sections through anterior region of right lobe of kidney, 6 µm, stained in Masson's trichrome.

(A) Section through posterior cardinal vein, with haemopoietic tissue containing many renal elements. Mostly on its ventral side the vein is lined with adrenocortical and chromaffin cells. Scale line corresponds to 200 µm.

(B) Same section, showing the posterior cardinal vein lined with adrenocortical and chromaffin cells, chiefly on its ventral surface. Scale line corresponds to 50 µm.

(C) Similar section posterior to (A). Scale line corresponds to 200 µm.

(D) Similar section to (B). Scale line corresponds to 50 µm.

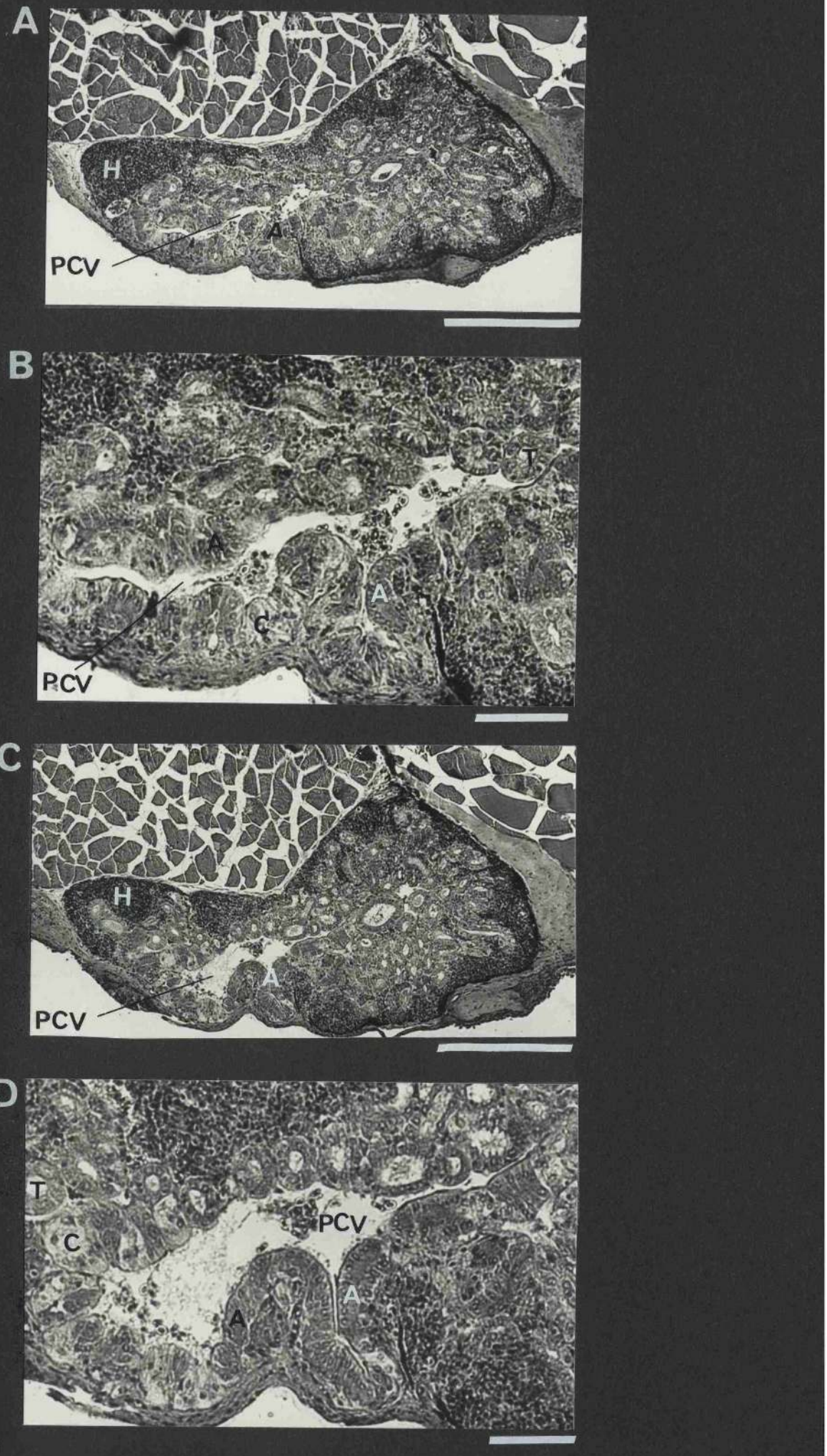
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Plate 13. (E-I). Thalassoma amblycephalus. Transverse sections through anterior region of left lobe of kidney and posterior part of the anterior region of right lobe, 6 µm, stained in Masson's trichrome.

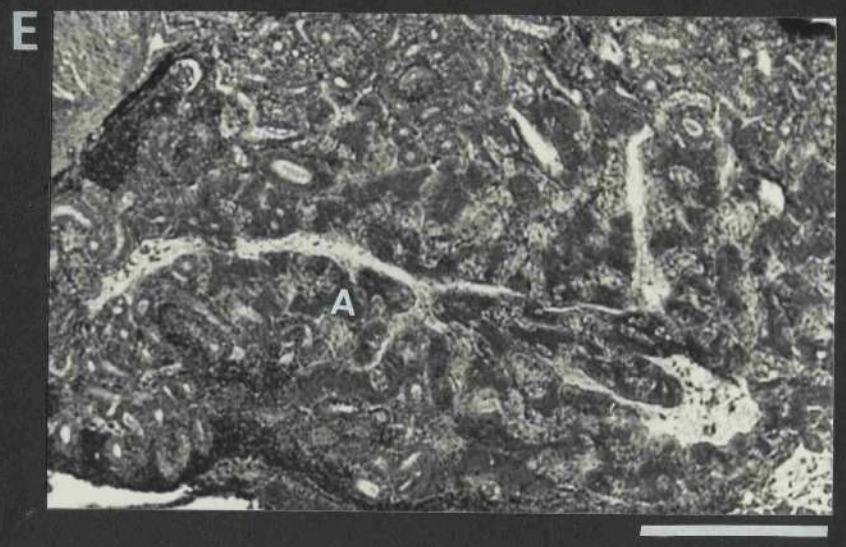
(E) Section through left posterior cardinal vein, showing haemopoietic tissue containing many renal elements. Posterior cardinal vein is small and branches extensively, lined with adrenocortical and chromaffin cells which extend into the haemopoietic tissue. Scale line corresponds to 200 µm.

(F) Same section as (E) showing the posterior cardinal vein lined with adrenocortical and chromaffin cells. Scale line corresponds to 50 µm.

(G) Section through right posterior cardinal vein, posterior to (C), showing chromaffin cells only. Scale line corresponds to 50 µm.

(H) Section posterior to (G), showing much enlarged posterior cardinal vein, with chromaffin cells in wall. Scale line corresponds to 200 µm.

(I) Same section as (H), showing chromaffin cells lining wall of vein and absence of adrenocortical cells. Scale line corresponds to 50 µm.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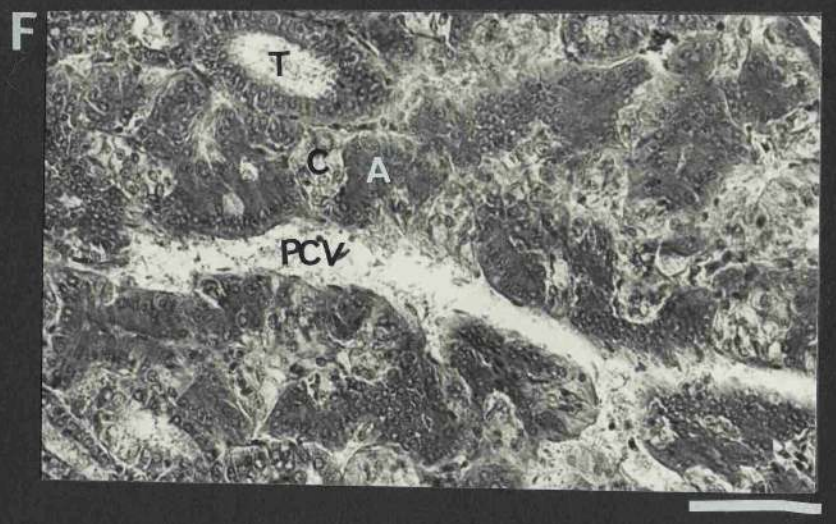
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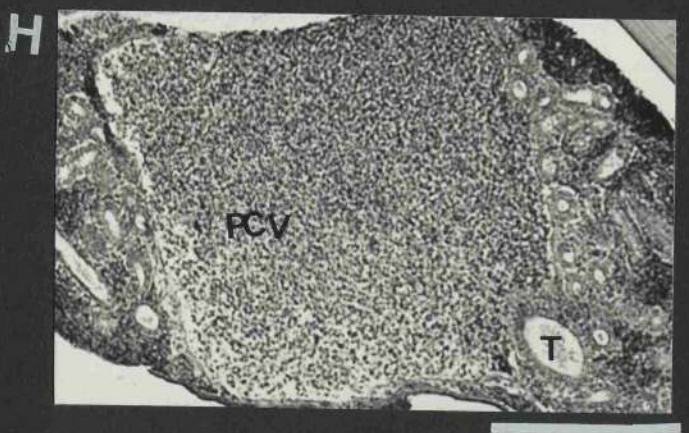
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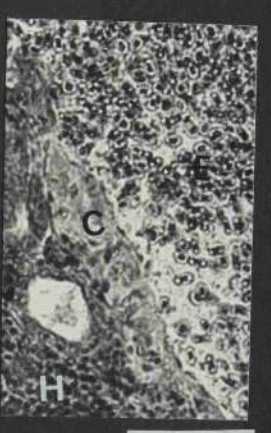
I
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Plate 14. Thalassoma lunare. Transverse sections, 5 µm, stained in Masson's trichrome.

(A) Section through anterior region of kidney, showing posterior cardinal vein with adrenocortical and chromaffin tissue. There are some renal tubules in the haemopoietic tissue. Scale line corresponds to 200 µm.

(B) Section in the same region as (A), with groups of adrenocortical and chromaffin cells in a framework of connective tissue and extensive sinusoids. Scale line corresponds to 50 µm.

(C) Section posterior to (A) showing main posterior cardinal vein with groups of chromaffin cells in its walls. Adrenocortical cells are absent, many renal elements are present. Scale line corresponds to 50 µm.

(D) Similar section to (C). Scale line corresponds to 50 µm.

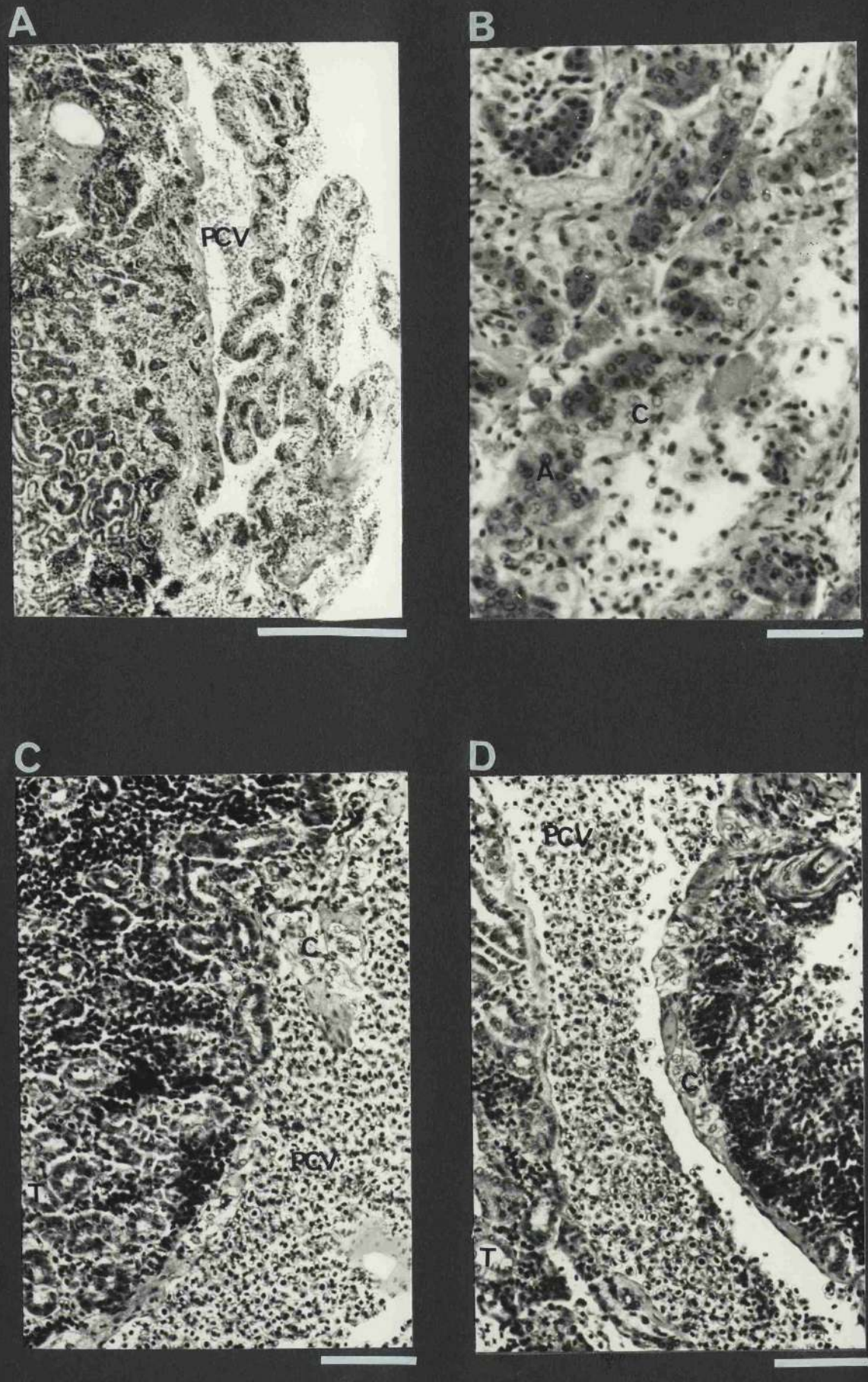
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Plate 15. Variola louti. Transverse sections through anterior regions of head-kidney, 6 µm, stained in Masson's trichrome.

(A) Section through branches of posterior cardinal vein, showing adrenocortical tissue round veins, melanophores, and rare kidney elements. Scale line corresponds to 200 µm.

(B) Section anterior to (A), showing adrenocortical tissue separated from vein by haemopoietic tissue. Scale line corresponds to 50 µm.

(C) Section posterior to (A), showing adrenocortical cells in the wall of the vein. Scale line corresponds to 50 µm.

(D) Section through main posterior cardinal vein showing rare chromaffin cells, and some adrenocortical cells, at the point where the vein is joined by major branches. Kidney duct in wall of vein. Scale line corresponds to 50 µm.

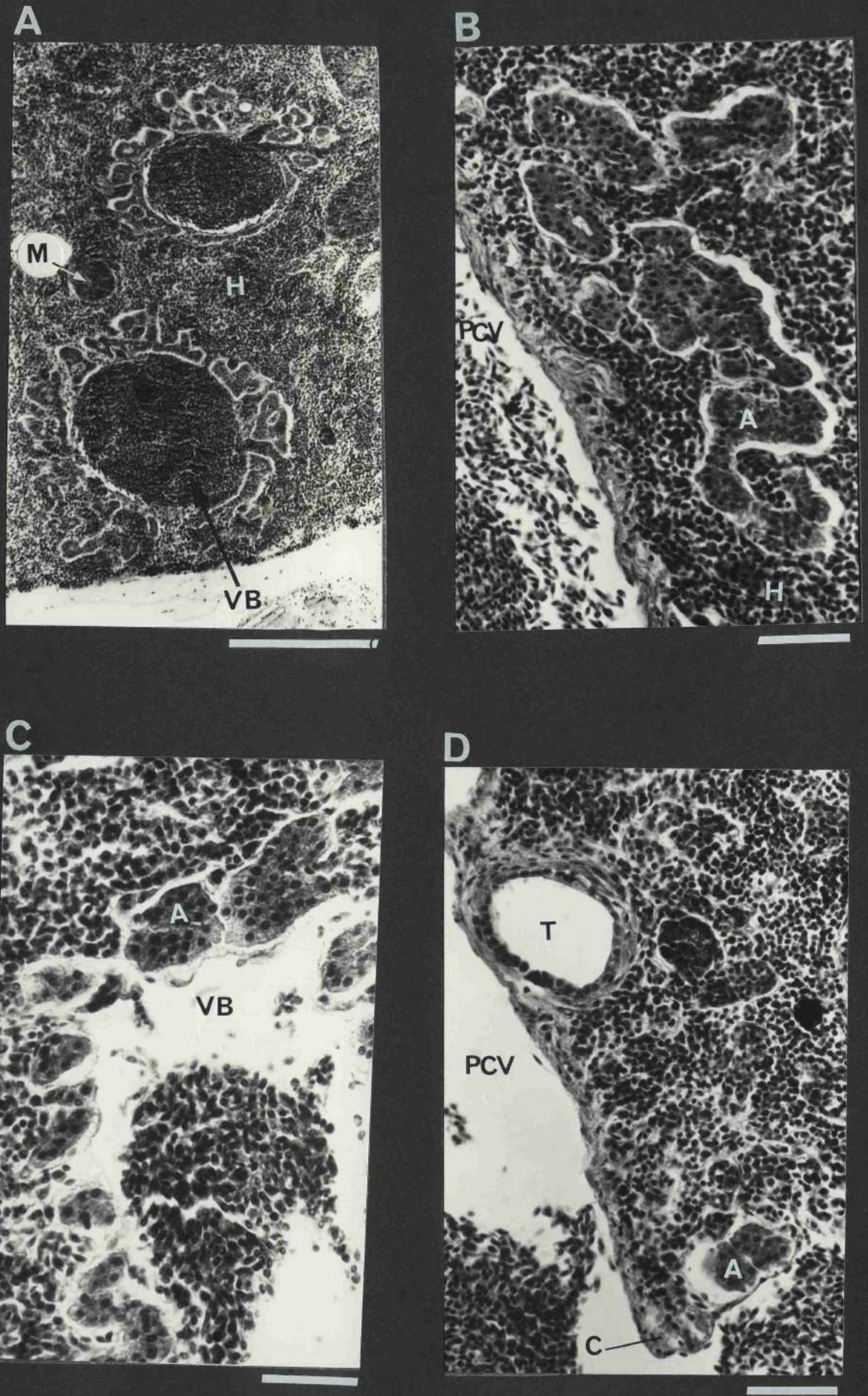
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Plate 16. Cephalopholis miniatus. Transverse sections through head-kidney, 6 µm, stained in Masson's trichrome.

(A) Section through posterior cardinal vein, showing adrenocortical tissue surrounding sinusoids in haemopoietic tissue behind cardinal vein. Melanophores are present. Scale line corresponds to 200 µm.

(B) Section through branch of posterior cardinal vein, showing adrenocortical cells, arranged in follicles separated by sinusoids. Some adrenocortical follicles lie away from the vein in the haemopoietic tissue. Scale line corresponds to 50 µm.

(C) Similar section, posterior to (B), showing chromaffin cells in wall of vein. Scale line corresponds to 50 µm.

(D) Section through branch of posterior cardinal vein, showing chromaffin cells embedded in its wall. Scale line corresponds to 50 µm.

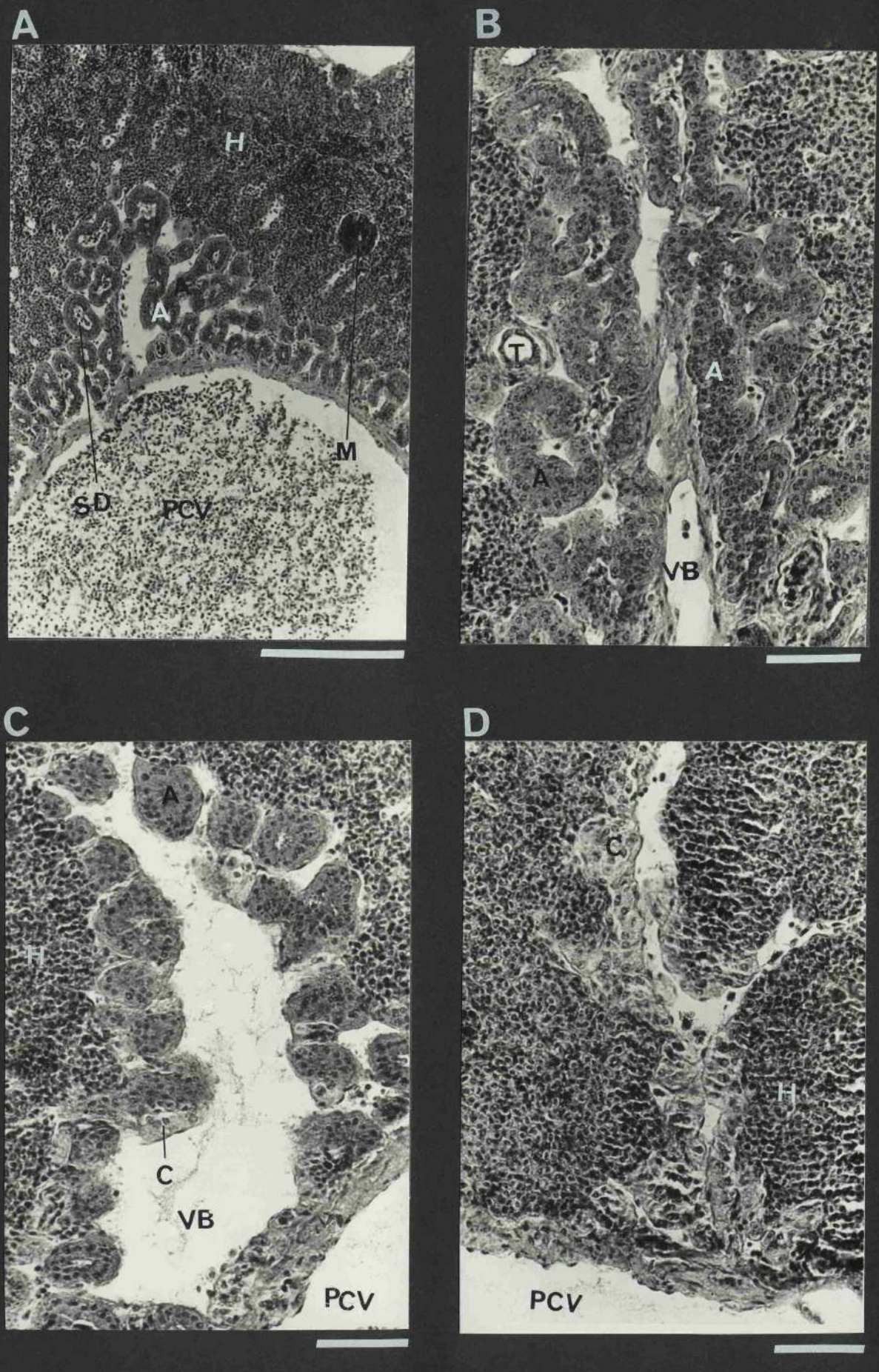
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Plate 17. Gaterin gaterinus. Longitudinal sections through anterior region of kidney, 6 µm, stained in Masson's trichrome.

(A) Section near anterior end of posterior cardinal vein, showing renal elements concentrated on one side of the vein, and haemopoietic tissue on the other, with adrenocortical tissue in the haemopoietic tissue. Scale line corresponds to 200 µm.

(B) Section through posterior cardinal vein, with renal elements on left of photograph, and adrenocortical cells on both sides. A few chromaffin cells are visible in the vein wall. Scale line corresponds to 50 µm.

(C) Section similar to (B) showing adrenocortical cells behind vein wall, and group of chromaffin cells protruding into lumen of vein. Scale line corresponds to 50 µm.

(D) Section along posterior cardinal vein near posterior end of head-kidney. Adrenocortical cells absent, and chromaffin cells present in the walls of vein. Kidney elements and haemopoietic tissue are also present on both sides. Scale line corresponds to 50 µm.

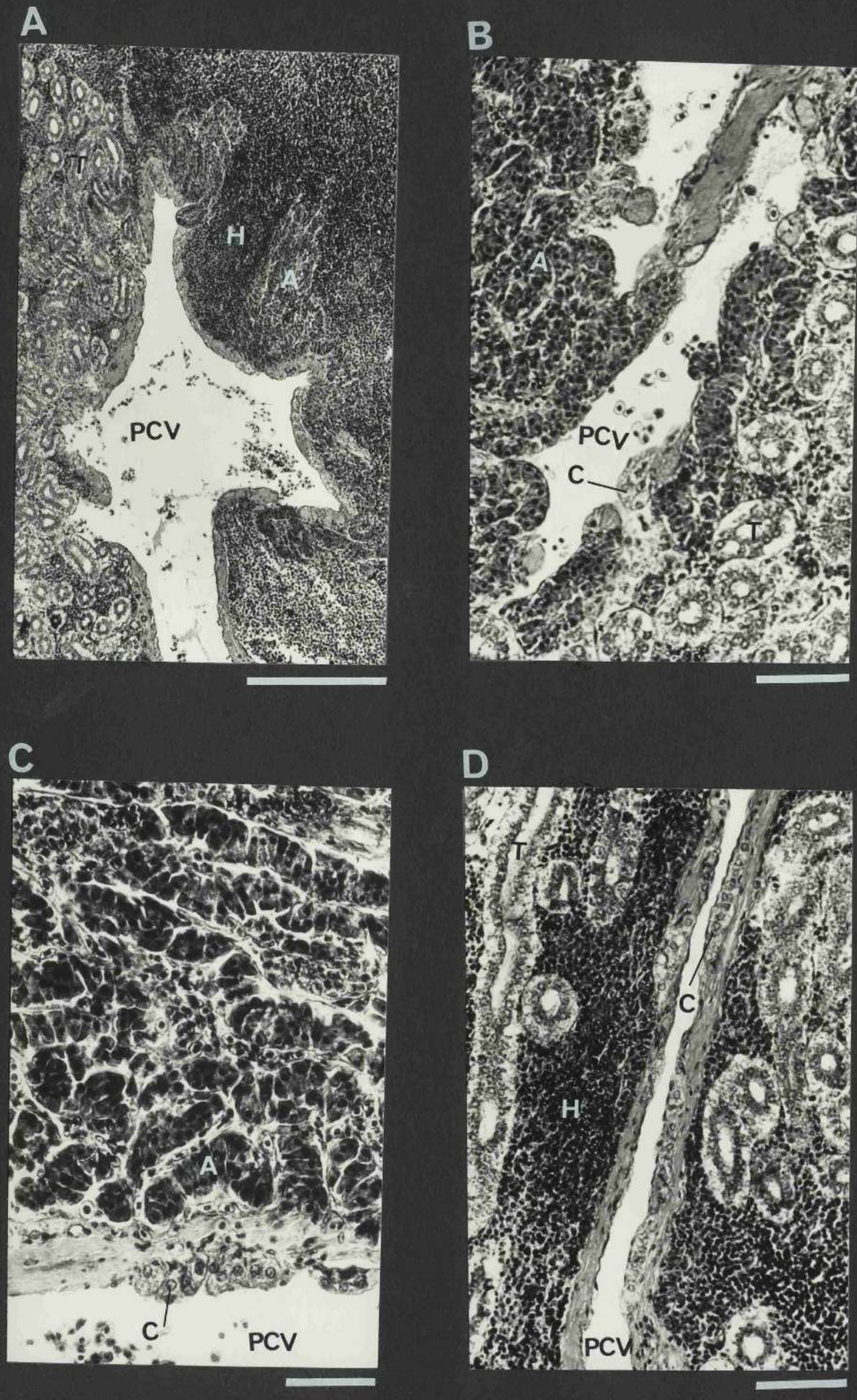
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Plate 18. Lethrinus species. Transverse sections trhough anterior region of kidney, 6 µm, stained in Masson's trichrome.

(A) Section through posterior cardinal vein, showing follicles of adrenocortical cells in haemopoietic tissue, and chromaffin tissue in and immediately behind vein walls. Scale line corresponds to 200 µm.

(B) Similar section to (A) showing adrenocortical follicles and renal tubules and chromaffin cells in vein wall. Note extensive sinusoids amongst adrenocortical follicles. Adrenocortical follicles and renal tubules are similar, but red blood cells are sometimes visible in adrenocortical follicles, and adrenocortical cells stain uniformly more darkly than tubule cells, and have a more swollen appearance. Scale line corresponds to 50 µm.

(C) Similar section to (B). Scale line corresponds to 50 µm.

(D) Section near posterior end of head-kidney, showing absence of adrenocortical cells, and vein wall containing many chromaffin cells. Scale line corresponds to 50 µm.

