

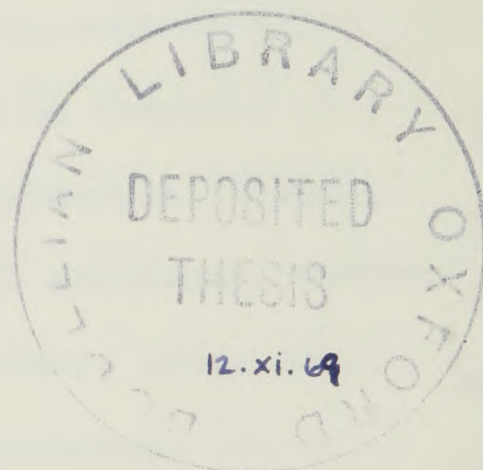
Contents

The Cytology of the Pronephros of Lampreys

by

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Trinity Term, 1969



A thesis submitted in fulfilment of the requirements of the
degree of Doctor of Philosophy in the University of Oxford.

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Abstract

The cytology of the pronephros of the three species of lamprey larvae, Lampetra planeri, L. fluviatilis and Petromyzon marinus, at the pronephric stage of excretion and during its subsequent degeneration, has been investigated with the optical and electron microscopes. The pronephros is a paired, segmental organ located in the cardiac region, each pronephros consisting of two functional units: a single large glomus and an aggregation of 3-8 tubules. Ultra-structurally, both have been found to be strikingly similar to their homologues in opisthonephric, mesonephric and metanephric kidneys.

The principal dissimilarities between the functional lamprey pronephros and the kidneys of the higher vertebrates are, in general, due to the less complex structure and arrangement of the cells and the tissue of the pronephros. The glomus is composed of the usual three cell types found in all vertebrate glomeruli, although in the glomus they exhibit a less complex internal structure. There is no juxtaglomerular apparatus in the glomerular artery, which arises from the dorsal aorta. Multiple filtration slit membranes are found between the foot processes of the epithelium, and the endothelial fenestrae lack membranes. There is no Bowman's capsule. Filtrate from the glomus passes into the coelom and coelomic fluid is collected by ciliated nephrostomes which lead to the tubular portion, which is enveloped by the anterior cardinal vein. The nephrostome is composed of an external funnel and a short, internal neck leading to the proximal tubule, which is the longest of the tubular sections and is

divided into two segments. The proximal tubule is connected to the shorter distal tubule by a short, undifferentiated intermediate segment. Each pronephros is drained by a duct which empties into the cloaca. The fine structure of the proximal and distal tubules, while exhibiting minor differences, is almost identical to the homologous tubules of the mammalian metanephros, but the tubules do not display the greater degree of subdivision found in other vertebrates, particularly mammals. The ultrastructure of the pronephric duct is very similar to that of the preceeding distal tubule.

Degeneration of the glomus begins in the latter part of the first year in all species. The capillaries become occluded with a dense, amorphous material and cellular material derived from proliferating endothelial and mesangial cells. The axial portion of the glomus increases in volume by further mesangial proliferation, and numerous reticular and collagenous fibres are deposited in the mesangial matrix. In the year before metamorphosis, the endothelial and mesangial cells begin to appear necrotic, but the epithelial cells are unaffected, and the glomus does not disappear entirely until metamorphosis.

The tubules begin to atrophy in the first year, the proximal tubules of the most anterior segments being the first to show signs of degeneration. Lysosome-like bodies appear in the cytoplasm, many cells rupture and the lumens become blocked with cellular debris and subsequently with invading macrophages. This occlusion is followed by a swelling of the tubules, phagocytic invasion increases, the

swollen tubules collapse and the remaining nephric cells are resorbed. The distal tubules and the tubules of the posterior segments follow in sequence, and by the last year of larval life all traces of the tubules have