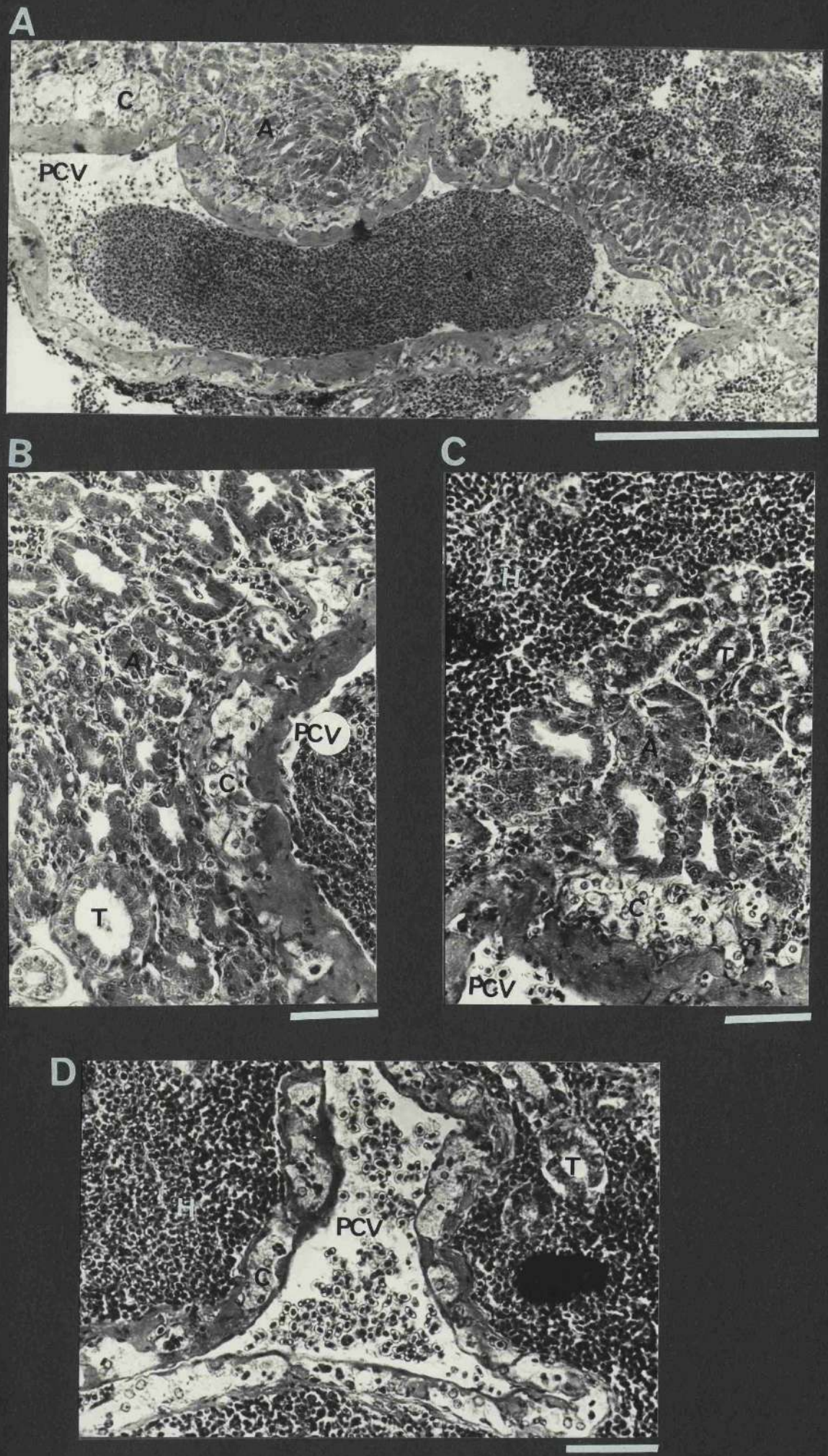
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Plate 19. Hepsetia pinquis. Transverse sections through whole fish, 6 µm, stained in Masson's trichrome.

(A) Section near posterior end of anterior kidney (head-kidney), showing dorsal aorta right posterior cardinal vein with a branch from left lobe of kidney. Kidney lobes are fused over dorsal aorta. Melanophores are present. Scale line corresponds to 300 µm.

(B) Section posterior to (A). Kidney lobes are separate, but fusion ventral to dorsal aorta occurs slightly further back. Renal elements are present throughout kidney. Scale line corresponds to 300 µm.

(C) Section through right anterior cardinal vein, showing vein wall lined with adrenocortical cells. Renal tubules are scattered amongst the haemopoietic tissue. Scale line corresponds to 50 µm.

(D) Section at junction of anterior and posterior cardinal veins, showing adrenocortical and chromaffin cells. Scale line corresponds to 50 µm.

(E) Section posterior to (D) through posterior cardinal vein, showing absence of adrenocortical tissue and presence of chromaffin tissue in wall of vein. Scale line corresponds to 50 µm.

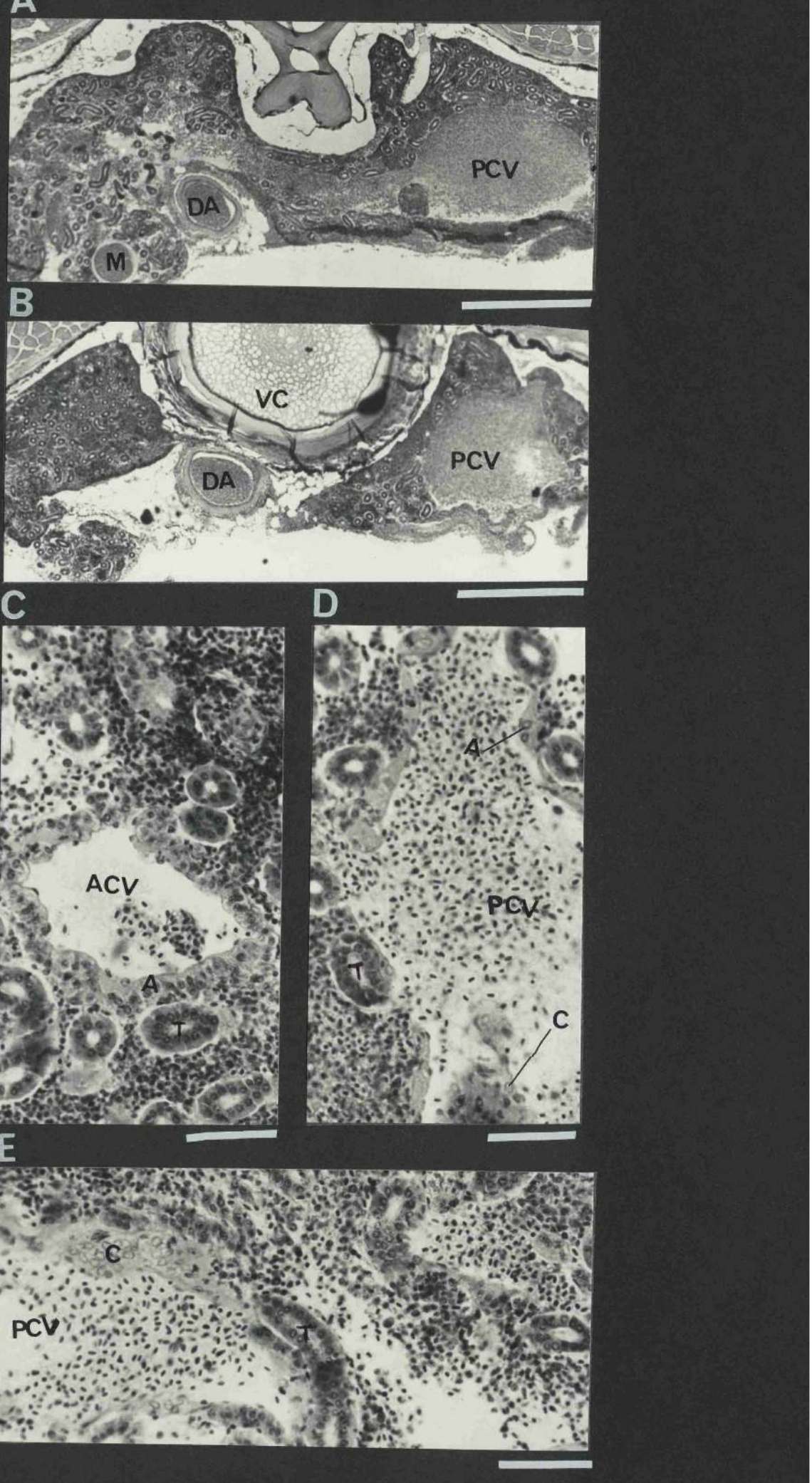
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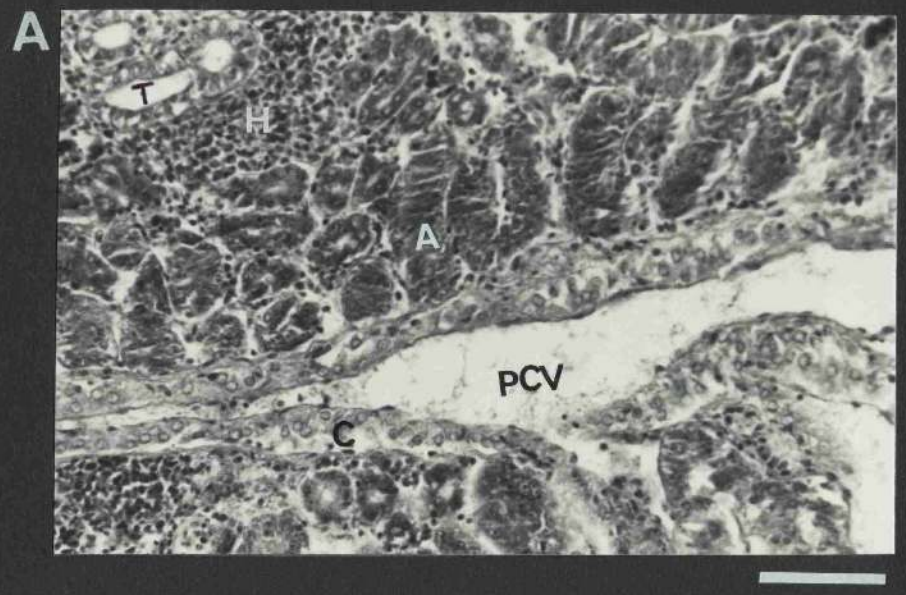
Plate 20. *Siganus* species. Longitudinal section through anterior region of kidney, 6 µm, stained in Masson's trichrome.
Scale line corresponds to 50 µm.

(A) Section through anterior region showing strands of adrenocortical cells in haemopoietic tissue and chromaffin cells in wall of vein, Renal elements are present.

(B) Section posterior to (A) showing few adrenocortical cells, and chromaffin cells in wall of vein.

(C) Section posterior to (B), showing absence of adrenocortical cells, and chromaffin cells in wall of vein.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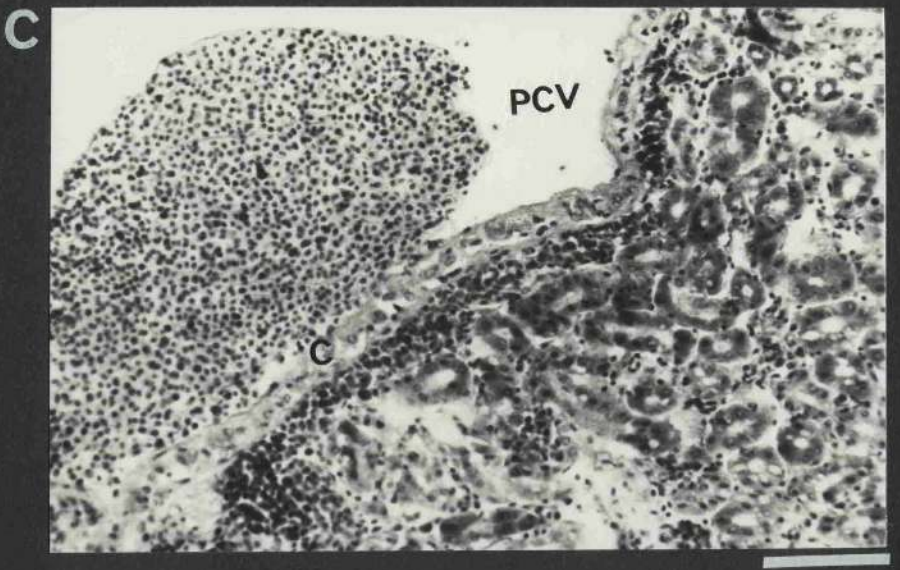
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Plate 21. *Monodactylus argenteus*. Transverse sections through anterior region of kidney, 6 µm, stained in Masson's trichrome.

(A) Posterior cardinal vein with thick wall and branch, in which lie groups of adrenocortical cells. Scale line corresponds to 50 µm.

(B) Similar section to (A), showing adrenocortical cells and a kidney duct. Scale line corresponds to 50 µm.

(C) Section posterior to (A), showing thin walled posterior cardinal vein, and adrenocortical and chromaffin cells in wall of vein. Scale line corresponds to 50 µm.

(D) Similar section to (C), but more posteriorly. Scale line corresponds to 50 µm.

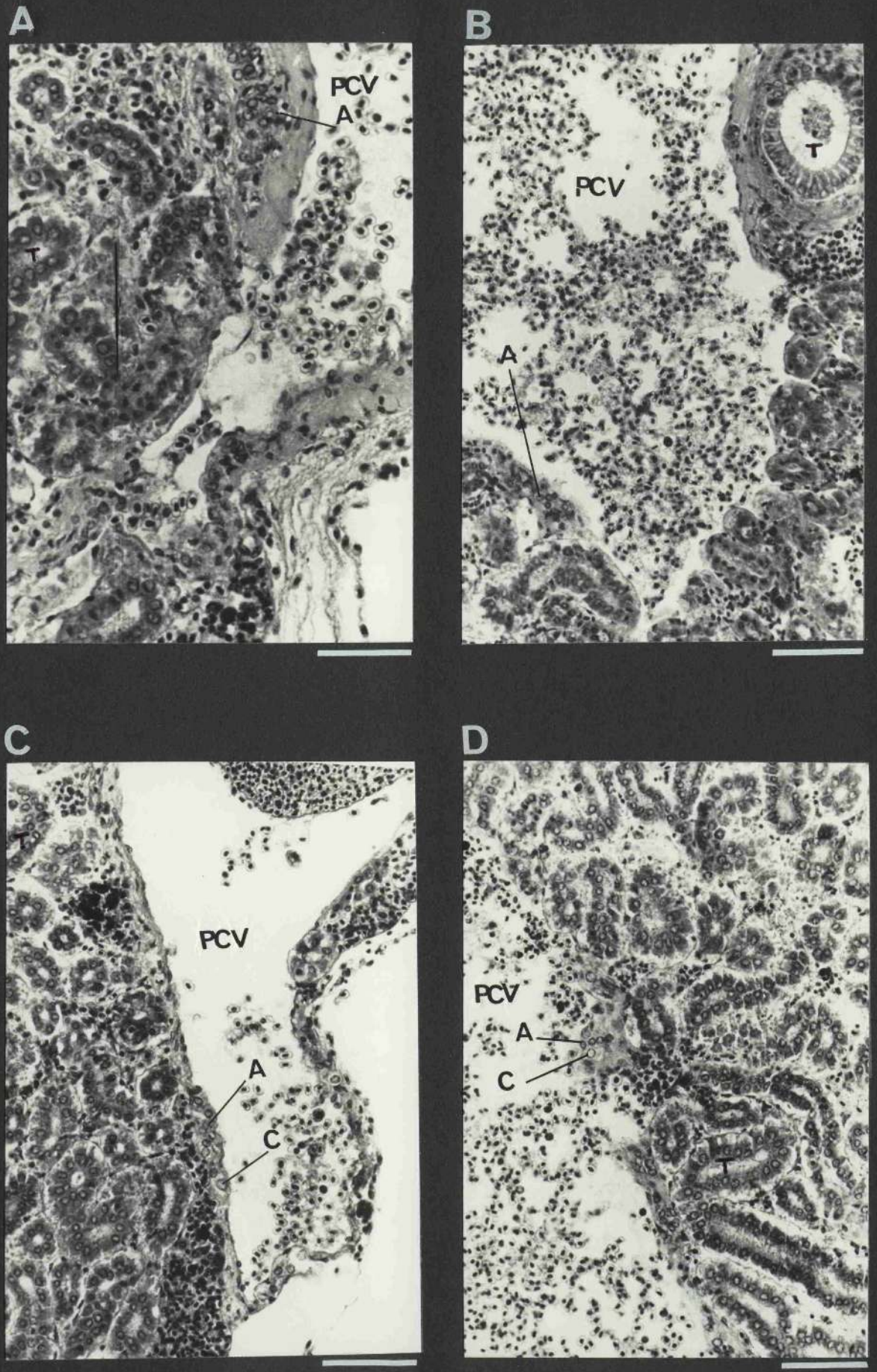
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Plate 22. *Zebrasoma veliferum*. Transverse sections through anterior region of kidney, 6 µm, stained in Masson's trichrome.
Scale line corresponds to 50 µm.

(A) Section near anterior end of posterior cardinal vein showing wall of vein lined with adrenocortical cells. Renal elements are present amongst the haemopoietic tissue.

(B) Section posterior to (A) showing chromaffin cells ih the wall of the posterior cardinal vein.

(C) Section about level of (A), showing adrenocortical cells in wall of posterior cardinal vein.

(D) Section posterior to (C), showing adrenocortical and chromaffin cells in wall of vein.

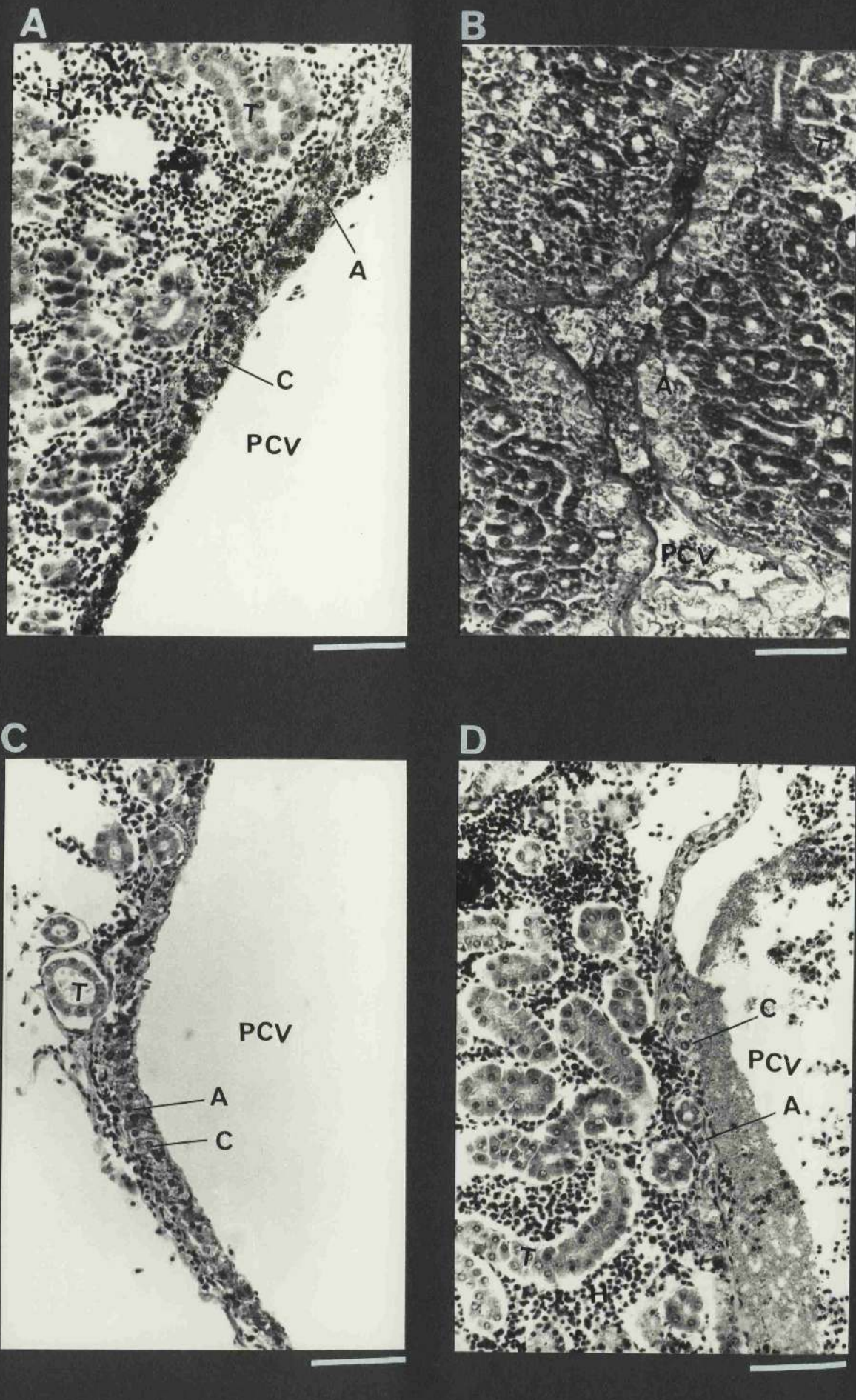
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Plate 23. Phoxinus phoxinus Longitudinal sections through ovary, 5 µm, stained in Masson's trichrome.

(A) April: showing: (i) primary oocytes (PO) with uniform purple cytoplasm and large clear nucleus containing peripheral nucleoli.

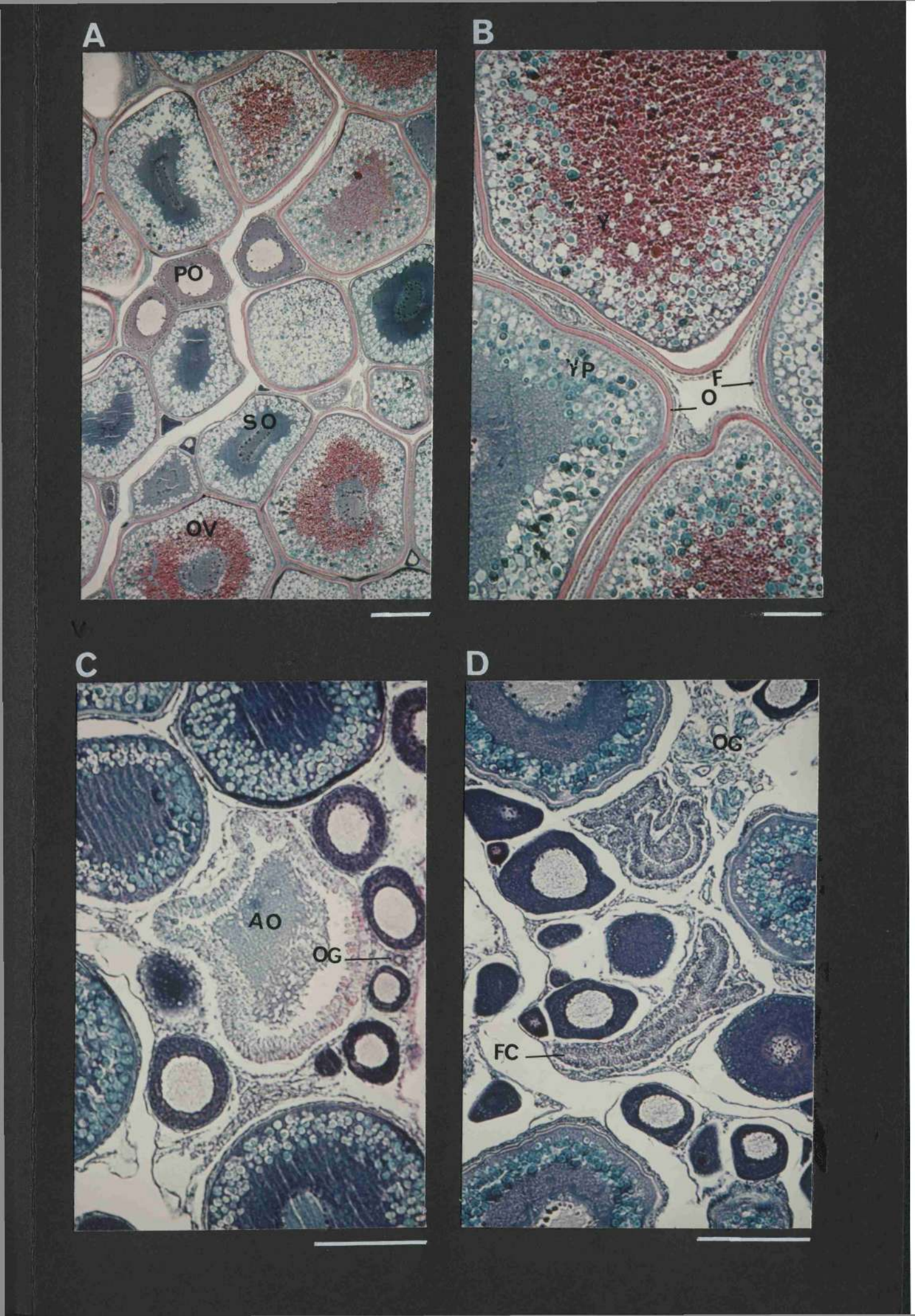
(ii) Secondary oocytes (SO) with blue-green cytoplasm containing yolk-precursor globules peripherally and red-staining oolemma. The nucleus, where visible, stains blue-green with dark red perpheral nucleole.

(iii) Ovum (OV) with red-staining yolk (Y) in central region of cell and pale green yolk-presursor vacuoles (YP) peripherally. Scale line corresponds to 400 µm.

(B) similar section showing ova and secondary oocytes. Note red-staining oolemma (O) and double layered follicle (F). Scale line corresponds to 100 µm.

(C) August, showing oogonia (OG), primary oocytes, young secondary oocytes and an atretic oocytes (AO). Scale line corresponds to 200 µm.

(D) Similar section, showing nest of oogonia, primary and young secondary oocytes, and two empty follicular calyces (FC). Scale line corresponds to 200 µm.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OG
FC

Plate 24. Phoxinus phoxinus. Longitudinal sections through testis, 5 µm, stained in Masson's trichrome.

(A) June, showing the seminiferous tubules (SF), sperm duct (on the left of photograph) and spermatozoa (S) free in their lumen. Scale line corresponds to 100 µm.

(B) June, Showing various spermatogenic stages:

Primary spermatogonia	(PG)
Secondary "	(SG)
Primary spermatocytes	(PS)
Secondary "	(SS)
Spermatids	(ST)
Spermatozoa	(S)

Scale line corresponds to 50 µm.

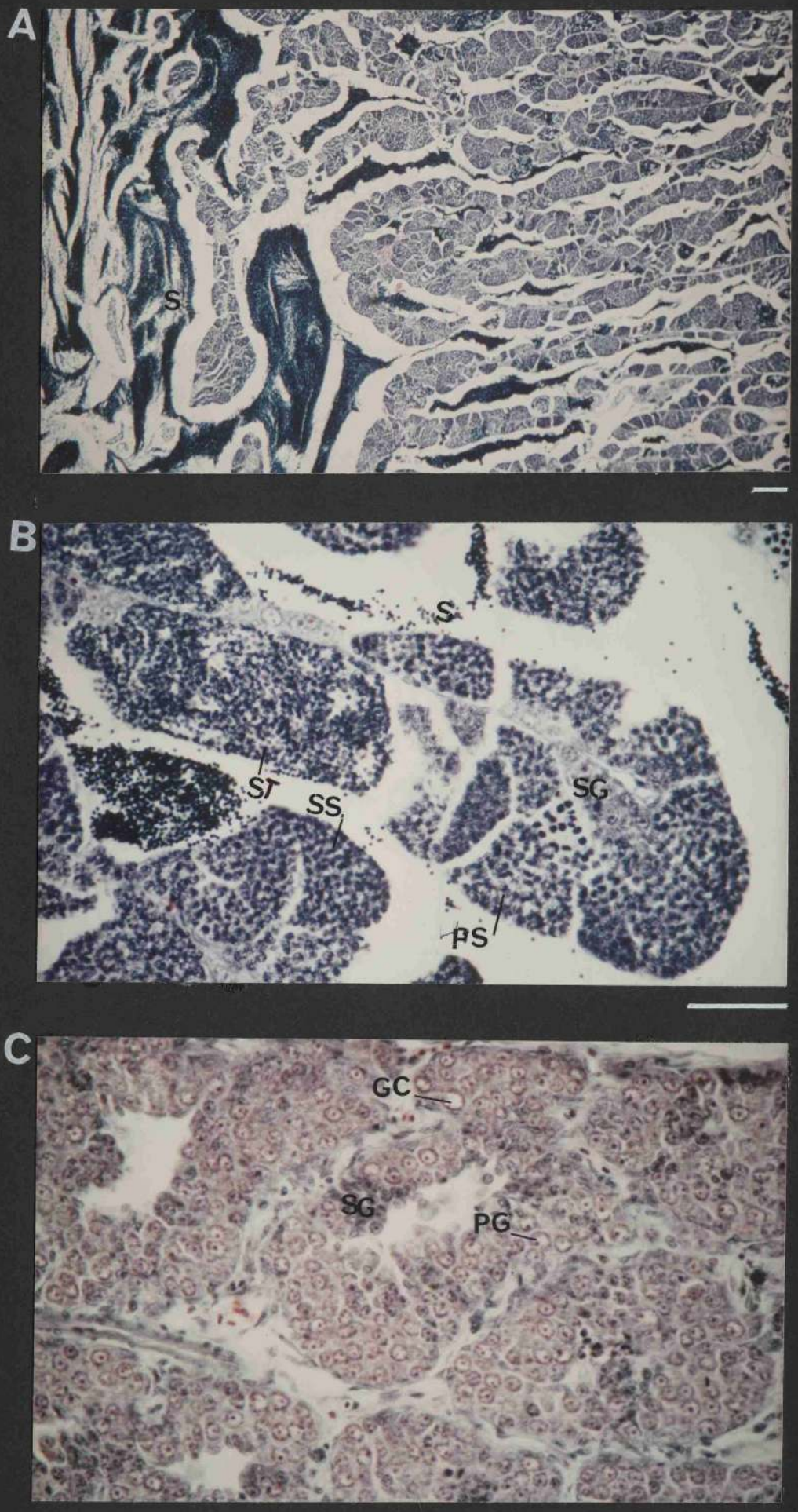
(C) August, showing various early spermatogenic stages:

Germ cells (GC)

Primary spermatogonia

Secondary spermatogonia

Scale line corresponds to 50 µm.


A
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S
ST
SS
SG
PS
C
GC
SG
PG

Plate 25. Phoxinus phoxinus Male and female showing nuptial colours.

Plate 26. (A–D) Phoxinus phoxinus Longitudinal sections through ovaries, 6 µm, stained in Masson's trichrome. Scale line corresponds to 400 µm.

(A) September: Ovary contains oogonia, primary oocytes, and a few secondary oocytes, and the remains of atretic oocytes which have been resorbed.

(B) November: Marked increase in the proportion of secondary oocytes in various stages of development. A few ova appear at this time.

(C) March: There is an increase in the proportion of advanced secondary oocytes and ova.

(D) April: Yolk-formation accelerates, and ova become common.

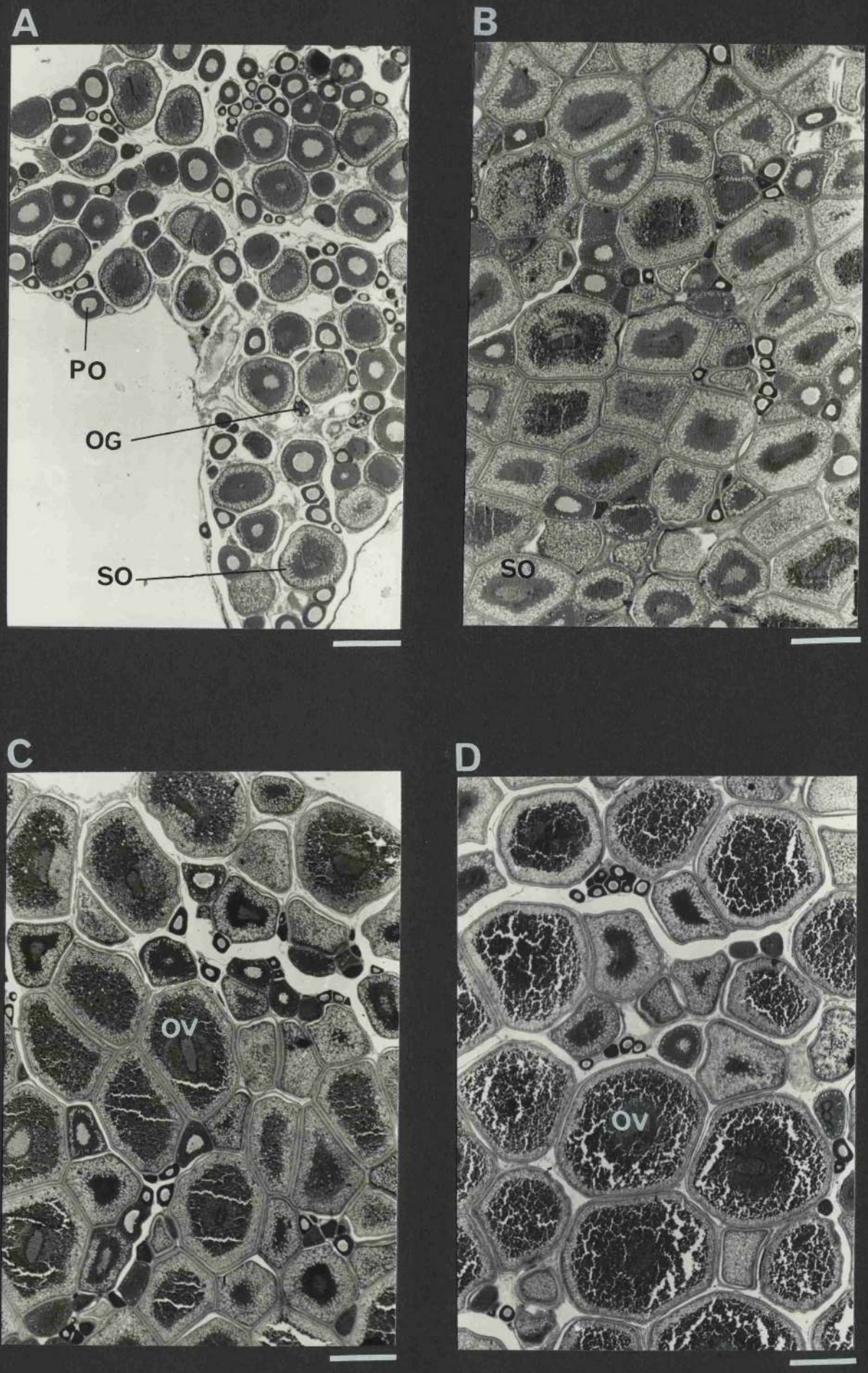
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Plate 26 (E–H) Phoxinus phoxinus Longitudinal sections through ovaries, 6 µm, stained in Masson's trichrome. Scale line corresponds to 400 µm.

(E) June The ovary is fully mature, with ripe ova predominating. A few secondary oocytes remain; they do not mature, but become atretic and are resorbed after spawning.

(F) July Spawning has taken place, and calyces of empty follicles are present. Unspawned ova and secondary oocytes are present.

(G) August Atretic oocytes are present, formed by the resorbing of ova and secondary oocytes.

(H) August Atretic oocyte.

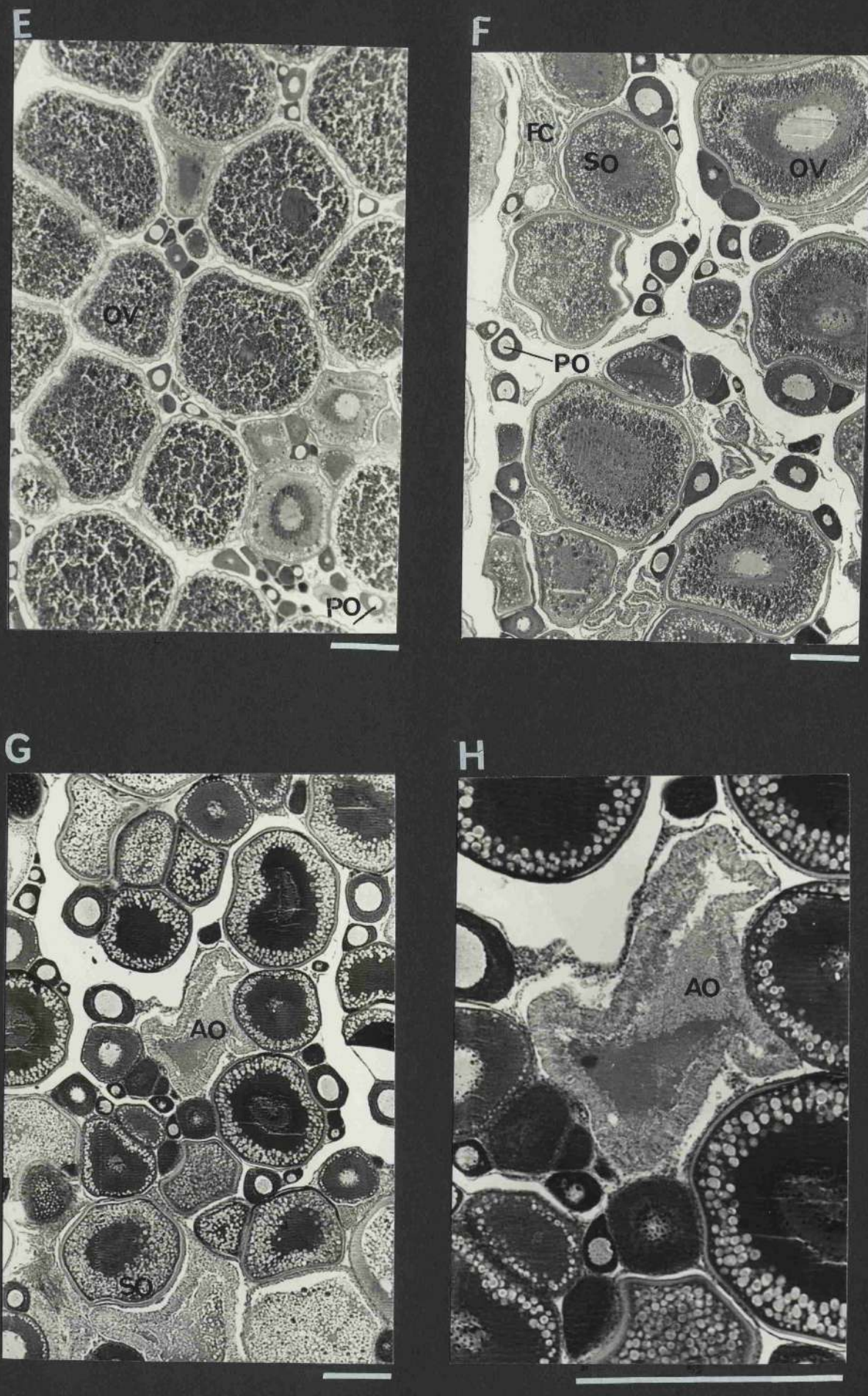
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Plate 27 (A-D) Phoxinus phoxinus Longitudinal sections through the testes, 5 µm, stained in Masson's trichrome. Scale line corresponds to 50 µm.

(A) February. Primary spermatocytes from the bulk of the walls of the seminiferous tubules.

(B) June Mature testis, with spermatozoa free in lumen of tubules. The sperm duct and the seminiferous tubules near it are packed with spermatozoa, leaving the tubules which lie further from the duct partly empty. Scale line corresponds to 400µm.

(C) June Showing tubules near the duct, full of spermatozoa.

(D) June Showing tubules further from the duct, partly empty.

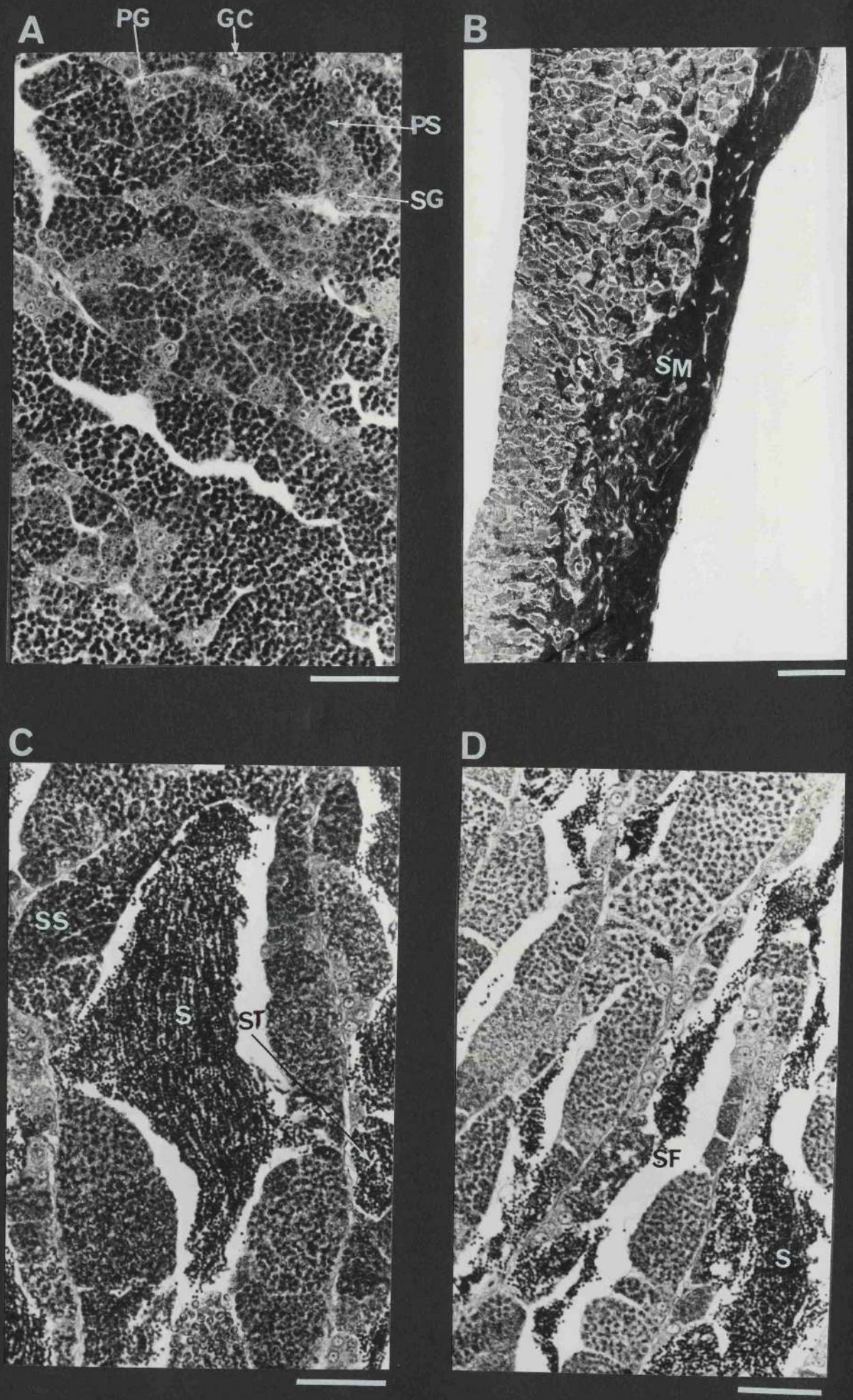
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Plate 27 (E-H) Phoxinus phoxinus Longitudinal sections through the testes, 5 µm, stained in Masson's trichrome. Scale line corresponds to 50 µm.

(E) August After spawning, testes are empty of spermatozoa, and other spermatocyte stages are resorbed.

(F) August Similar to section (E)

(G) September Primary spermatocytes reappear in the walls of the tubules.

(H) November Primary spermatocytes are plentiful in the walls of the tubules.

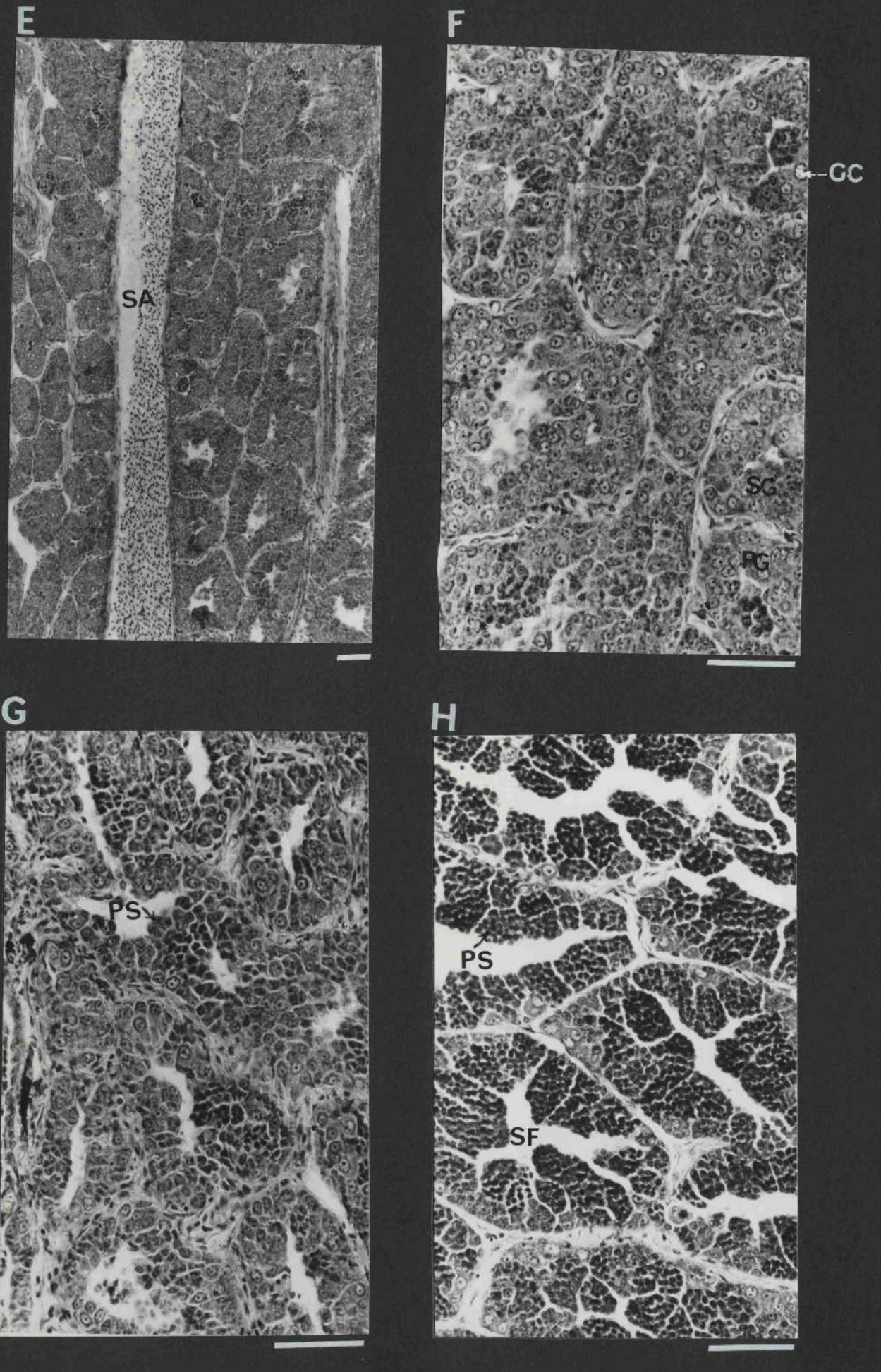
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Plate 28 (A–D) Phoxinus phoxinus Serial transverse sections (slightly oblique) through the whole fish, from anterior end of kidney posteriorly. A–L are 30 µm apart, M–S are about 60 µm apart; all sections are 6 µm thick stained in Masson's trichrome. Scale line corresponds to 150 µm.

(A) Section at anterior end of kidney, showing head-kidney, with glomerulus, lying immediately ventral to dorsal aorta. The left anterior cardinal vein is approaching the head-kidney.

(B) Section showing head-kidney splitting into two lateral lobes. The dorsal aorta is giving off a segmental artery on the left, and the gastromesenteric artery between the kidney lobes. The left anterior cardinal vein has entered the head-kidney, and the right anterior cardinal vein is travelling towards it. A ganglionated sympathetic nerve is visible above the right lobe of the head-kidney. Spinal nerves are visible amongst the muscle.

(C) Section showing head-kidney divided into two lateral lobes, each containing an anterior cardinal vein. Dorsal aorta branching as in (B). Spinal nerves are visible amongst the muscle.

(D) The head-kidney is bilobed and the gastromesenteric artery passed between the lobes. The roots of two segmental arteries are visible, and a small sympathetic nerve trunk lies dorsal to the left lobe of the head-kidney.

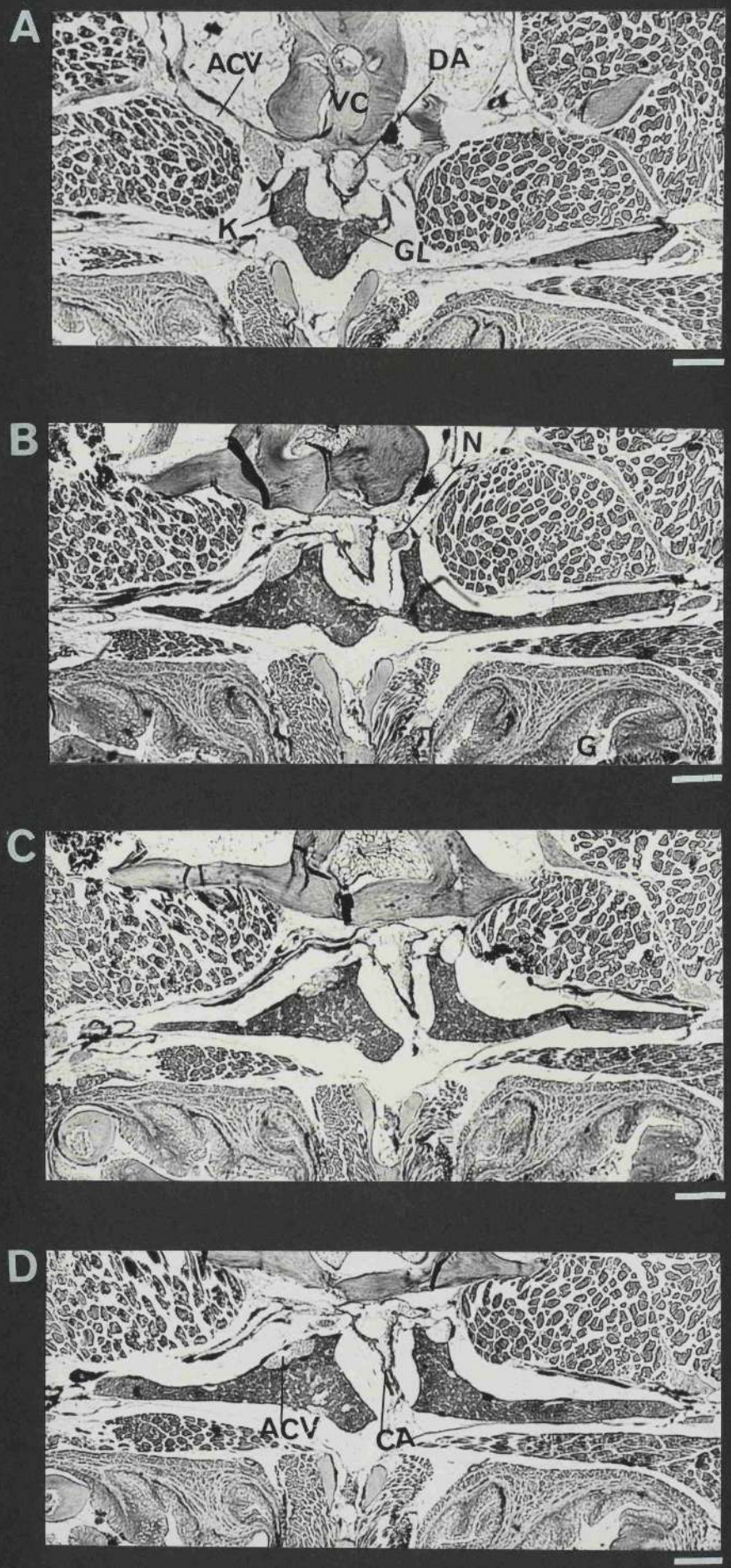
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Plate 28 (E–H) *Phoxinus phoxinus* Serial transverse sections (slightly oblique) through the whole fish, from anterior end of kidney posteriorly. A–L are 30 µm apart, M–S are about 60 µm apart; all sections are 6 µm thick stained in Masson's trichrome. Scale/line corresponds to 150 µm.

(E) Similar section to (D), but gastromesenteric artery now separate from dorsal aorta. The left anterior cardinal vein has divided into two main branches. A conspicuous sympathetic nerve trunk lies dorsal to the left head-kidney lobe; above the right head-kidney lobe the nerve trunk is fragmented.

(F) Similar section to (E), showing further brandhing of anterior cardinal veins into head-kidney tissue.

(G) The ducts of cuvier leave the head-kidney, passing towards the sinus venous. Sympathetic nerve trunks are present on either side of the dorsal aorta, and fine branches pass ventrally through the median space between the head-kidney lobes, along with the gastromesenteric artery.

(H) The branches from the sympathetic nerve trunks meet in the midline, close to the gastromesenteric artery. A little adrenocortical tissue lines the ducts of Cuvier where they leave the head-kidney.

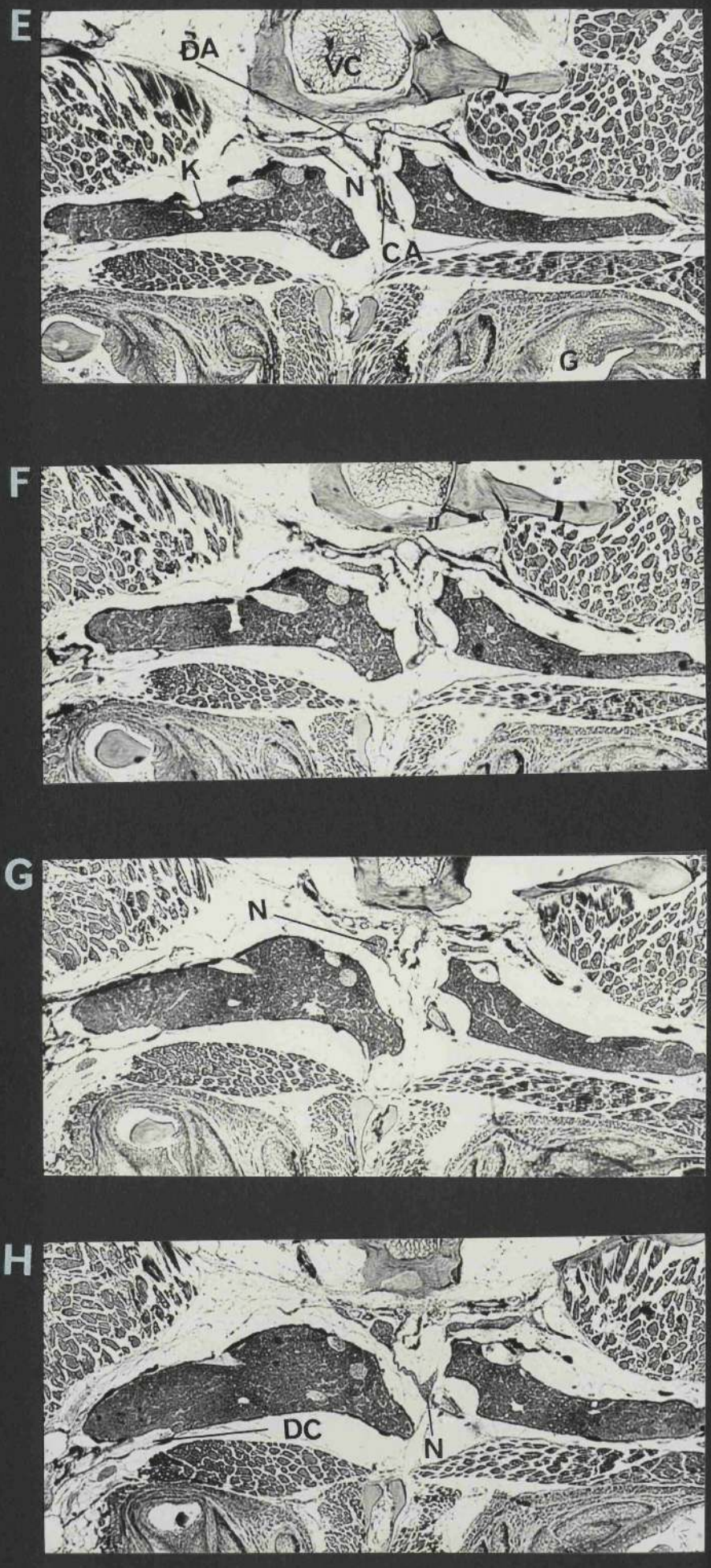
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Plate 28 (I–L) Phoxinus phoxinus Serial transverse sections (slightly oblique) through the whole fish, from anterior end of kidney posteriorly. A–L are 30 µm apart, M–S are about 60 µm apart; all sections are 6 µm thick stained in Masson's trichrome. Scale line corresponds to 150 µm.

(I) The ganglionated gastromesenteric sympathetic trunk is now separate from the paired trunks beside the dorsal aorta, and runs close beside the gastromesenteric artery. A spinal nerve trunk is visible on the left. The anterior cardinal veins have largely broken up into sinusoids in the head-kidney tissue. The tributary veins which form the left duct of cuvier are visible, with adrenocortical tissue lining the vein walls in this region. The right duct of Cuvier also appears, with adrenocortical tissue lining the walls. A renal duct is visible in the right lobe.

(J) A similar section to (I).

(K) The two lobes of the head-kidney have fused medially, and the gastromesenteric artery and nerve are running in a hollow on the ventral surface of the kidney.

(L) A similar section to (K). A spinal nerve trunk is visible on the right.

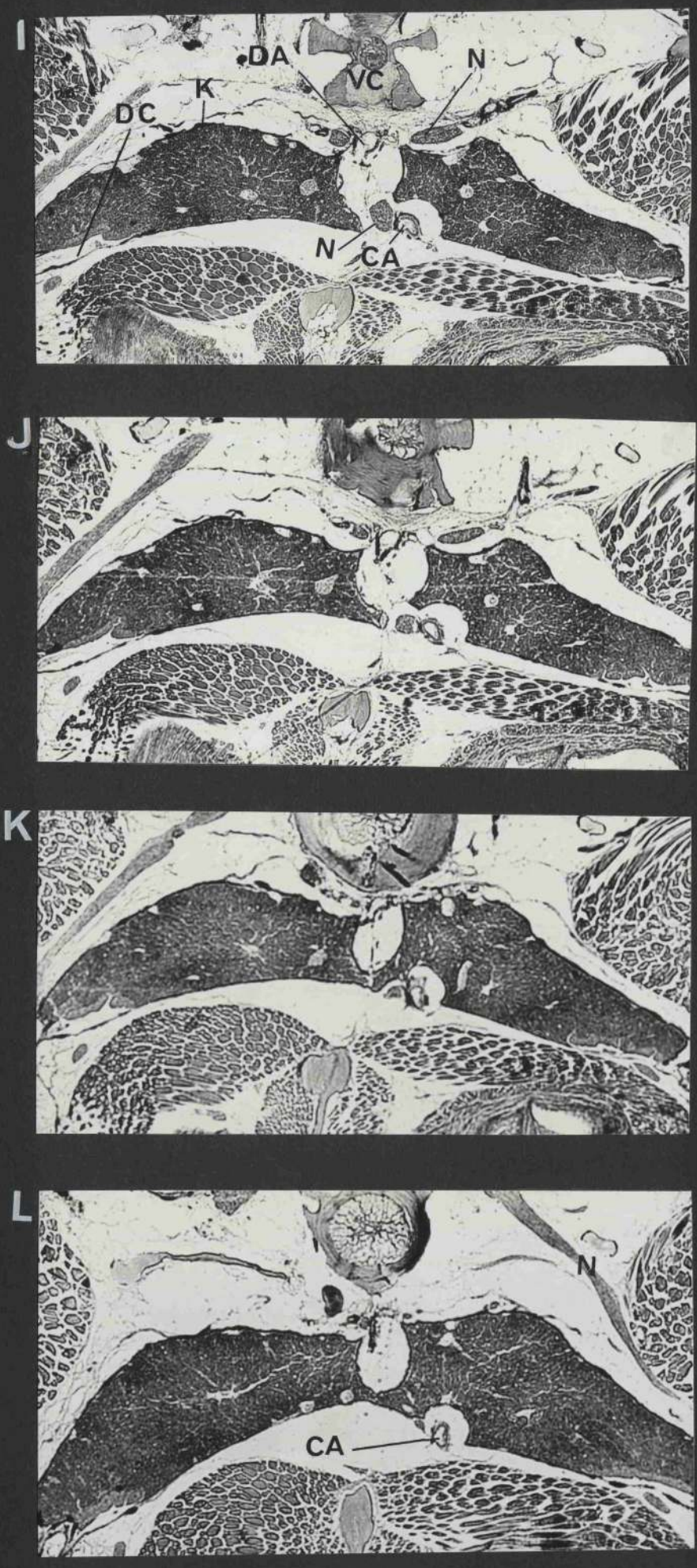
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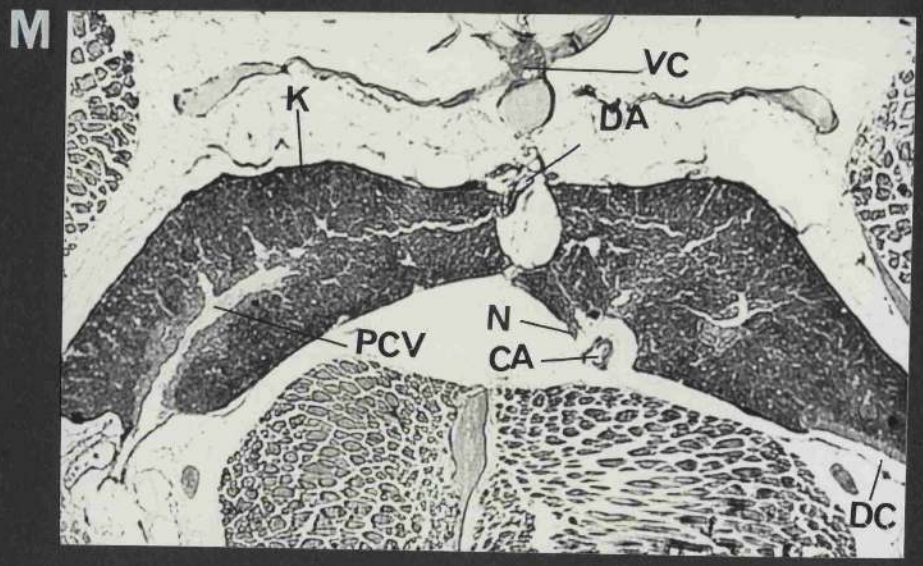
Plate 28 (M–O) Phoxinus phoxinus Serial transverse sections (slightly oblique) through the whole fish, from anterior end of kidney posteriorly. A–L are 30 µm apart, M–S are about 60 µm apart; all sections are 6 µm thick stained in Masson's trichrome. Scale line corresponds to 150 µm.

(M) The main trunk of the left duct of Cuvier is formed from an extensive network of tributaries in the left lobe of the head-kidney, with endocrine tissue lining the larger vessels.

(N) The corresponding main vessel of the right duct of Cuvier is visible, with endocrine tissue lining its walls.

(O) A similar section to (N).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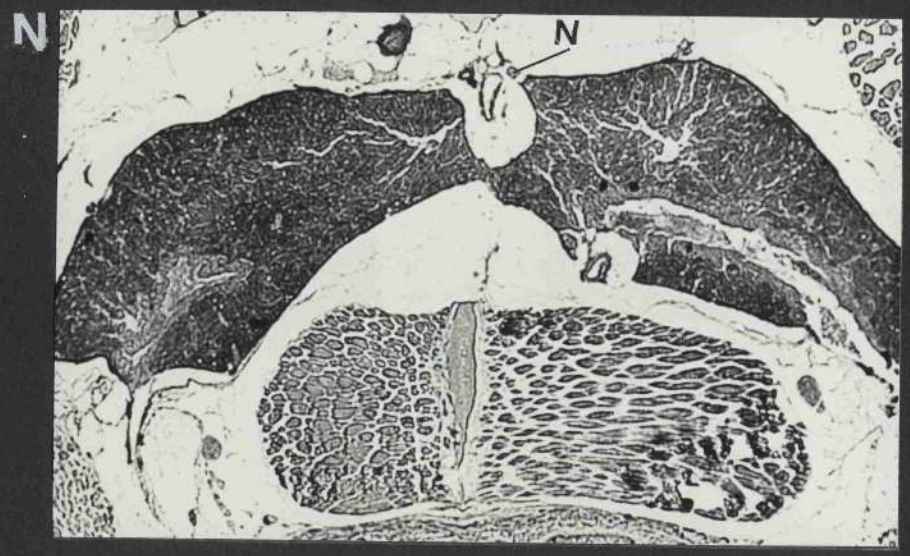
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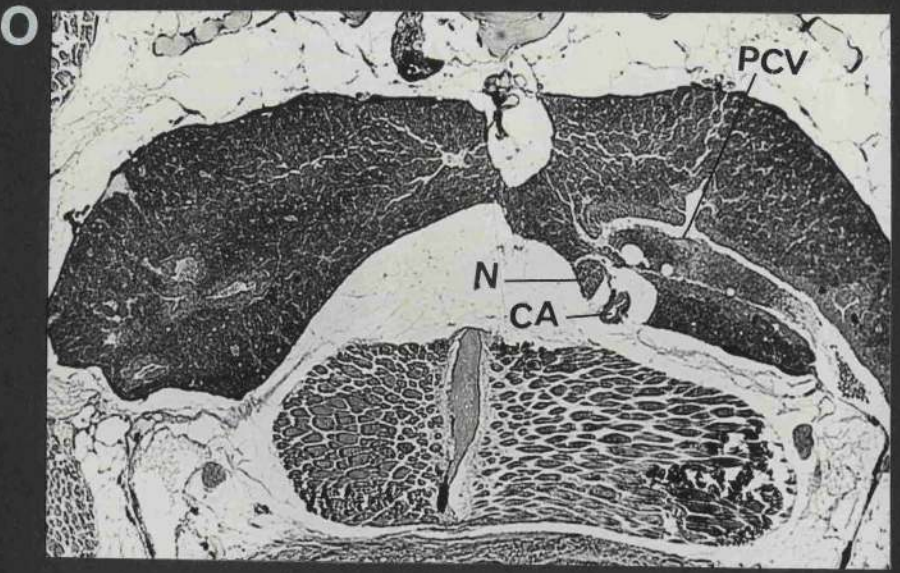
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Plate 28 (P–R) Phoxinus phoxinus Serial transverse sections (slightly oblique) through the whole fish, from anterior end of kidney posteriorly. A–L are 30 µm apart, M–S are about 60 µm apart; all sections are 6 µm thick stained in Masson's trichrome. Scale line corresponds to 150 µm.

(P) A simila r section to (O). Notice the large (ganglionated) gastromesenteric nerve. The right duct of Cuvier reaches nearly to the mid-line of the kidney.

(Q) The main vessel of the right duct of Cuvier emerges from the right lobe of the head-kidney, gathering tributaries from the entire right lobe, and also from the left lobe, so that it forms a much larger vessel than the left duct of Cuvier.

(R) The two posterior cardinal veins are visible, the right very much larger than the left, and separated from if by the dorsal hollow in which the dorsal aorta runs. Most of the blood from the kidneys drains into the right-hand side of the system. The gastromesenteric artery and nerve still run in a groove on the ventral surface of the head-kidney.

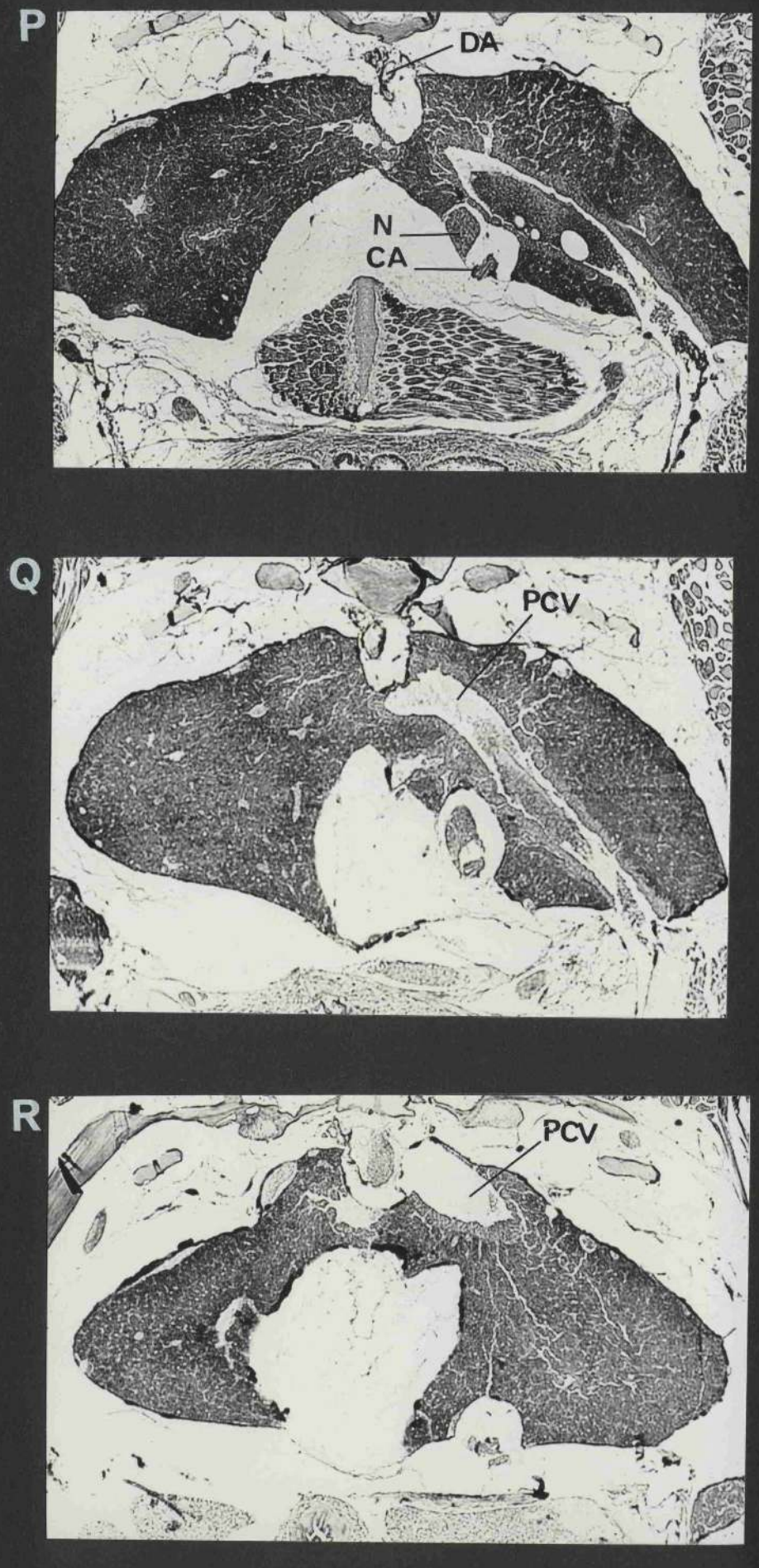

P
DA
N
CA
Q
PCV
R
PCV

Plate 29. Phoxinus phoxinus. Transverse sections through head-kidney region, 6 µm, stained in Masson's trichrome. Scale line corresponds to 100 µm.

(A) Section showing gastromesenteric artery branching off dorsal aorta, and passing bentrally between the right and left lobes of the head-kidney which at this level consists almost entirely of haemopoietic tissue. A pair of segmental arteries is also branching off the dorsal aorta. The two anterior cardinal veins have entered the head-kidney, one to each lobe. This section corresponds to 'D' of Plate 28.

(B) A section posterior to A, showing the gastromesentric artery now separate from the dorsal aorta, and running in a hollow in the right lobe of the head-kidney. Branches from sympathetic ganglia on either side of the dorsal aorta are descending through the gap between the lobes of the head-kidney and joining to form a ganglionated trunk alongside the gastromesenteric artery. The anterior cardinal veins are branching up into venules in the head-kidney tissue. A renal duct is visible. This section corresponds to 'H' of Plate 28.

(C) A section posterior to B, showing the gastromesentric artery and nerve running side by side in a hollow on ventral surface of the head-kidney. The posterior cardinal vein is lined with adrenocortical and chromaffin cells at this level, as are major tributaries. This section corresponds to 'P' of Plate 28.

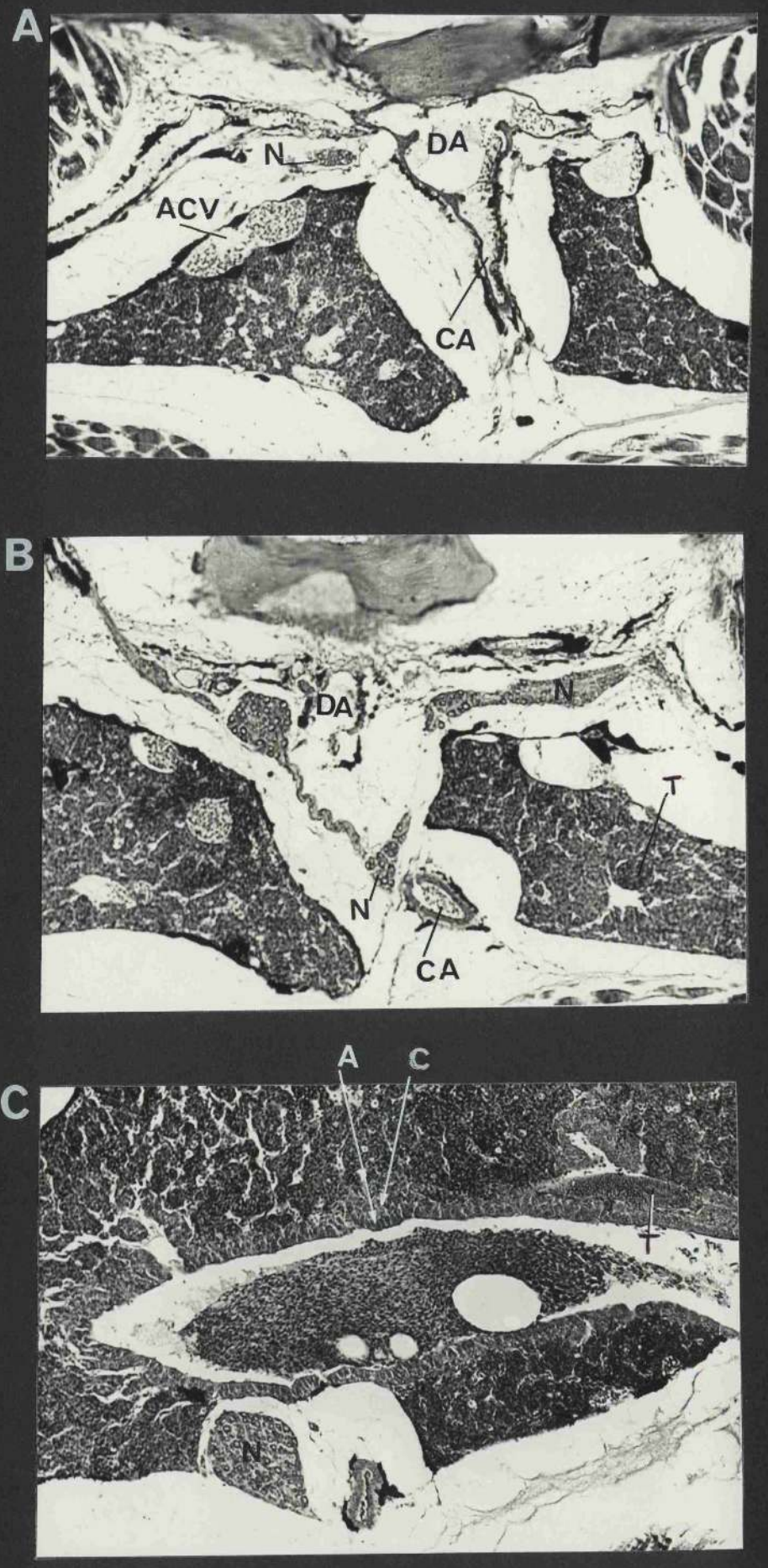
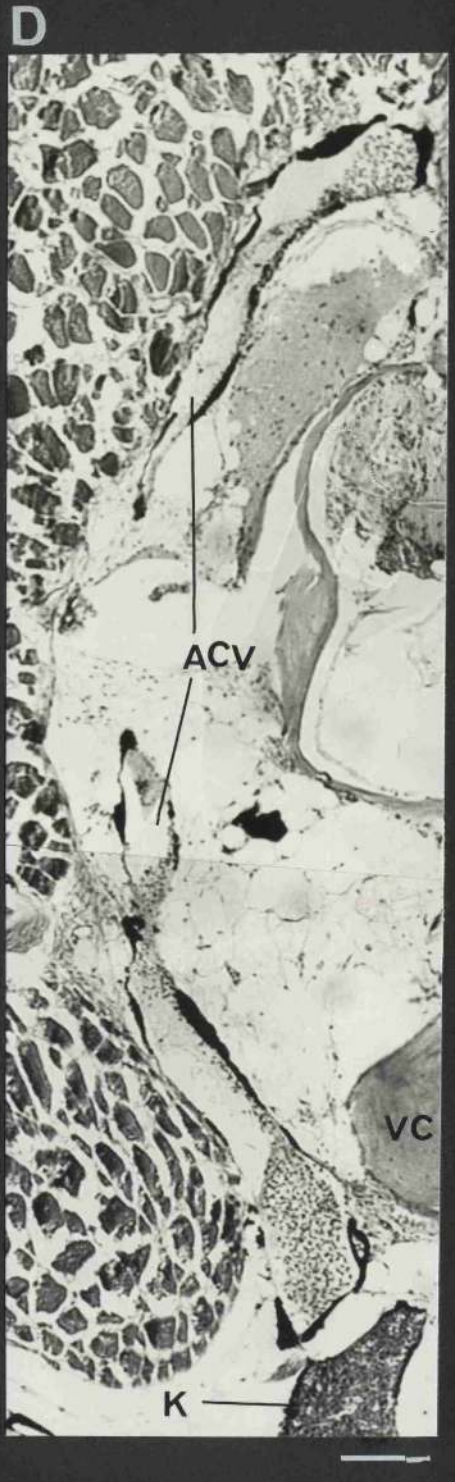

A
N
DA
ACV
CA
B
DA
N
T
N
CA
C
A
C
T
N

Plate 29 Phoxinus phoxinus. Transverse sections through head-kidney region, 6 µm, stained in Masson's trichrome. Scale line corresponds to 100 µm.

(D) Section near anterior end of head-kidney. The left anterior cardinal vein can be seen gathering from the dorso-lateral muscles and travelling ventrally past the vertebral column towards the head-kidney. This section corresponds to 'A' of Plate 28.

(E) Section showing emergence of left duct of Cuvier from left lobe of head-kidney. The duct and its major tributaries are lined with layers of adrenocortical and chromaffin cells. This section corresponds to 'M' of Plate 28.


D
ACV
VC
K

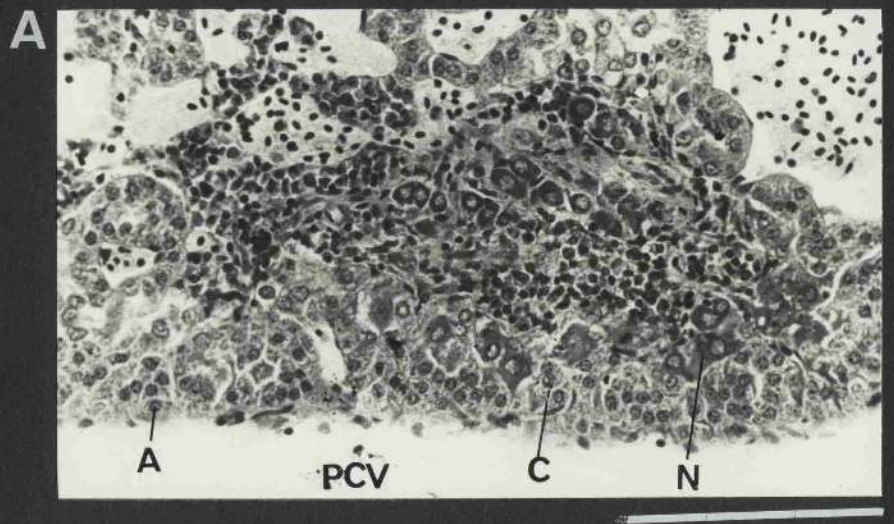

E
PCV
C
A
DC

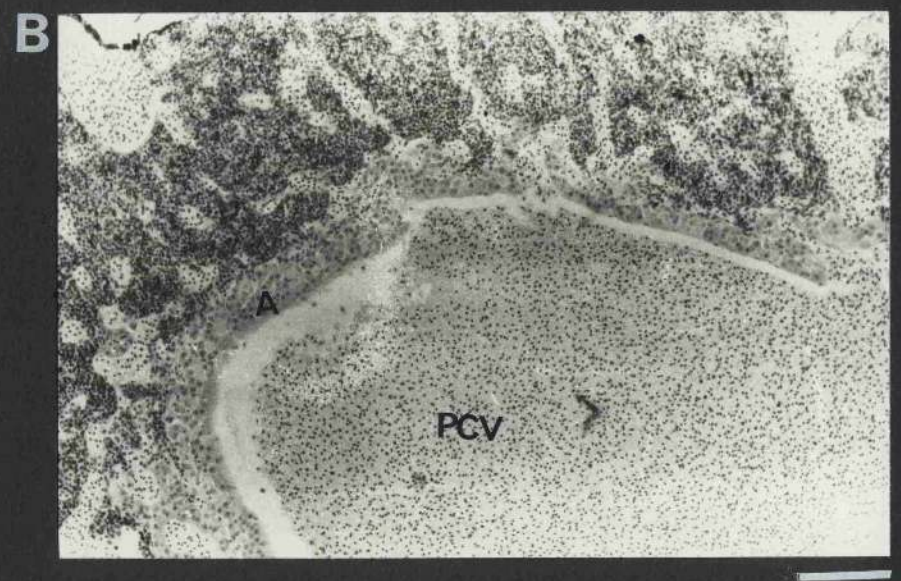
Plate 30. Phoxinus phoxinus. Transverse sections through head-kidney, 5 µm, stained in Masson's trichrome. At different times of year. Scale line corresponds to 100 µm.

(A) March. Showing adrenocortical and chromaffin cells in wall of posterior cardinal vein and its branches; and nerve cells.

(B) June Mature fish at spawning time, showing hyperplasia of adrenocortical cells, round posterior cardinal vein.

(C) June As 'B', showing increased size of adrenocortical and chromaffin cells.


A
A
PCV
C
N


B
A
PCV

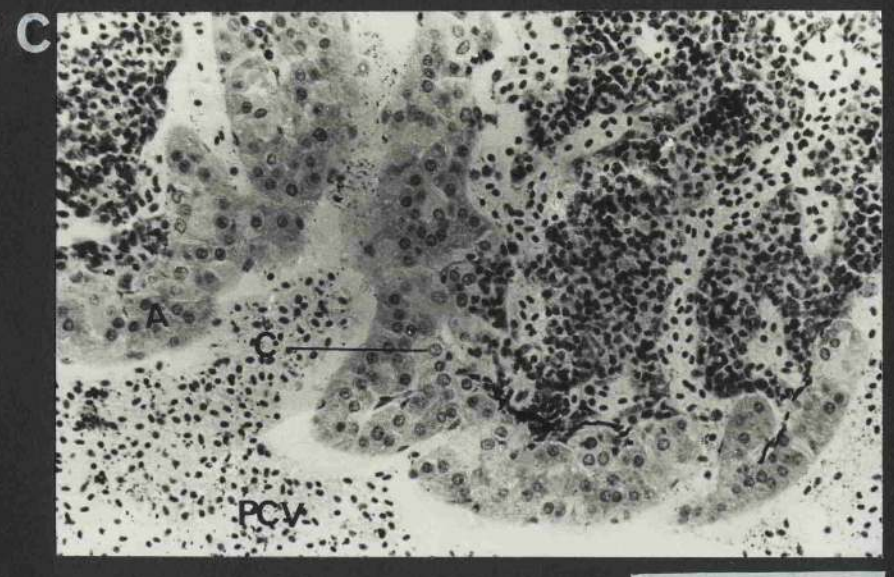
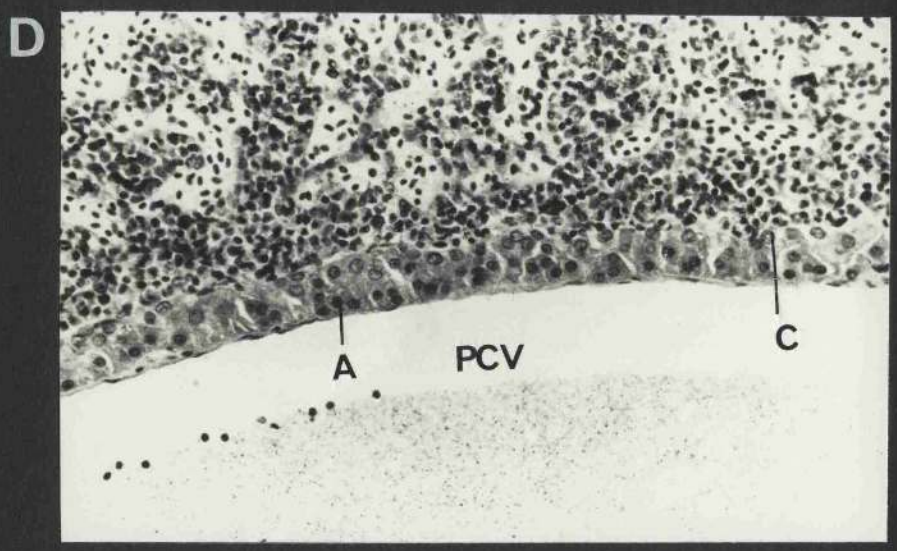

C
A
C
PCV

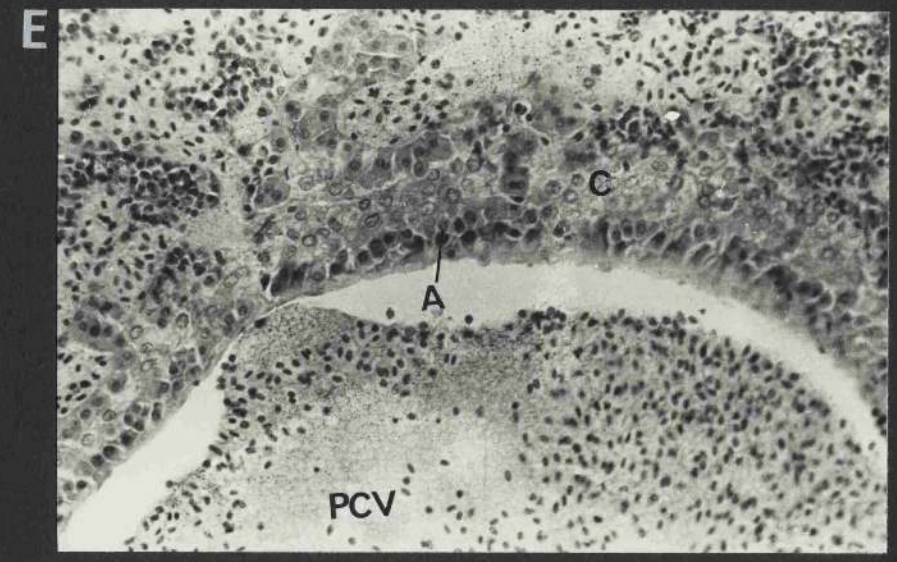
Plate 30. Phoxinus phoxinus. Transverse sections through head-kidney, 5 µm, stained in Masson's trichrome. At different times of year. Scale line corresponds to 50 µm.

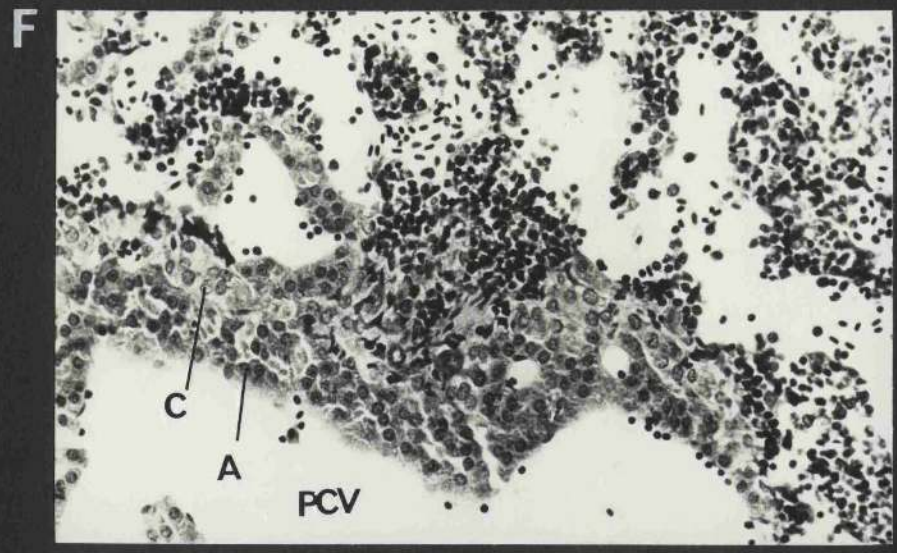
(D) July Spawned fish, showing reduction in number of layers of adrenocortical cells, and densely-staining cytoplasm.

(E) July Spawned fish, showing reduction in number of layers of adrenocortical cells, with densely-staining cytoplasm. The chromaffin tissue in this section forms an abundant layer behind the adrenocortical tissue.

(F) November Showing increase in cell-layers of adrenocortical tissue, and some chromaffin tissue.


D
A
PCV
C


E
C
A
PCV


F
C
A
PCV

ABBREVIATIONS

A	=	Adrenocortical cell
C	=	Chromaffin cell
CV	=	Chromaffin vesicle
CO	=	Core
E	=	Erythrocyte
ER	=	Endoplásmic reticulum
F	=	Fibroblast
G	=	Golgi complex
L	=	Lymphocytes
M	=	Mitchondrian
MB	=	Microbodies
MI	=	Microvilli
MN	=	Mylenated nerve
MT	=	Mitchondria tubule
N	=	Nucleus
NE	=	Nerve ending
NU	=	Nucleolus
PE	=	Peri-endothelial space
R	=	Ribosomes
S	=	Synaps
SD	=	Sinusoid
PCV	=	Posterior cardinal vein
V	=	Vacuole

PLATES 31 to 55

Plate 31. Phoxinus phoxinus. Electron micrograph of head-kidney, at the boundary between haemopoietic tissue (on left) and endocrine tissue (on right). At the boundary between the two zones are sinusoids containing erythrocytes, and also some connective tissue and fibroblasts. A row of adrenocortical cells lines the posterior cardinal vein, just visible at the bottom right. A single chromaffin cell lies behind them. Most of the lymphocytes contain ellipsoid granules, but agranular forms are also visible. Scale line corresponds to 4.4 µm.

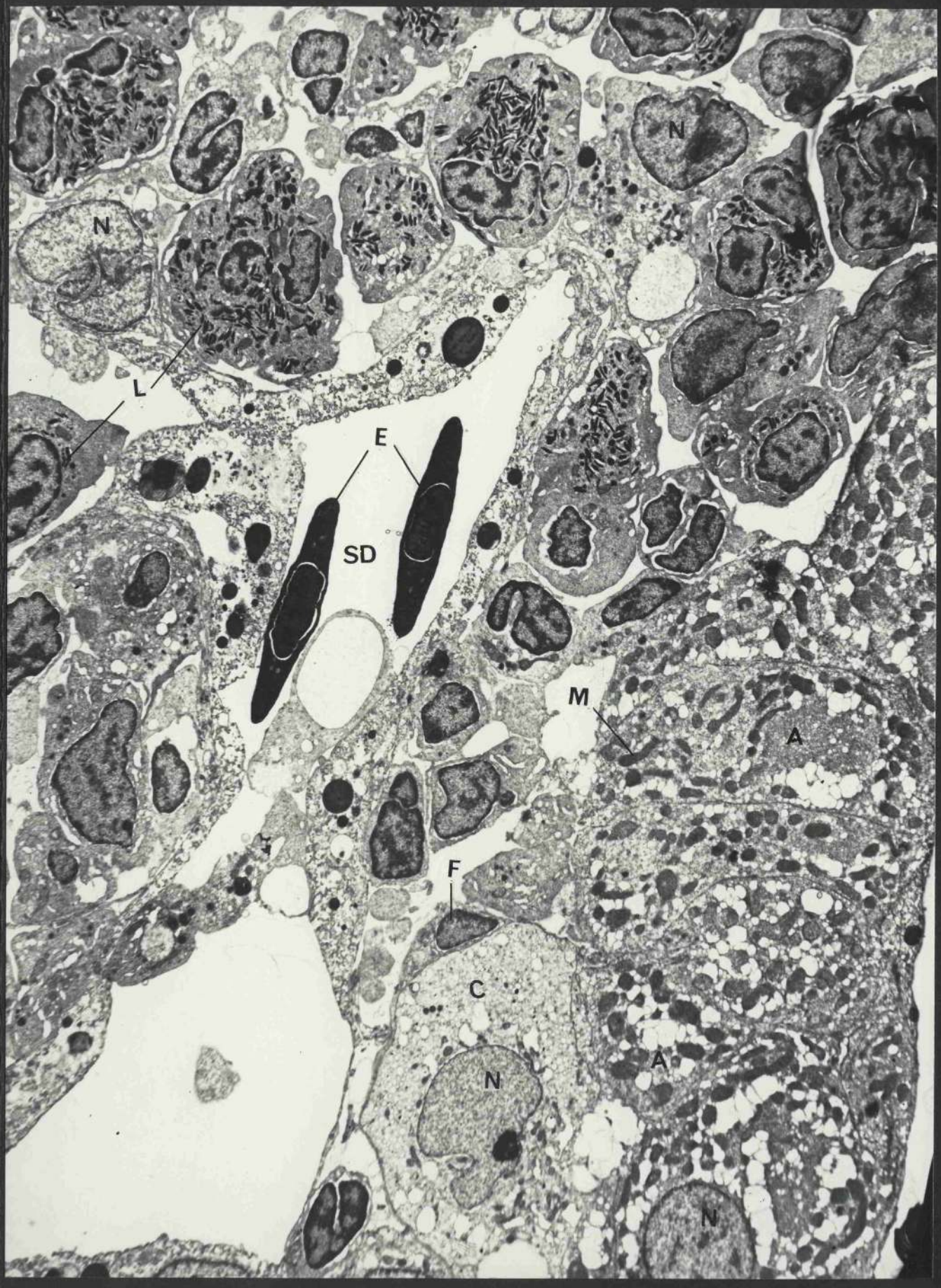

N
N
L
E
SD
M
A
F
C
N
A
N

Plate 32. Phoxinus phoxinus. A similar section to Plate 31, with the haemopoietic tissue (top right) separated from the endocrine tissues (bottom left) by connective tissue. Adrenocortical cells line the posterior cardinal vein, out of the field of view, bottom left. Several chromaffin cells lie distally, with varying degrees of granulation in their chromaffin vesicles. One can be seen with a long process extending towards the vein, and sections through similar processes from other cells are also visible. Nerve axons are visible amongst the chromaffin cells, with which they make synaptic contacts (cf. Plates 48,49,50). Scale line corresponds to 3.6µm.

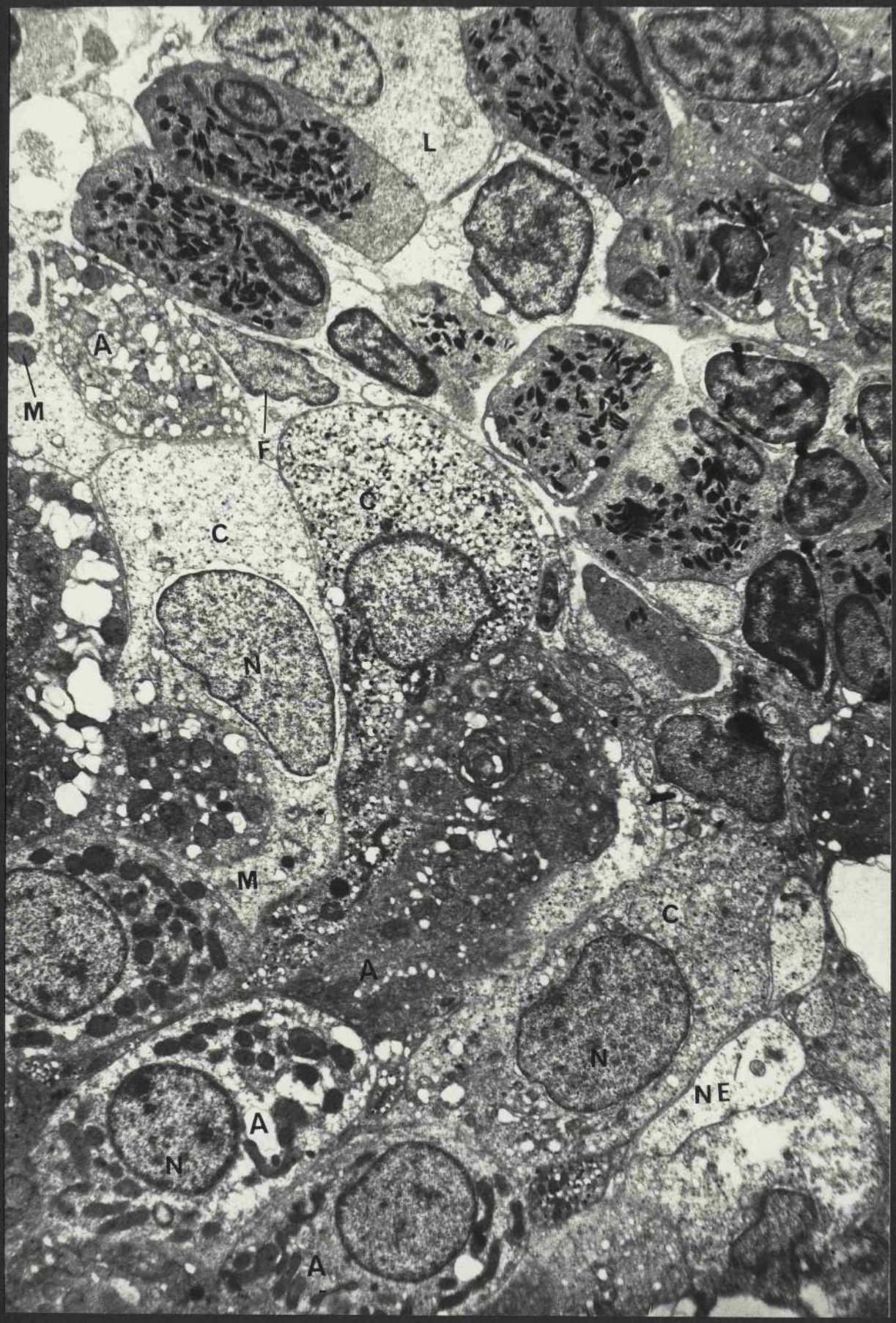

L
A
M
F
C
C
N
M
A
C
N
NE
A
N
A

Plate 33. Salmo gairdnerii. Electron micrograph of a section near the anterior end of the head-kidney. Haemopoietic tissue is at the bottom, adrenocortical tissue at the top of the photograph. There are no lymphocytes with ellipsoid granules as in Phoxinus, but a range of other types. Nerve axons are present in the boundary region, but it is not clear whether synapses occur with any adrenocortical cells. The mitochondria in different adrenocortical cells range from small elongate forms with a dense matrix to large circular forms with little internal structure (v. discussion, p. 74). Scale line corresponds to 2μm.

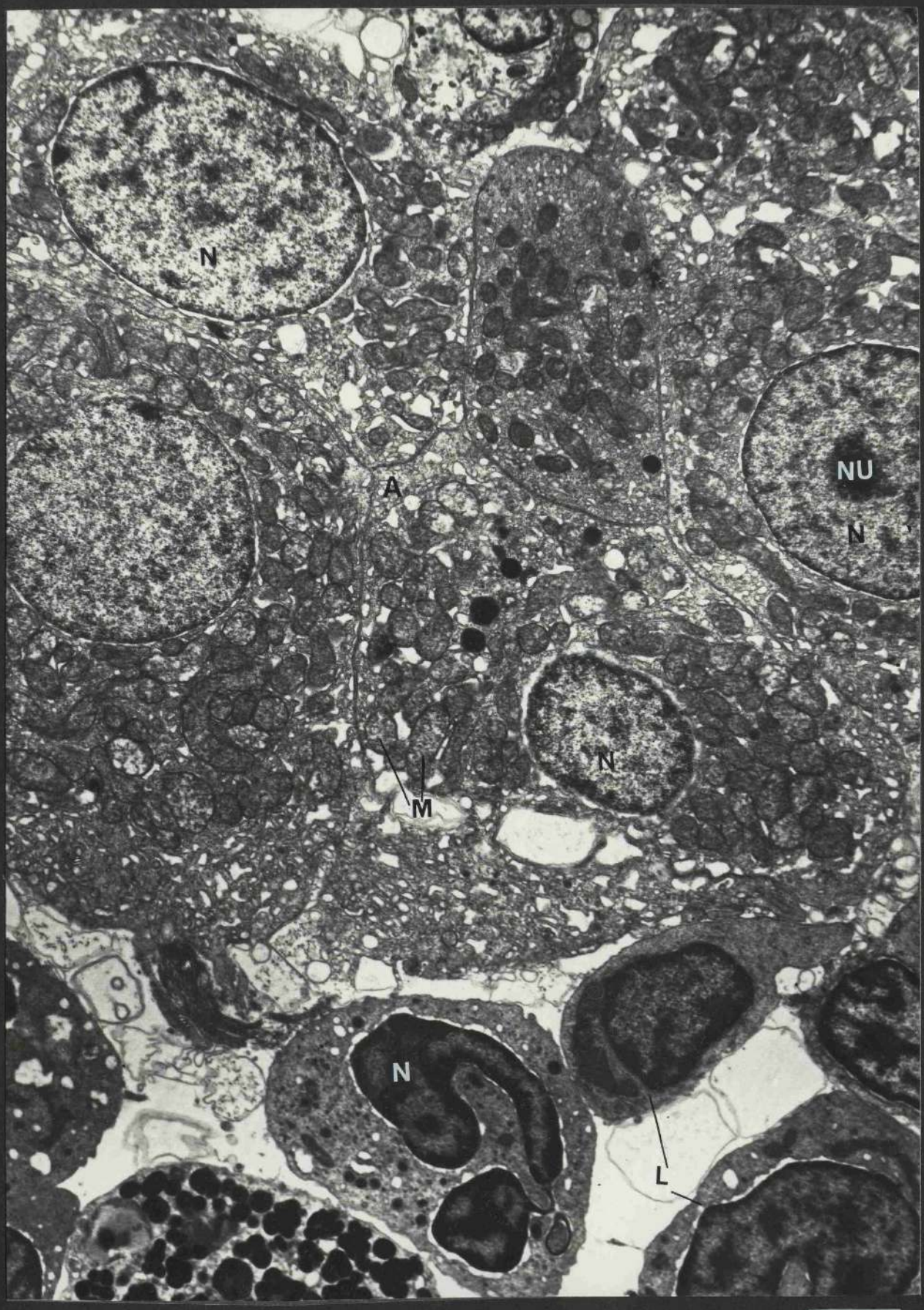

N
A
NU
N
N
M
N
L

Plate 34. Salmo gairdnerii. A similar section to Plate 33, but from the posterior region of the head-kidney where only chromaffin and no adrenocortical tissue occurs. Several types of white blood cells lie in the haemopoietic tissue (top), separated from the chromaffin tissue (bottom) by a broad band of connective tissue in which are embedded many nerve fibres. Axons can also be seen amongst the chromaffin cells, with which they make synaptic contacts (cf. Plates 46,51, 52,53). Scale line corresponds to 4.4µm.

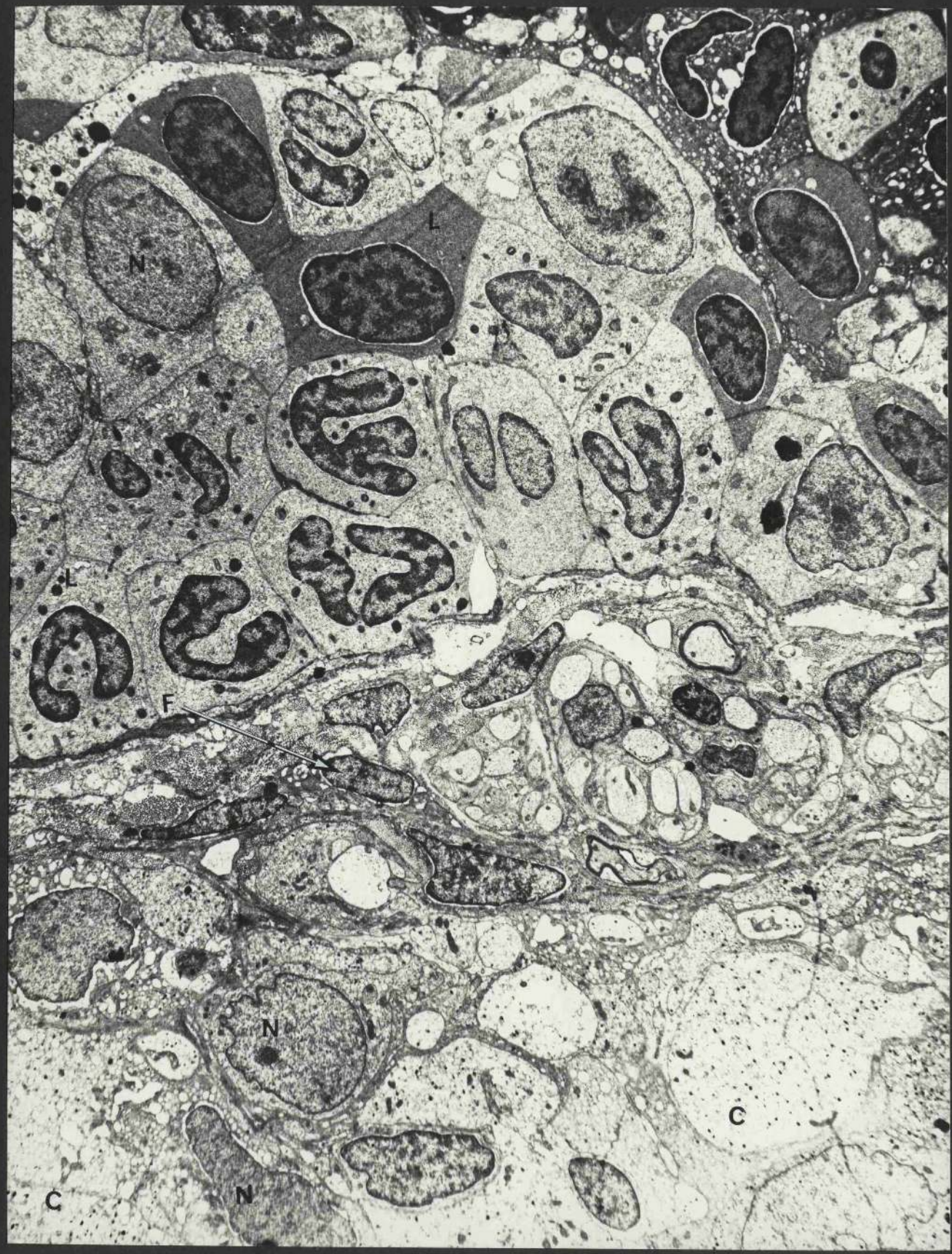

L
N
L
F
N
C
C
N

Plate 35. Salmo gairdnerii. A continuation of the section shown in Plate 34, towards the posterior cardinal vein, the lumen of which is visible at the bottom of the photograph containing erythrocytes. Chromaffin cells with a range of intensity of granulation are visible, with many nerve axons amongst them some forming synapses. Notice the irregular shape of the nuclei of the chromaffin cells, and the presence of few irregular mitochondria. The layer of chromaffin tissue is bounded by the connective tissue band containing nerve fibres below the haemopoietic tissue, and the connective tissue forming the wall of the posterior cardinal vein. Scale line corresponds to 4.4μm.

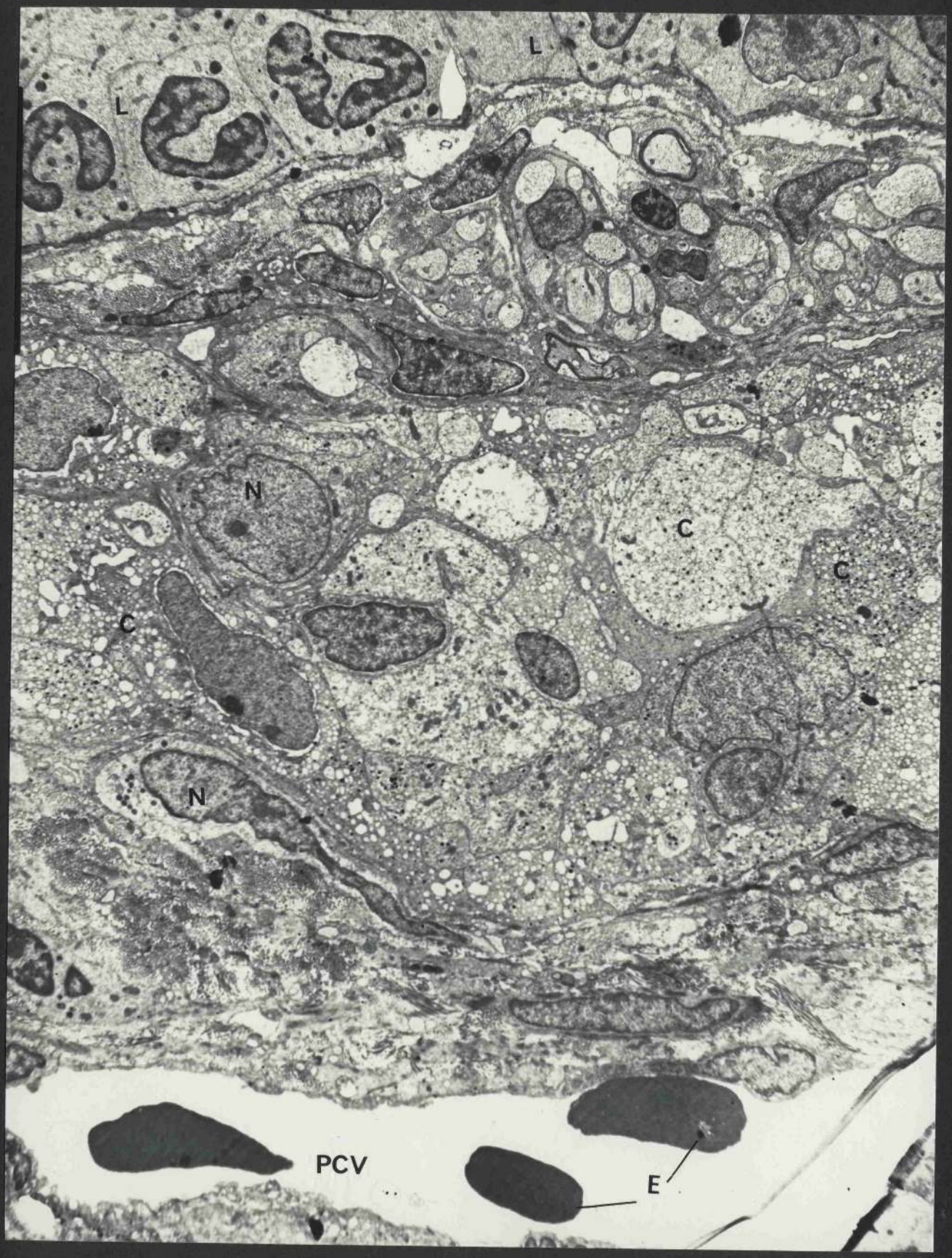

L
L
L
N
C
C
C
N
PCV
E

Plate 36. Phoxinus phoxinus. An aquarium-maintained specimen. A section through one of the tributaries of the posterior cardinal vein in the head-kidney. Lymphocytes and erythrocytes are present in the lumen of the vein. Adrenocortical cells in varying stages of activity surround the vein, and distal to them are several chromaffin cells with processes running towards the vein. Some of the chromaffin cells are heavily granulated, while in others the vesicles are almost empty. The nuclei of the adrenocortical cells are regular in outline. There is a range in shape, size, and electron opacity of the mitochondria, and in the density of the cytoplasm, and its degree of vacuolation, but there is no clear relationship between mitochondrial form and cytoplasm density in any given cell (v. discussion, p. 75). Scale line corresponds to 4.4µm.

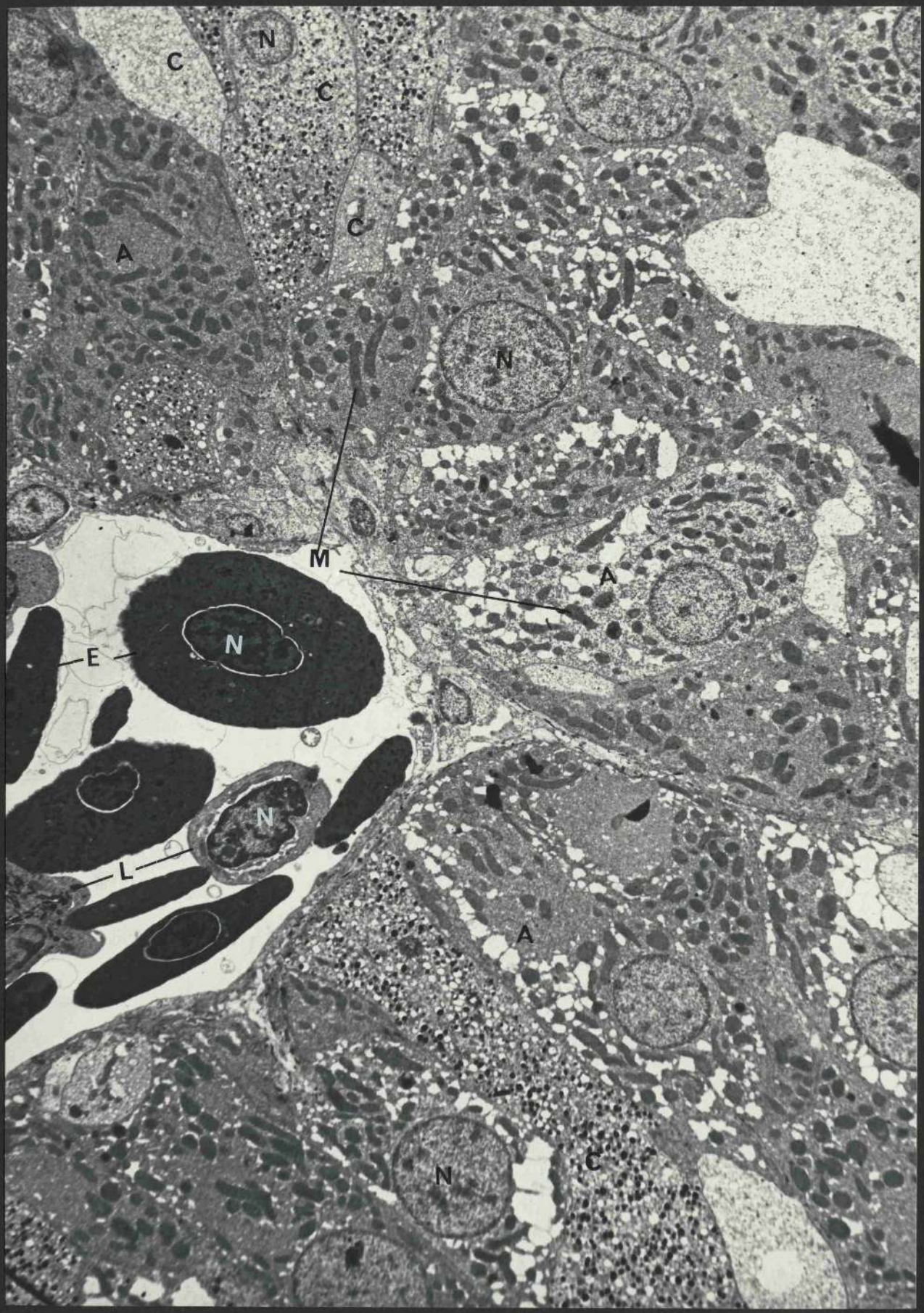

C
N
C
C
A
N
M
A
E
N
N
L
A
N
C

Plate 37. Phoxinus phoxinus. A similar section to Plate 36, showing the posterior cardinal vein on the left. All the adrenocortical cells shown here have highly vacuolated cytoplasm. The section is from an aquarium-maintained fish, which would be under stress. The adrenocortical cells abut on to a sub-endothelial space in a series of microvilli (v. Plate 38). Scale line corresponds to 1.1µm.

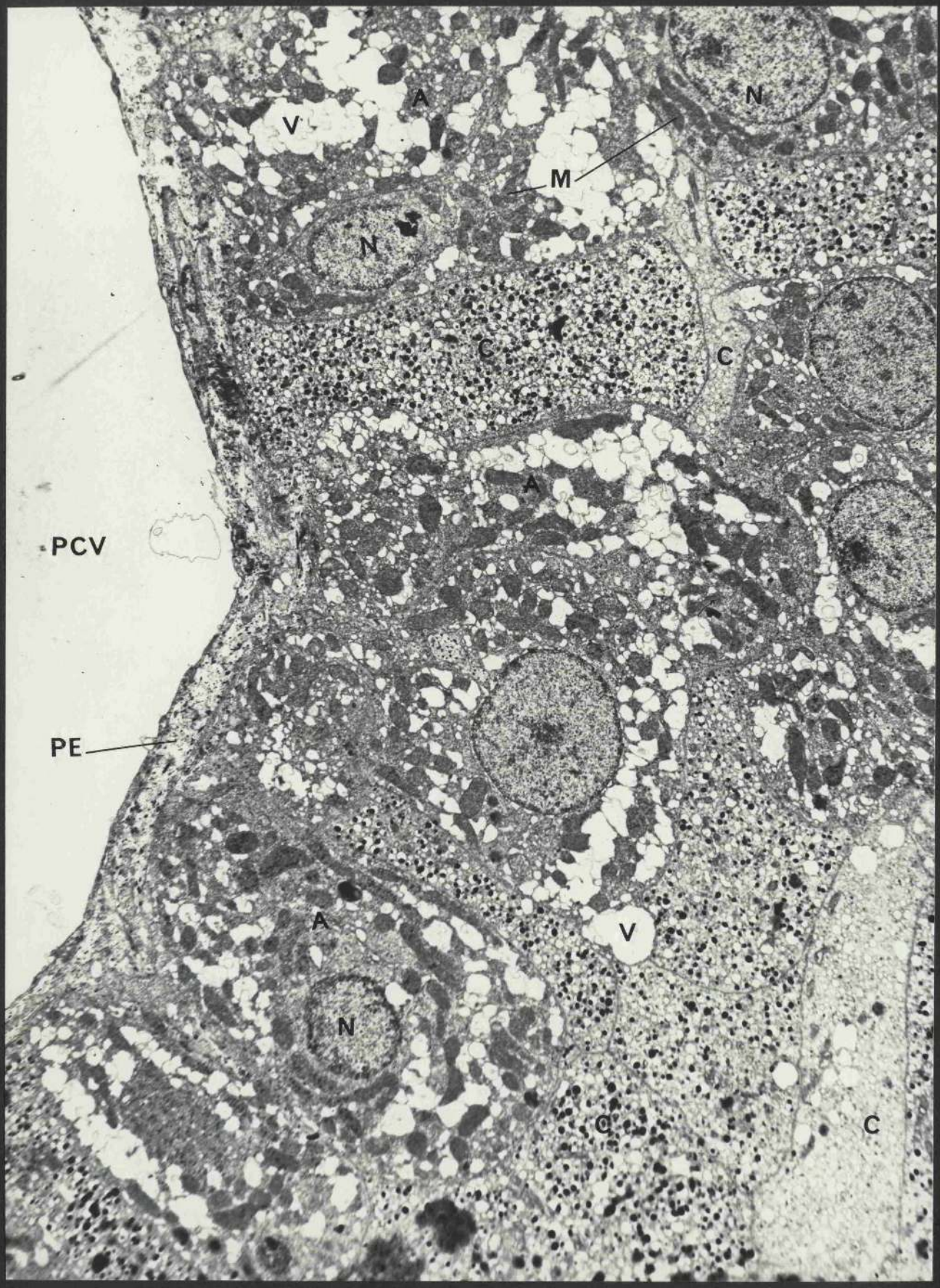

V
A
N
M
N
C
C
A
PCV
PE
A
V
N
C
C

Plate 38. Phoxinus phoxinus.

A. Section through head-kidney, showing endocrine tissues abutting posterior cardinal vein. Extensive microvilli are visible on the walls of the adrenocortical cells and the single chromaffin cell in contact with the vein, or with a subendothelial space. Scale line corresponds to 1.8µm.

B. Similar section to A.
Scale line corresponds to 1.8µm.

C. Similar section to A.
Scale line corresponds to 1.4µm.

D. Salmo gairdneri. Similar section through anterior region of head-kidney, showing adrenocortical tissue only, with microvilli in contact with lumen of vein. A group of three adrenocortical cells at bottom shows small, elongate mitochondria with a dark matrix in uniformly dense cytoplasm. Two cells show large circular mitochondria with disintegrating matrix in light vacuolated cytoplasm (v. discussion, p. 75). Scale line corresponds to 4.6µm.

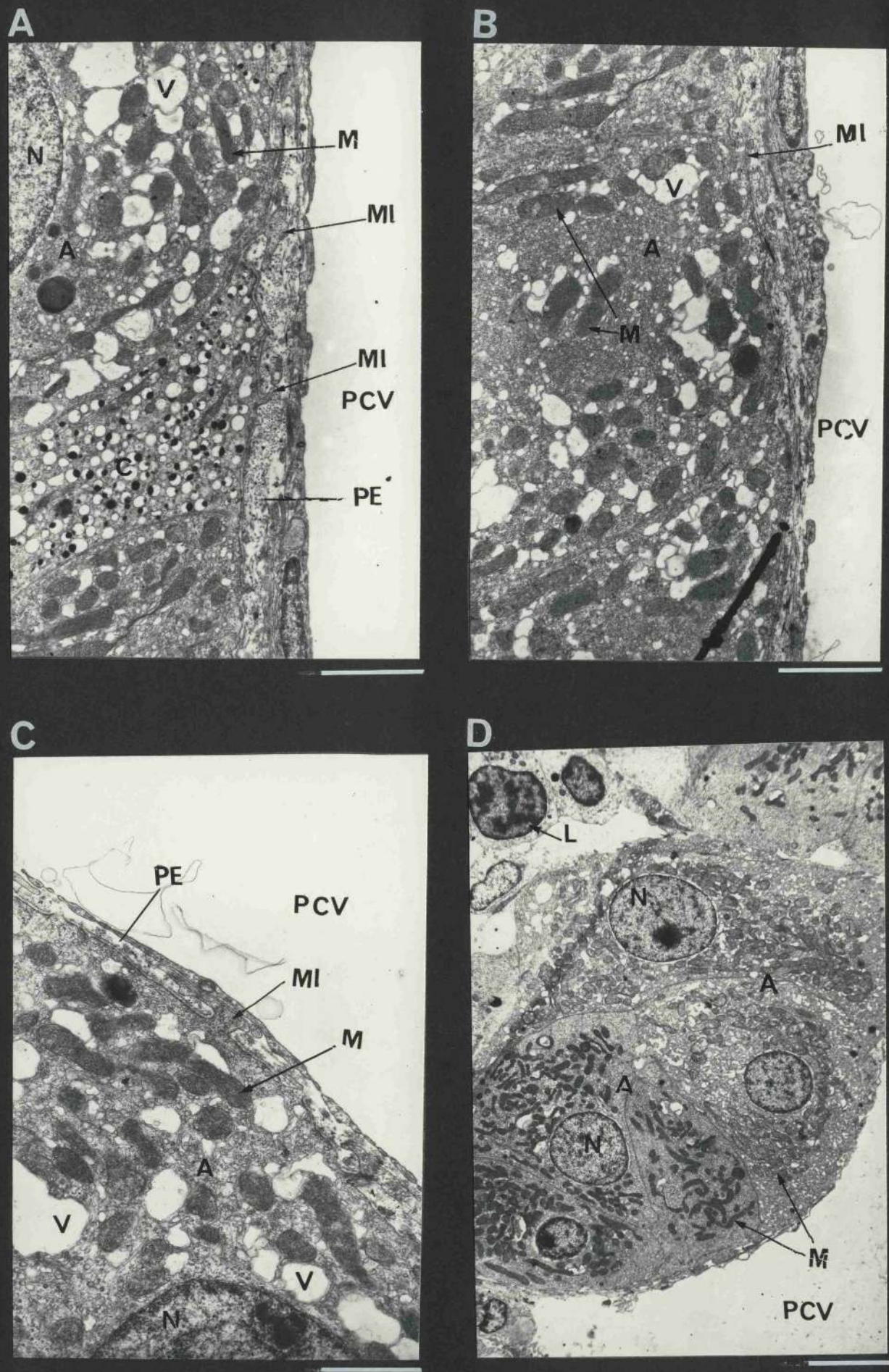

A
V
N
M
Ml
A
Ml
PCV
C
PE
B
Ml
V
A
M
PCV
C
PE
PCV
Ml
M
A
V
V
N
D
L
N
A
A
N
M
PCV

Plate 39. Phoxinus phoxinus.

A. Adrenocortical cells (with a small part of a chromaffin cell on left). To show structure of nucleus, notice large, circular, pale nuclei and somewhat vacuolated cytoplasm. Scale line corresponds to 1.8µm.

B. Adrenocortical cells (with a small part of a chromaffin cell bottom right), to show conspicuous Golgi complex in a cell with highly vacuolated cytoplasm and large, pale mitochondria top left (v. discussion, pp. 75). Scale line corresponds to 2.1µm.

C. Adrenocortical cells, showing part of nucleus (bottom right) with nuclear membrane and peripheral granules. The tubulo-vesicular form of the mitochondria is visible, ribosomes, smooth endoplasmic reticulum and golgi complex with a large vacuole. Scale line corresponds to 0.8µm.

D. Adrenocortical cell (top) and chromaffin cell (bottom). The adrenocortical cell shows conspicuous golgi complex and smooth endoplasmic reticulum in a cell with pale cytoplasm. Scale line corresponds to 1.1µm.

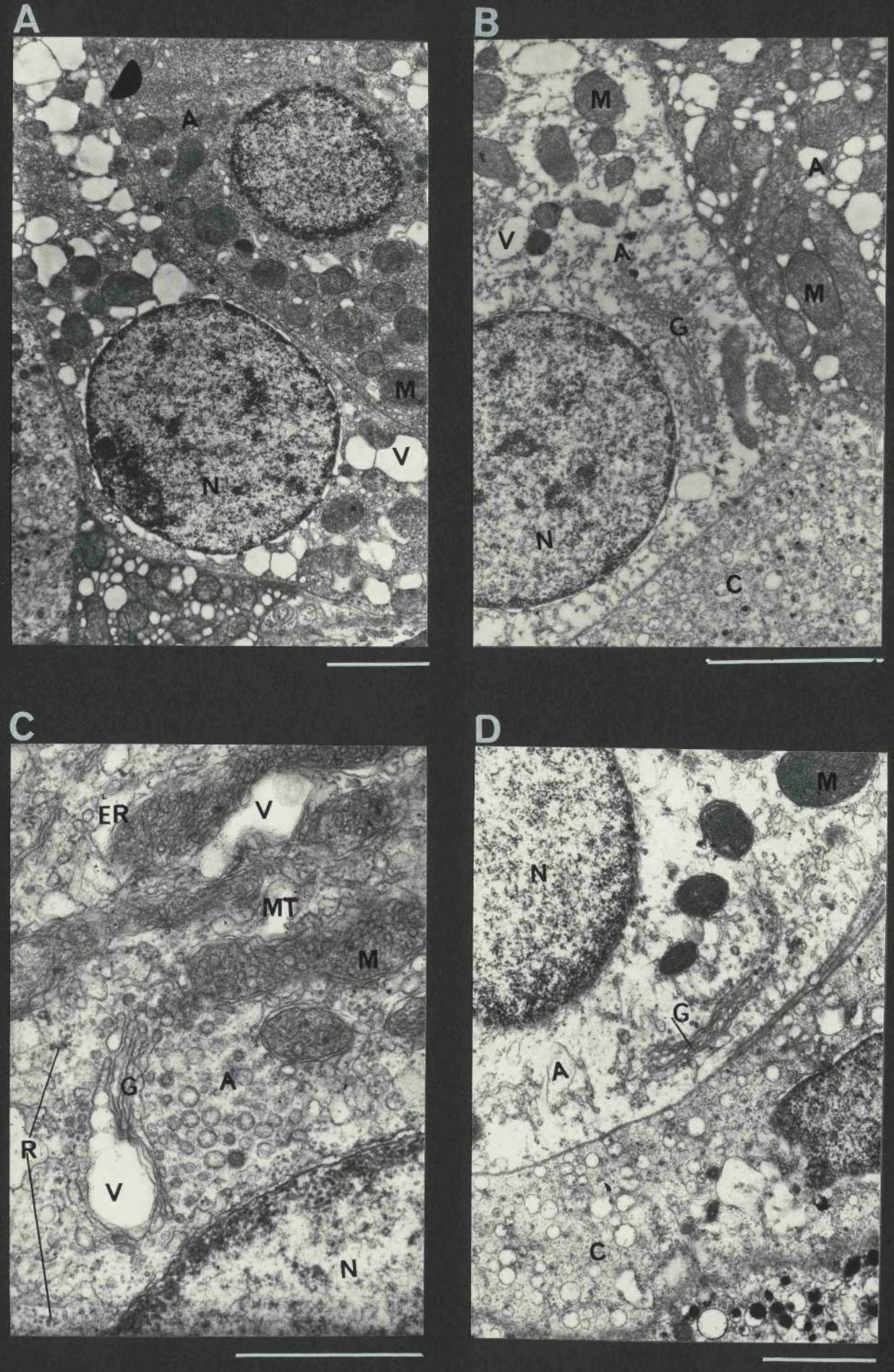
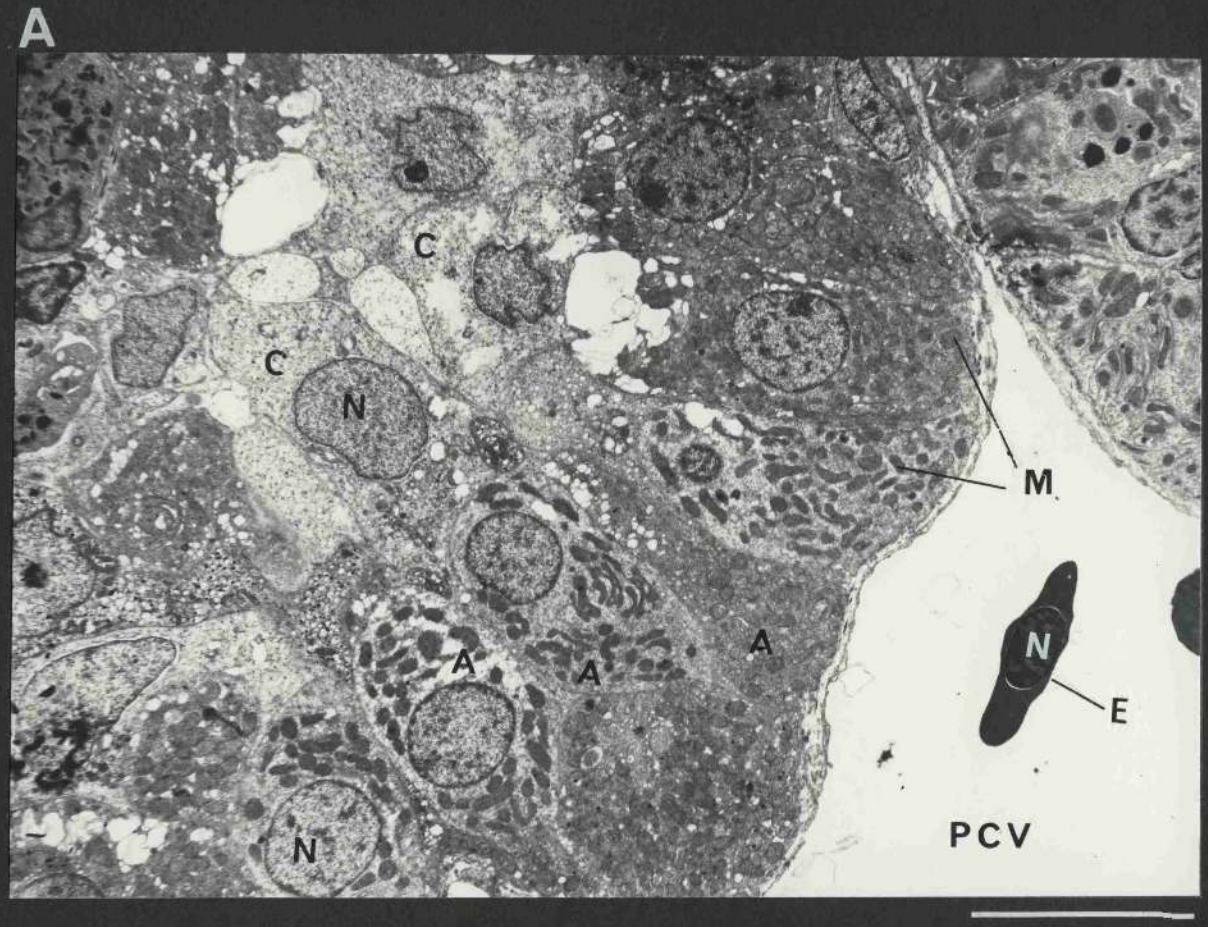

A
A
M
V
N
B
M
A
V
A
M
G
N
C
C
ER
V
MT
M
G
A
R
V
N
D
M
N
G
A
C

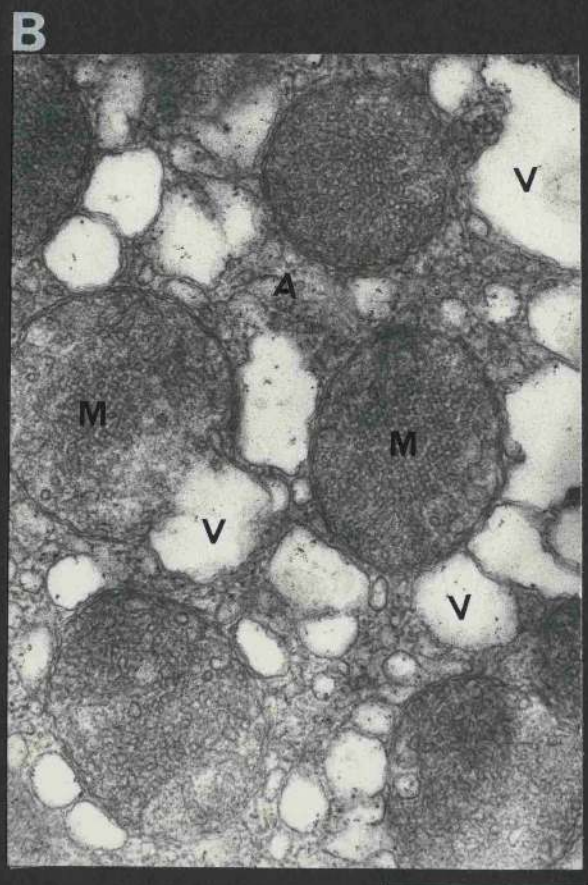
Plate 40. Phoxinus phoxinus.

A. An unstressed specimen section through posterior cardinal vein in head-kidney. Adrenocortical cells line the vein, with chromaffin cells and nerve fibres distally. Some of the adrenocortical cells contain large, relatively pale, circular mitochondria, while others contain more irregular, dark mitochondria. In some cells the cytoplasm is uniformly dark, in others pale and more or less vacuolated. In this section, the dark mitochondria are associated with light cytoplasm, but this is not invariably so (v. discussion p. 75). Scale line corresponds to 7.9µm.

B. Mitochondria of adrenocortical cell, showing internal tubulo-vesicular structure, ribosomes, smooth endoplasmic reticulum, and vacuoles. Scale line corresponds to 0.8µm.

C. Boundary between two adrenocortical cells, that on the left showing less vacuolation, more ribosomes and more extensive smooth endoplasmic reticulum than that on the right, and therefore presumed to be highly active (v. discussion, p. 76). Scale line corresponds to 0.4µm.


A
C
C
N
M
A
A
A
N
N
E
PCV


B
V
A
M
M
V
V

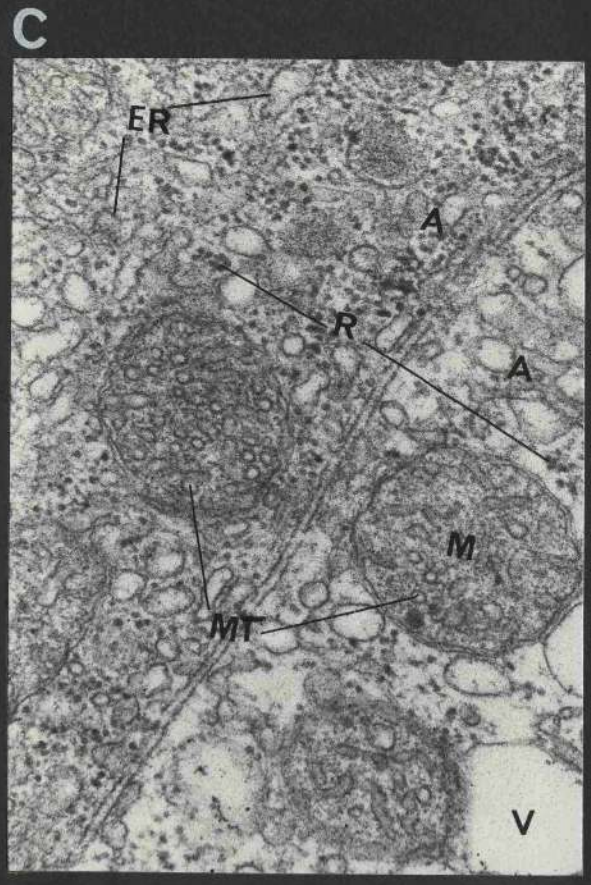

C
ER
A
R
A
M
MT
V

Plate 41. Salmo gairdneri. Adrenocortical-cells, showing mitochondria with varying degrees of breakdown of internal structure. In some cases, nothing remains but the empty outline (v. discussion, p. 75). Scale line corresponds to 1.5µm.

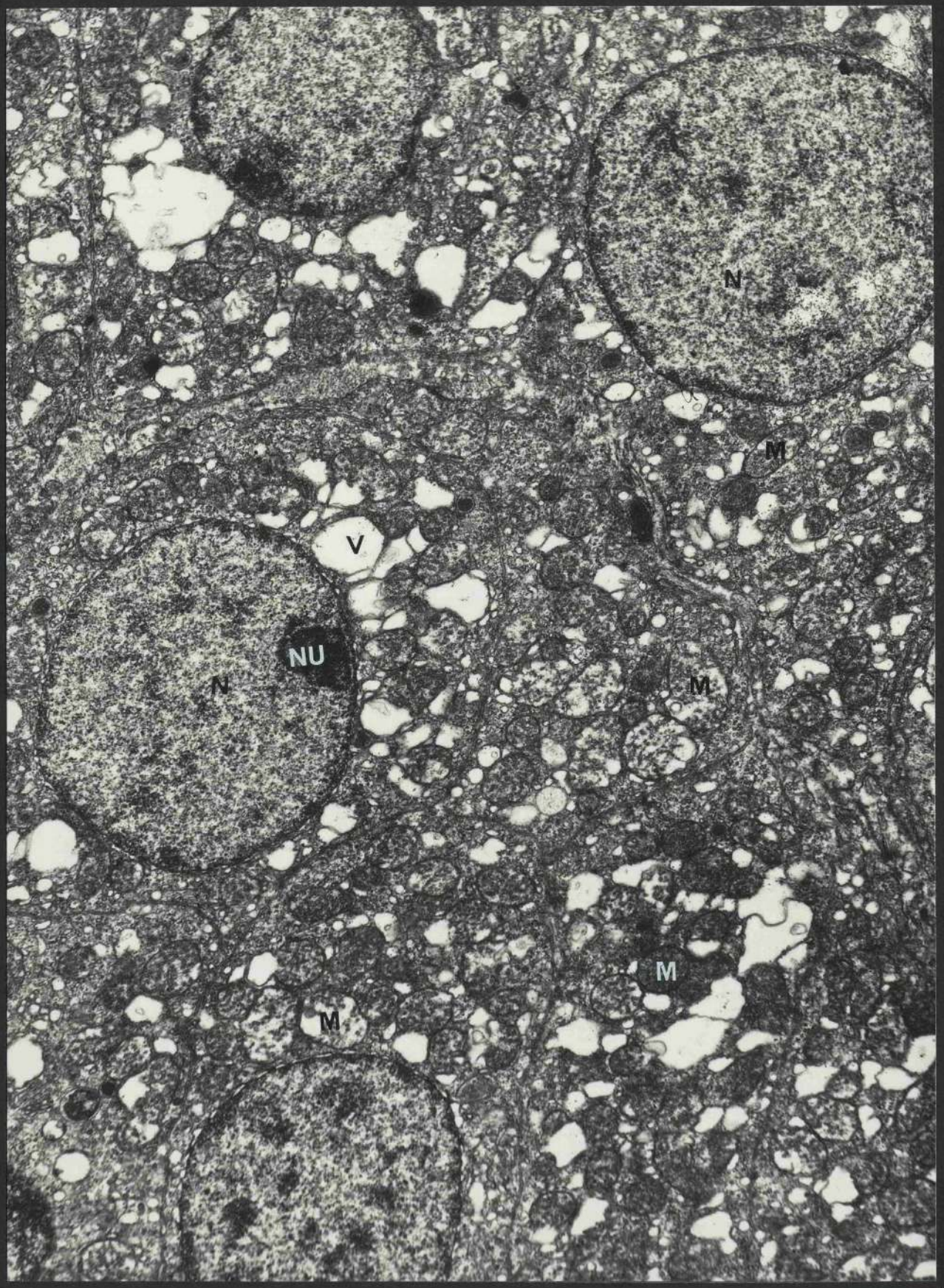

N
M
V
NU
N
M
M
M

Plate 42. Phoxinus phoxinus. Adrenocortical cells (top right and bottom left) separated by processes from one or more chromaffin cells. Many of the mitochondria of the main adrenocortical cell are losing or have lost all their internal structure, leaving large vacuoles. Microbodies are visible which may be an early stage in the development of mitochondria. Scale line corresponds to 0.6µm.

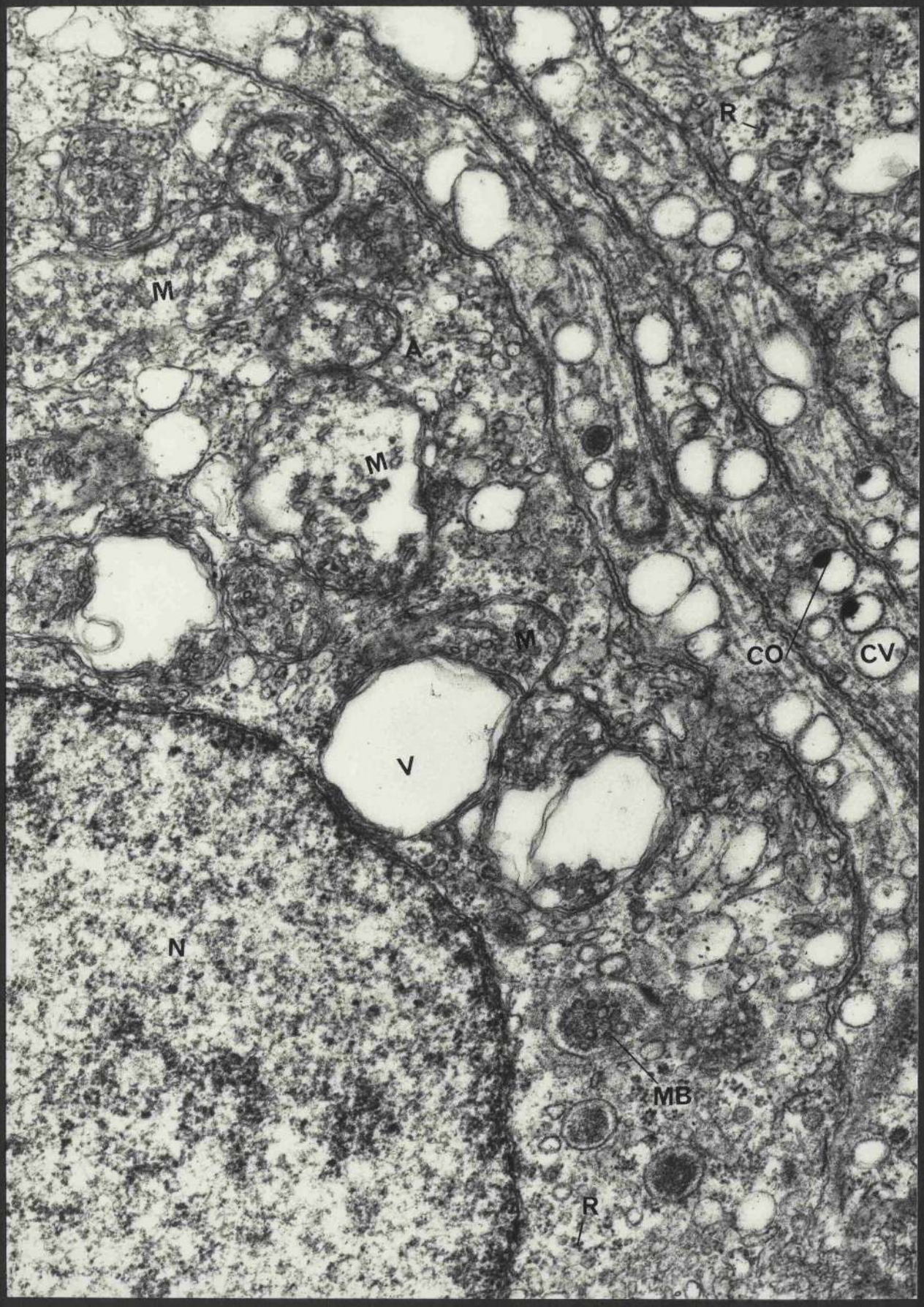

R
M
A
M
M
CO
CV
V
N
MB
R

Plate 43. Salmo gairdnerii. Adrenocortical cells showing range of types of mitochondria, from small dark forms relatively widely dispersed in the cell, to large pale forms closely packed within the cell. Scale line corresponds to 1.6µm.

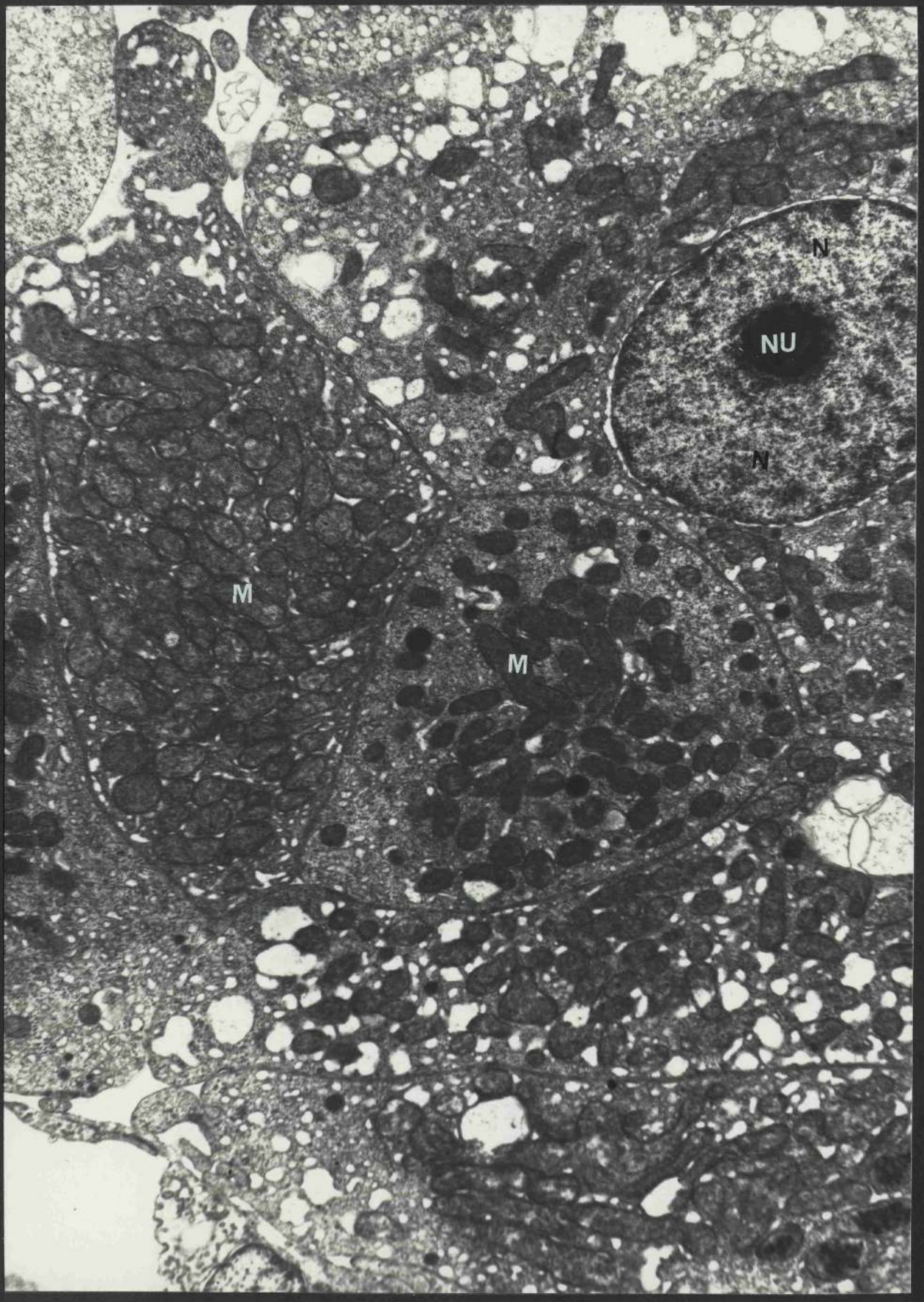
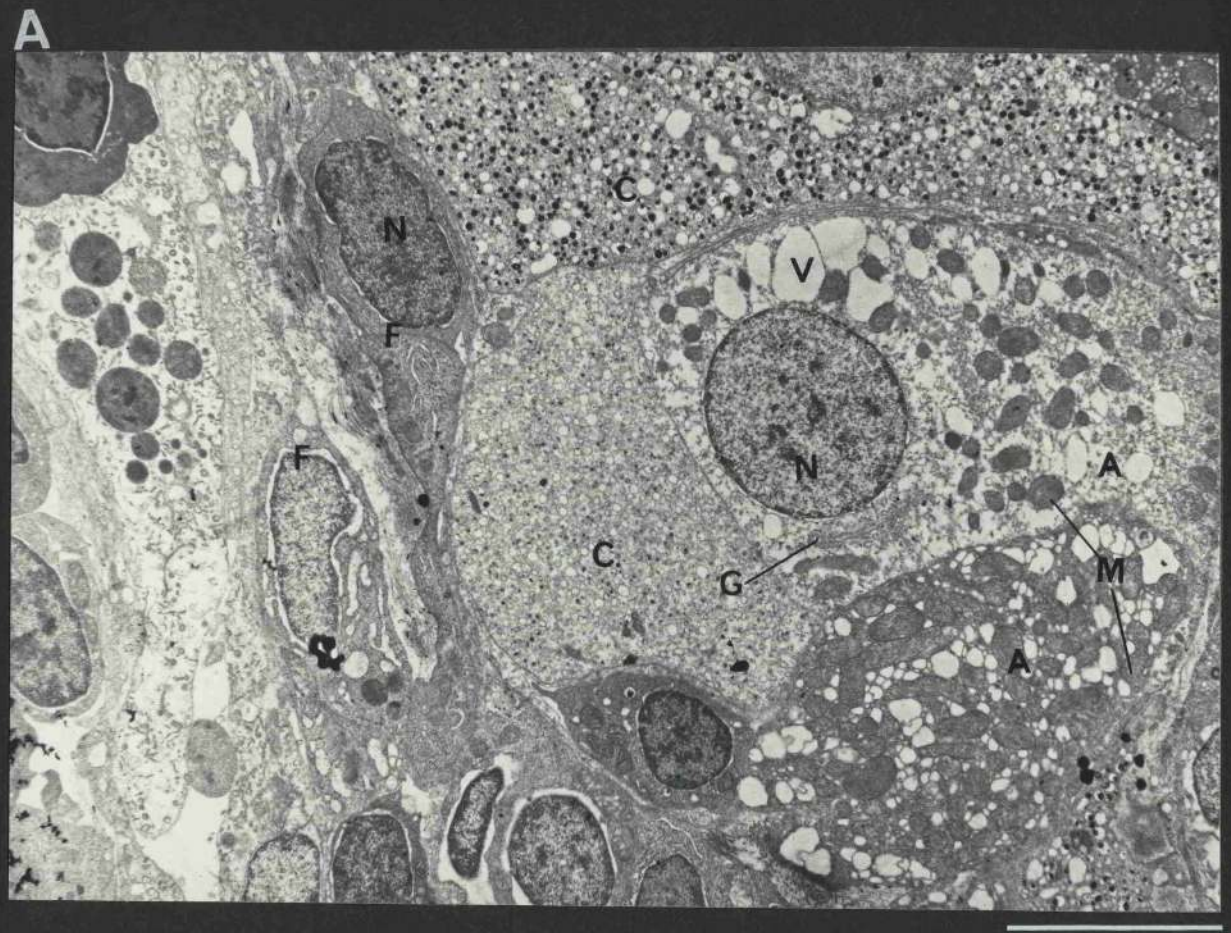

N
NU
N
M
M

Plate 44. Phoxinus phoxinus.

A. Section through head-kidney at junction of haemopoietic tissue (left) and endocrine tissues (right). A chromaffin cell with almost empty vesicles adjoins one in which the vesicles contain a considerable amount of granular material. Two adrenocortical cells are also visible, one with a conspicuous golgi complex and pale, highly vacuolated cytoplasm containing few mitochondria; the other with a greater number of mitochondria. Scale line corresponds to 4.3µm.

B. Two chromaffin cells. That on the right has vesicles almost empty of granules, that on the left has vesicles ranging from empty to full of electron-opaque material. Mitochondria are much sparser than in adrenocortical cells, and very variable in form. Scale line corresponds to 0.8µm.


A
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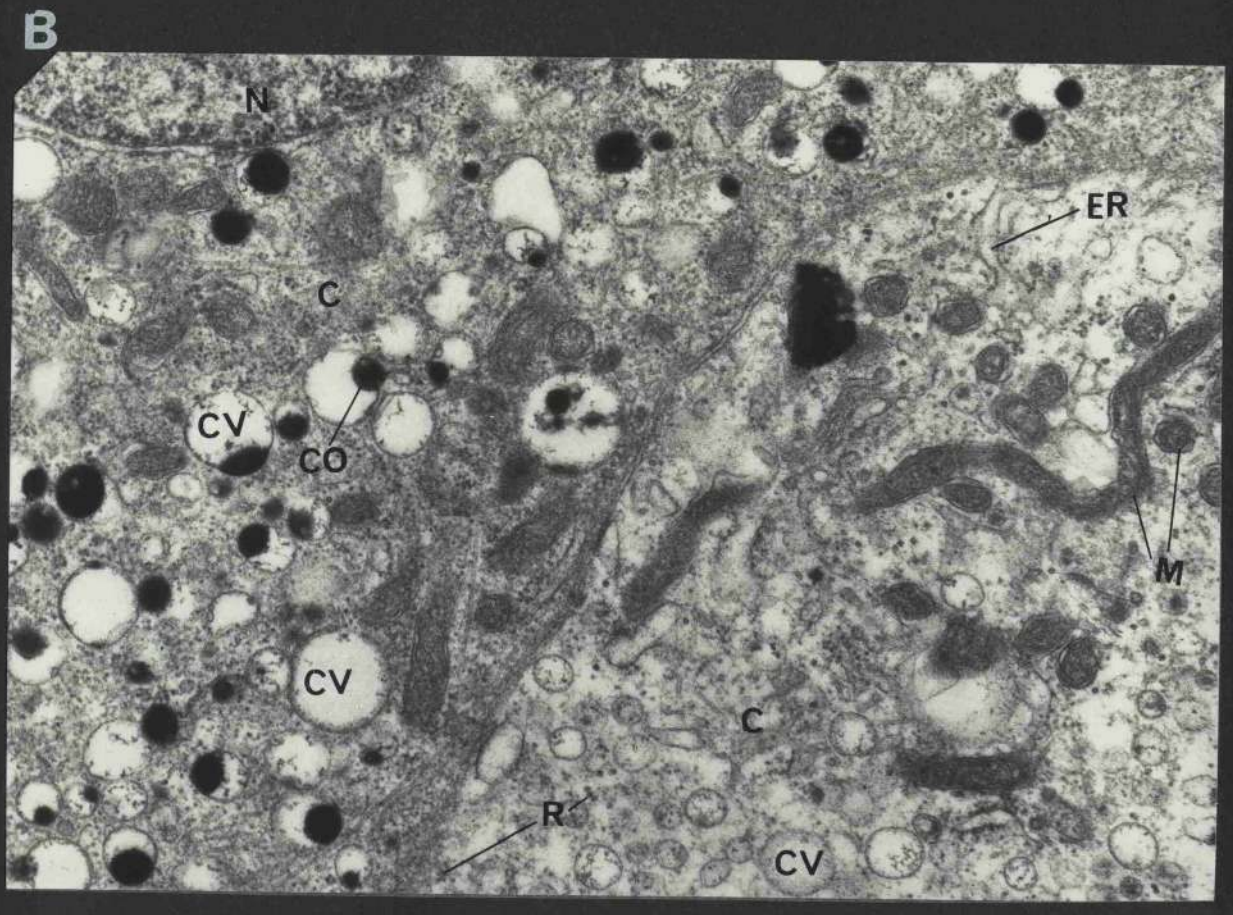
C
V
F
F
N
A
C
G
M
A


B
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ER
C
CV
CO
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CV
C
R
CV

Plate 45. Phoxinus phoxinus.

A. Chromaffin cell (left) and adreno-cortical cell (right) abutting posterior cardinal vein. Microvilli at surface of chromaffin cell. Scale line corresponds to 1.8µm.

B. Chromaffin cell flanked by an adreno-cortical cell (above) and a second chromaffin cell (below). Scale line corresponds to 0.5µm.

C. Detail of chromaffin cell, showing chromaffin vesicles, mitochondria, endoplasmic reticulum and many ribosomes. Scale line corresponds to 0.7µm.

D. Golgi complex in chromaffin cell. Scale line corresponds to 0.7µm.

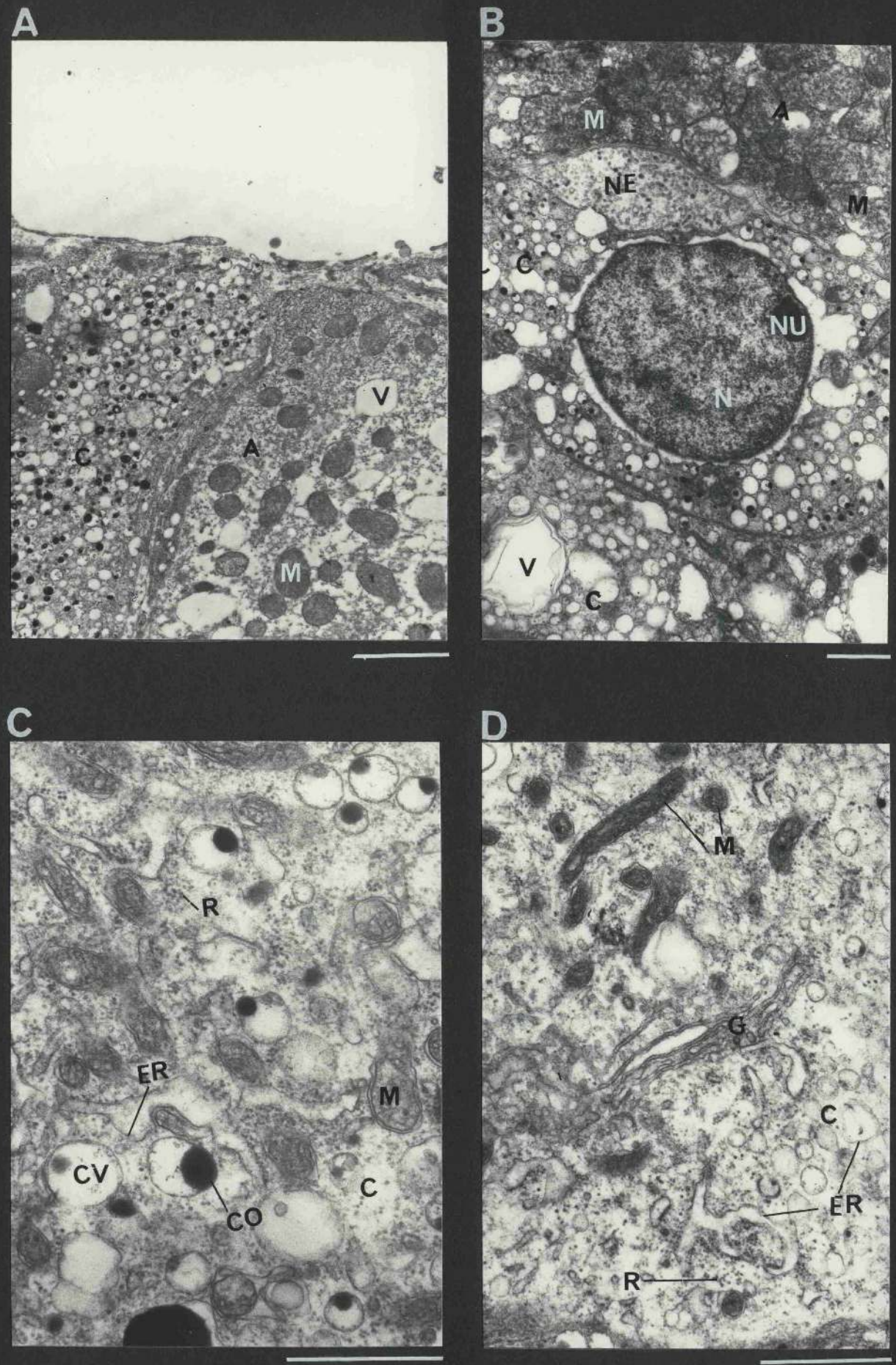
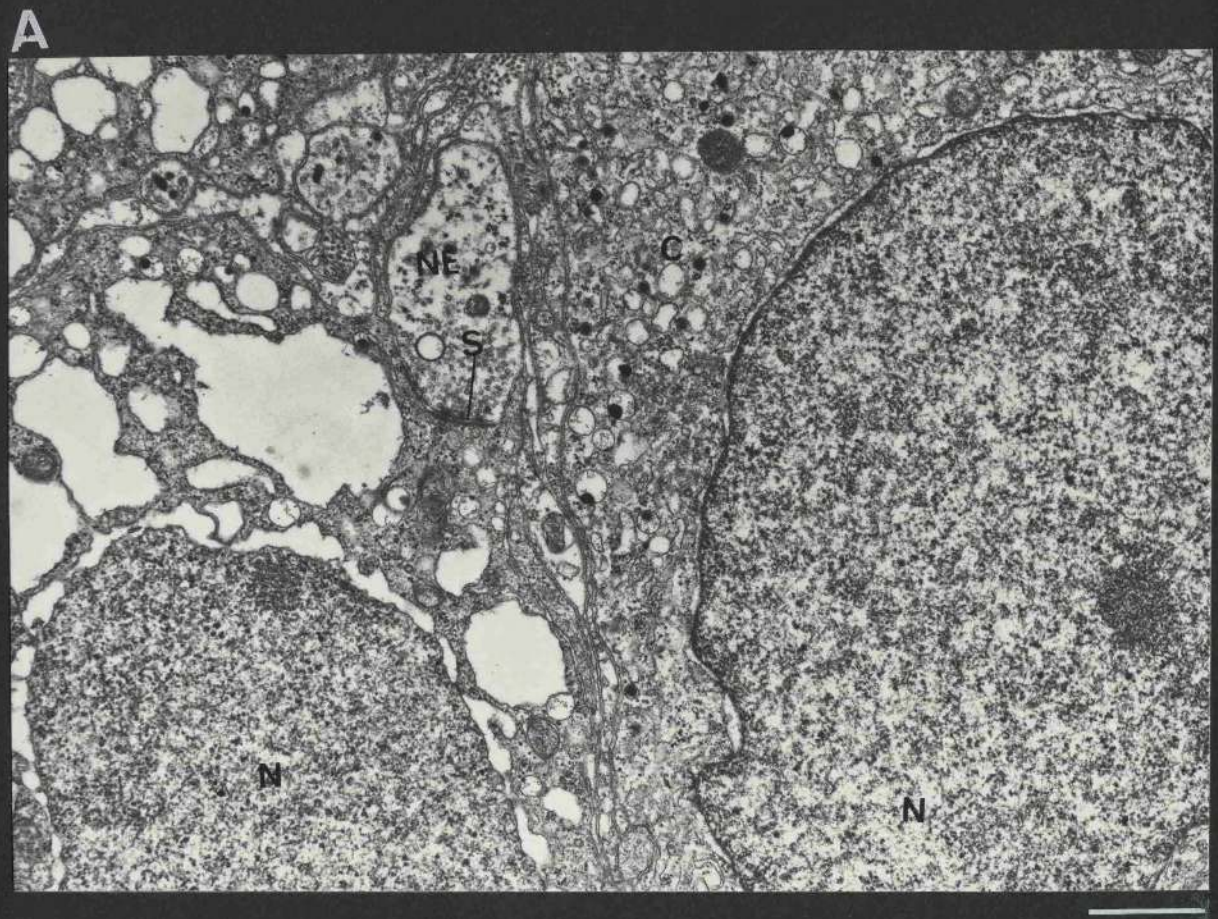

A
V
C
A
M
B
M
A
NE
M
C
NU
N
V
C
C
R
ER
M
CV
C
CO
D
M
G
C
ER
R

Plate 46. Salmo gairdnerii.

A. Chromaffin cell in posterior region of head-kidney, showing nucleus (right) with nucleolus. Nerve axons are associated with the cell on the left, and synaptic thickening is visible (v. Plates 51,52,53). Scale line corresponds to 0.8µm.

B. Chromaffin cells in posterior region of head-kidney, showing varying degrees of granulation of chromaffin vesicles. Nerve ending with synaptic thickening is visible on the right. Scale line corresponds to 0.8µm.


A
NE
S
C
N
N

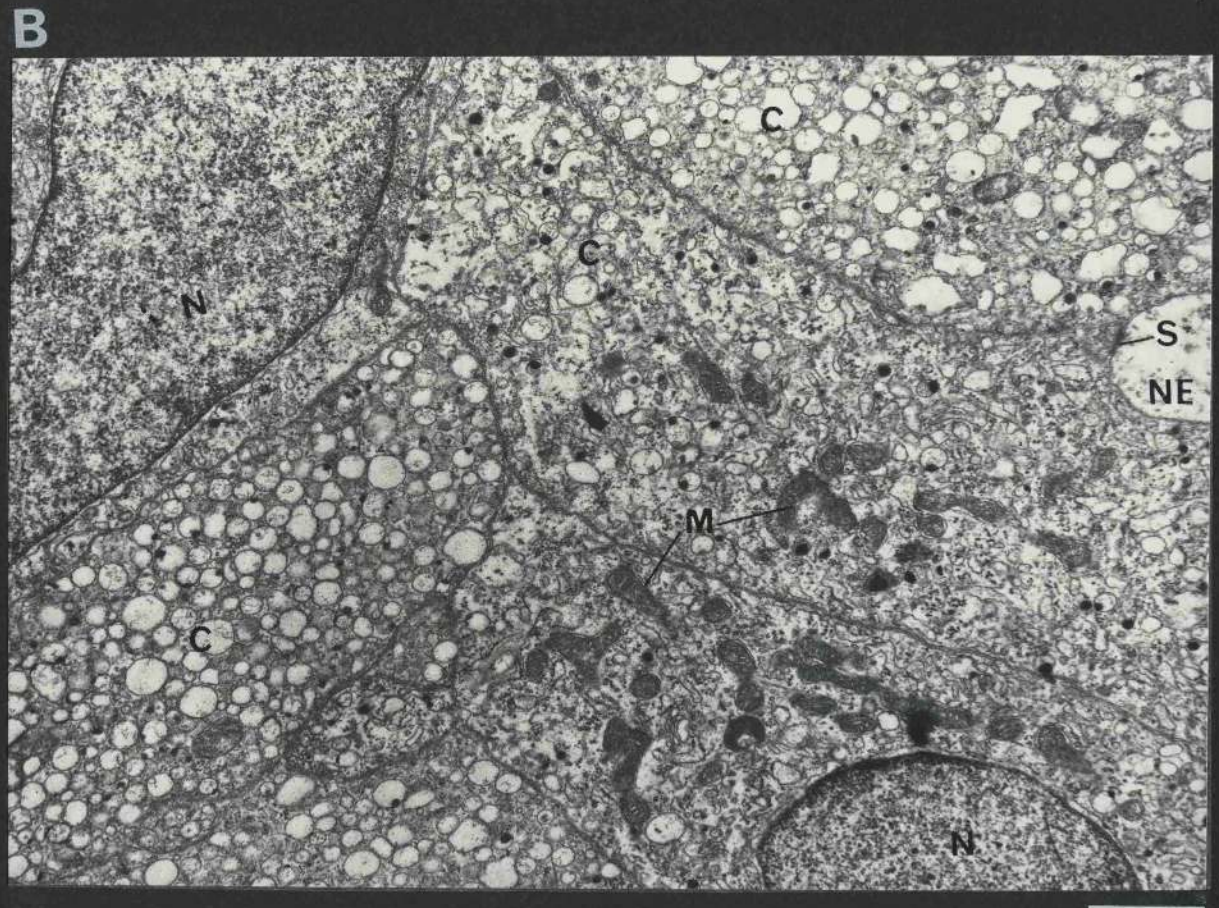

B
C
C
N
S
NE
M
C
N

Plate 47. *Salmo gairdnerii*.

A. Golgi complex in chromaffin cell. Scale line corresponds to 0.6µm.

B. Detail of chromaffin cell, showing mitochondria, groups of ribosomes and chromaffin vesicles with sparse granular contents. Scale line corresponds to 0.3µm.

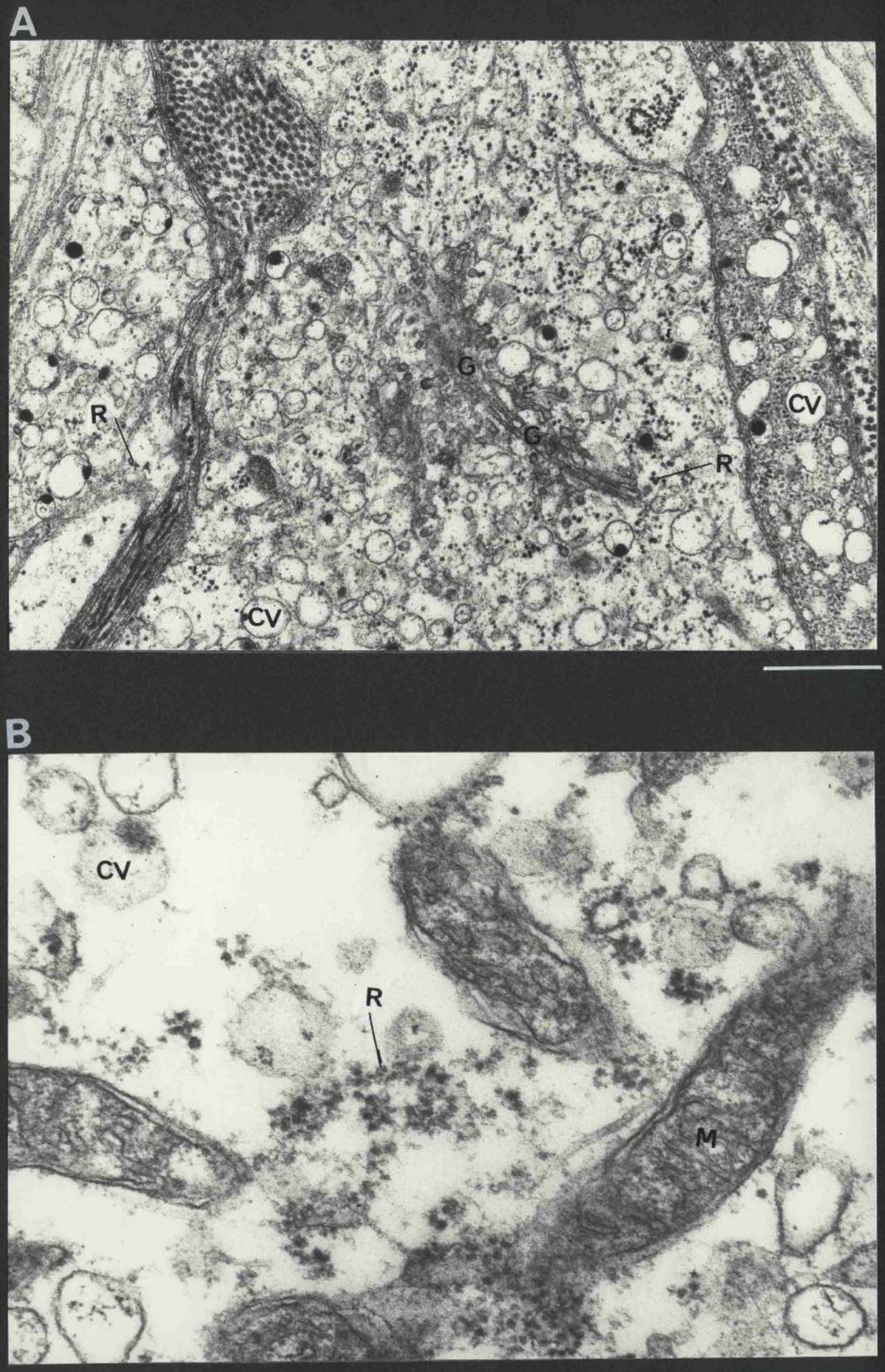
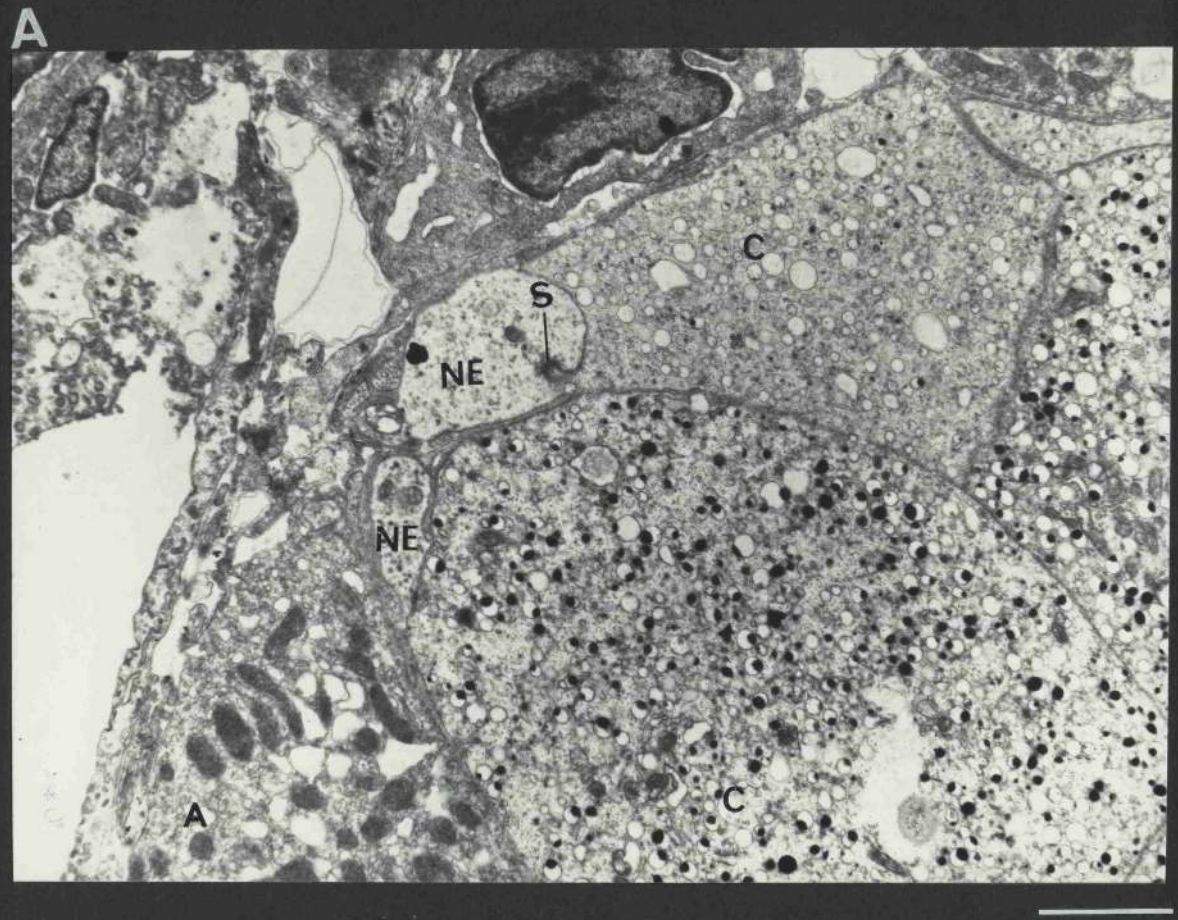

A
R
G
G
CV
R
CV
B
CV
R
M

Plate 48. Phoxinus phoxinus.

A. Cholinergic nerve axons synapsing on two adjacent chromaffin cells, one with heavily granulated vesicles, the other with empty vesicles. On the left is an adrenocortical cell. Scale line corresponds to 1.6µm.

B. Section close to (A). showing synaptic thickenings. Scale line corresponds to 1.3µm.

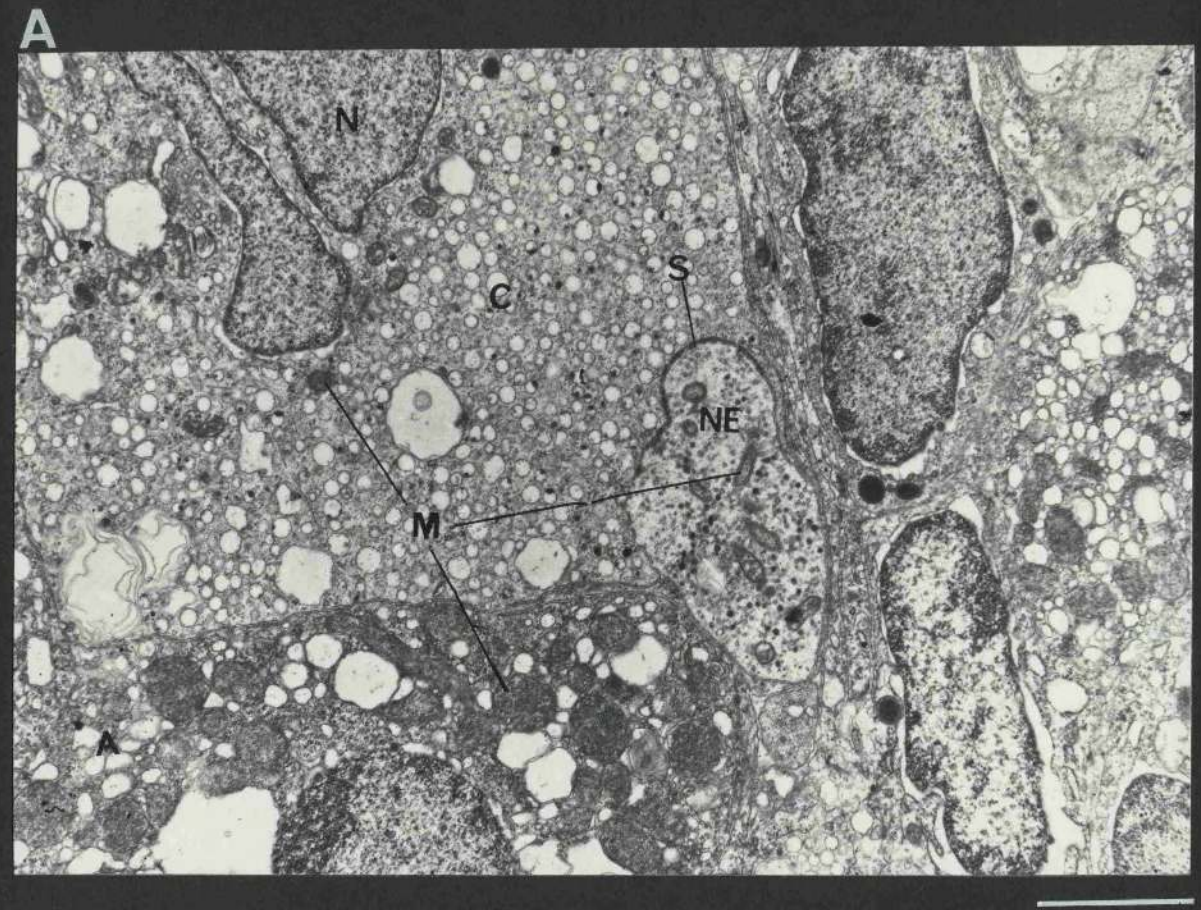

A
C
S
NE
NE
A
C


B
NE
A
NE
S
S
C
C

Plate 49. Phoxinus phoxinus.

A. Cholinergic nerve ending on chromaffin cell, showing synaptic thickenings and vesicles, and mitochondria in axon. Scale line corresponds to 1.6µm.

B. Cholinergic nerve ending on chromaffin cell. Synaptic thickenings are apparent both on the surface of the button, and also on two invaginations of the cell into the exon. Synaptic vesicles and larger, dense-cored vesicles are present. Scale line corresponds to 0.8µm.


A
N
C
S
NE
M
A


B
M
NE
A
V
S
V
CV
C

Plate 50. Phoxinus phoxinus.

A. Cholinergic nerve ending on chromaffin cell. Note synaptic thickenings on both surface of button, and on invaginations of cell into axon. Small synaptic vesicles and larger dense-cored vesicles are present. Scale line corresponds to 0.6µm.

B. A similar section to (A). Scale line corresponds to 0.4µm.

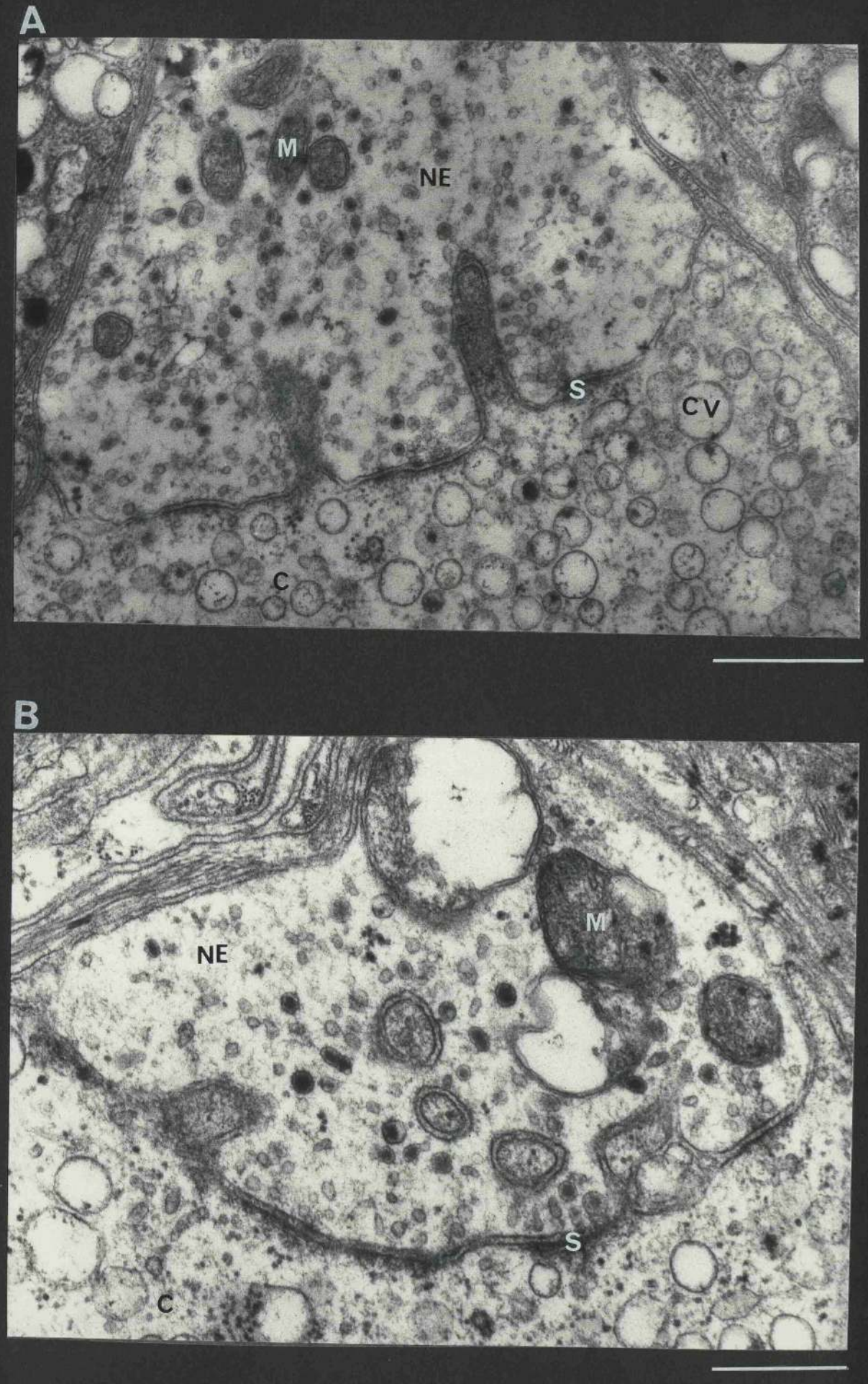

A
M
NE
S
CV
C
B
NE
M
S
C

Plate 51. *Salmo gairdnerii*. Section of chromaffin cells (bottom) adjacent to the connective tissue layer dividing them from the haemopoietic tissue (top). Abundant nerve fibres run in the connective tissue layer, and axons are visible synapsing with the chromaffin cells. Scale line corresponds to 2μm.

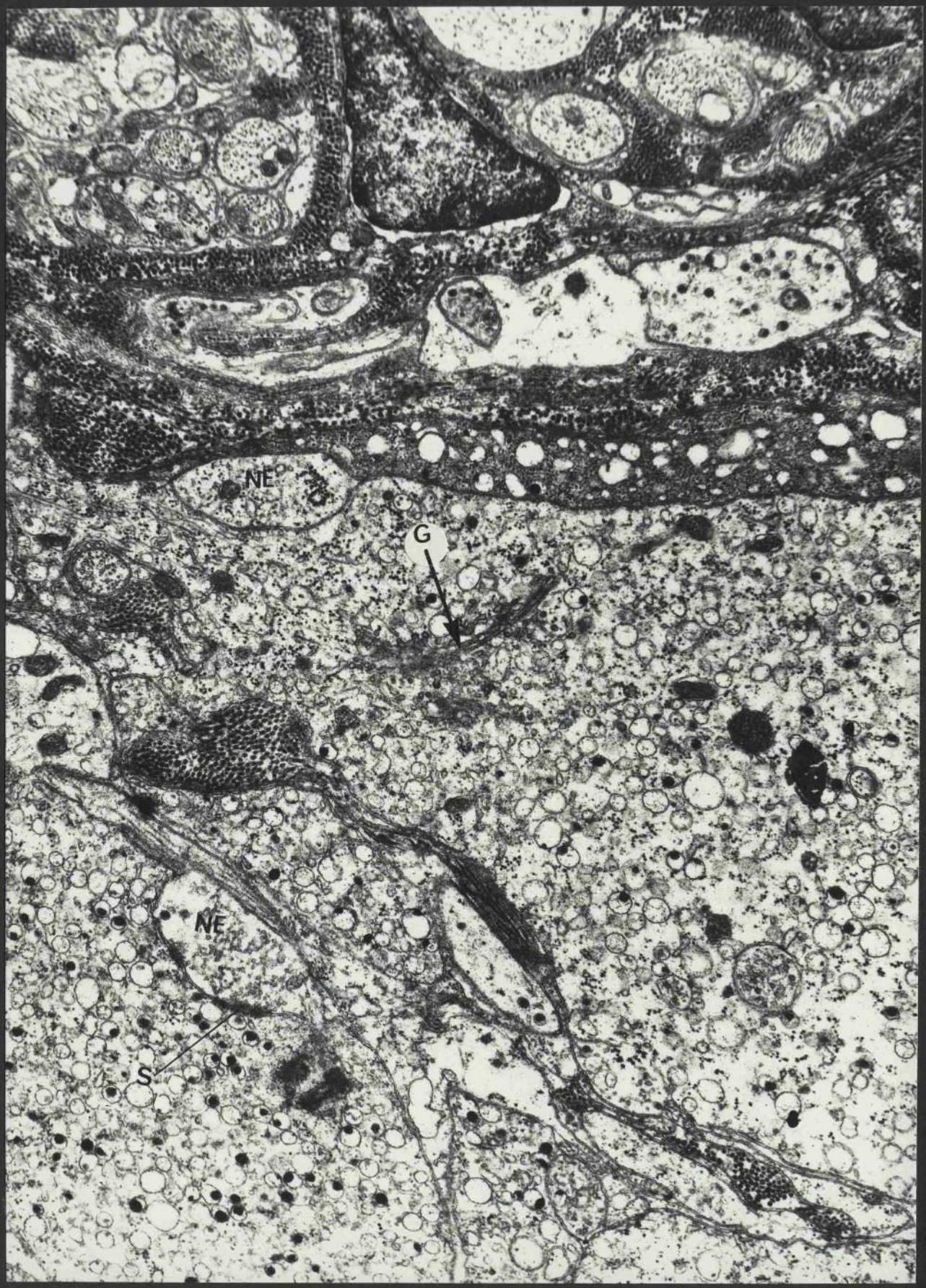

NE
G
NE
S

Plate 52. Salmo gairdnerii. Cholinergic synapses with chromaffin cells. Scale line corresponds to 1.3μm.

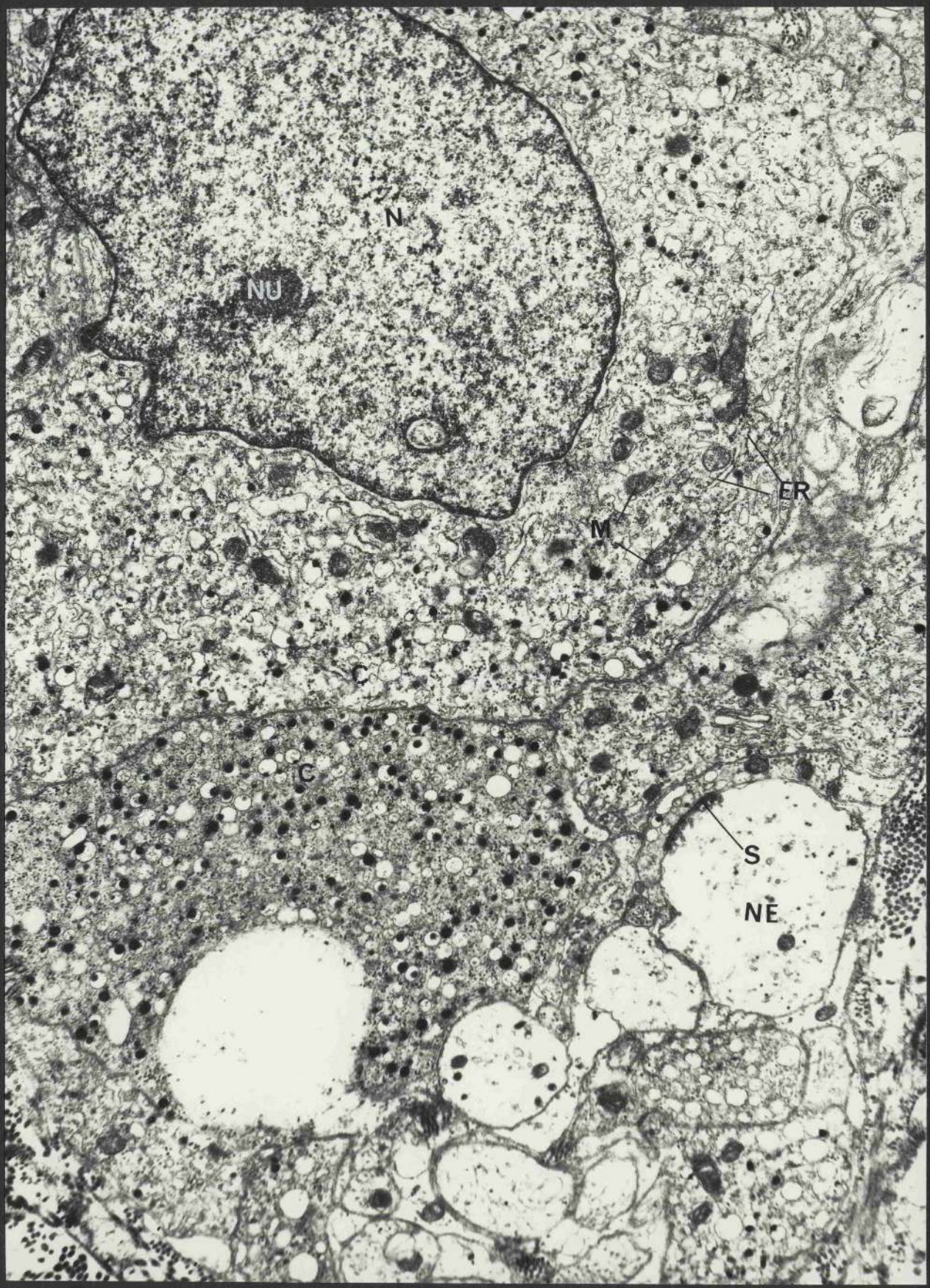

N
NU
ER
M
C
C
S
NE

Plate 53. Salmo gairdnerii. Detail of cholinergic nerve ending on chromaffin cell synaptic thickenings are visible, synaptic vesicles and large dense-cored vesicles. Scale line corresponds to 0.9µm.

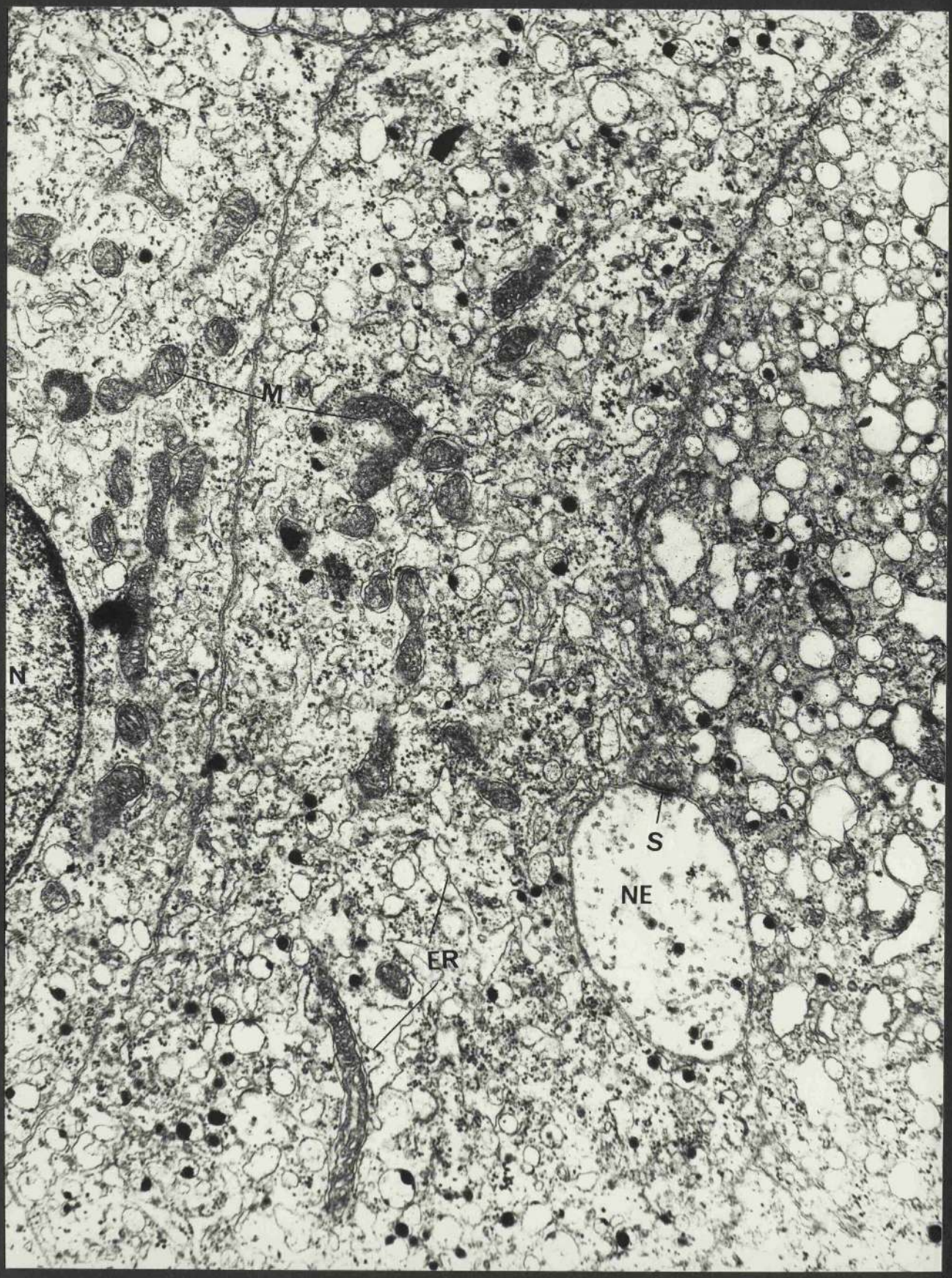
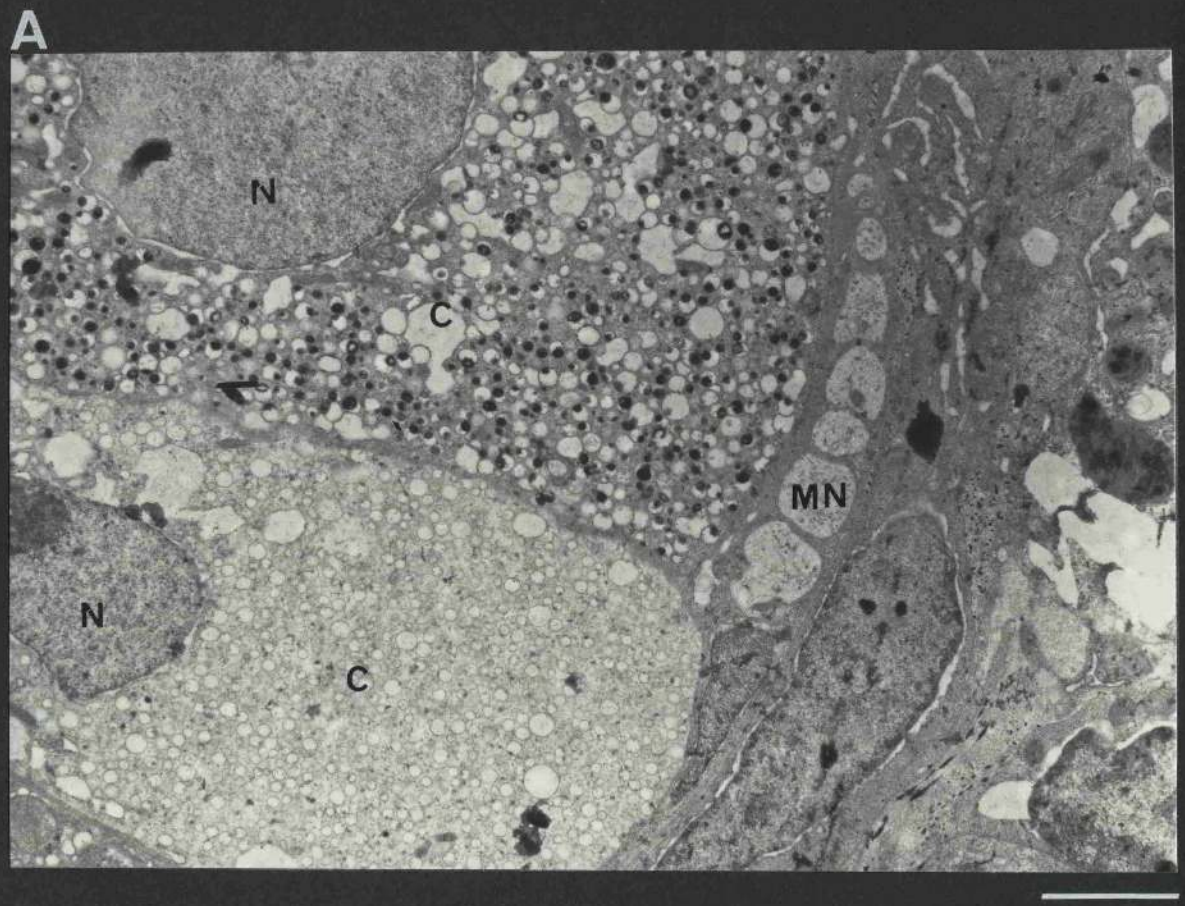

M
N
S
NE
ER

Plate 54. Phoxinus phoxinus.

(A) Section through two chromaffin cells at the junction with the haemopoietic tissue. A mylenated nerve fibre runs in the connective tissue, showing a series of varicosities. Note the contrast between the amount of electron-dense core in the chromaffin vesicles of the two chromaffin cells.
Scale line corresponds to 1.9 µm.

(B) A similar section, showing detail of nerve structure. Note multi-layered nature of nerve wall.
Scale line corresponds to 0.7 µm.


A
N
C
MN
N
C

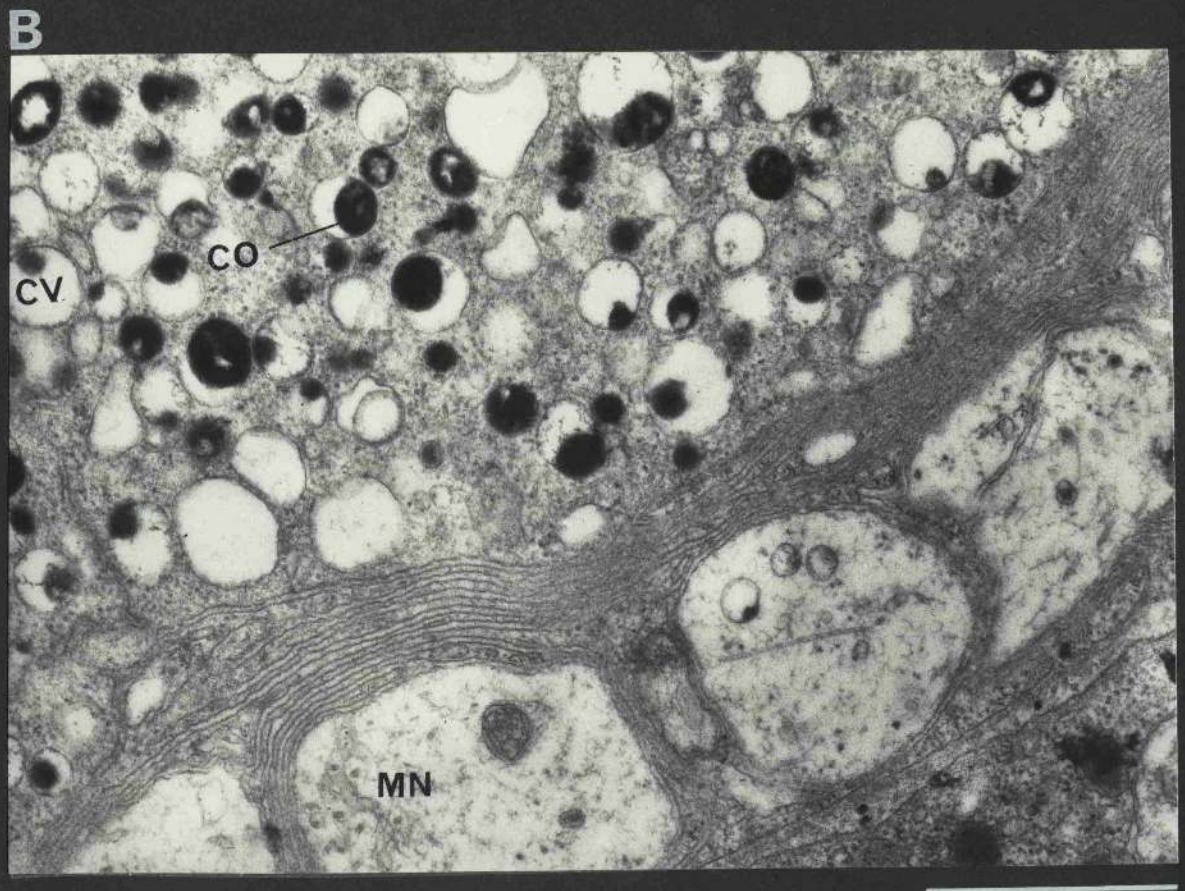
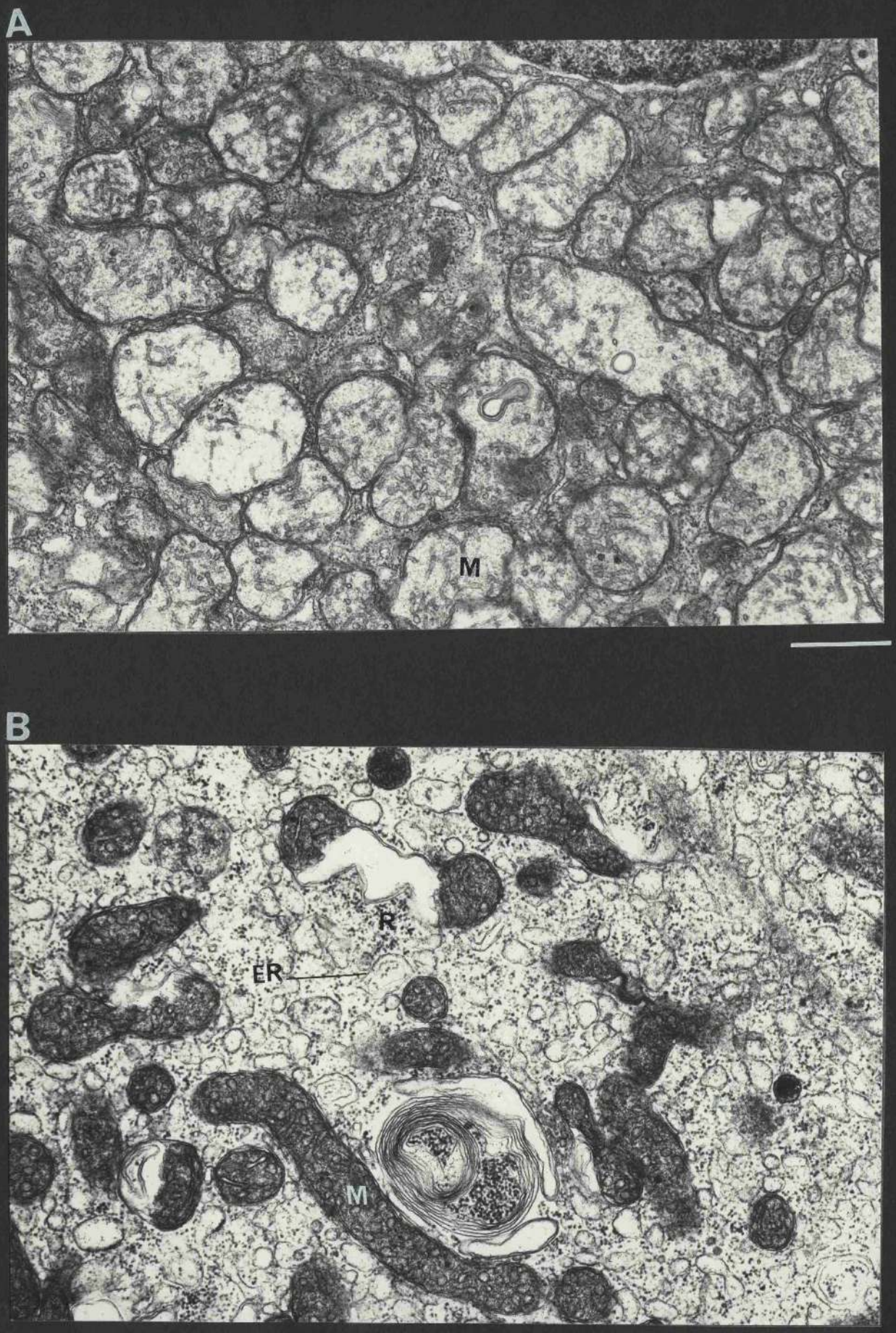

B
CO
CV
MN

Plate 55. Salmo gairdnerii.

A. Detail of adrenocortical cell, showing mitochondria with internal tubulo-vesicular structure breaking down. Scale line corresponds to 0.5µm.

B. Detail of adrenocortical cell, showing multi-layered circinate structure, dark mitochondria, ribosomes, and endoplasmic reticulum.. Scale line corresponds to 0.5µm.


A
M
B
R
ER
M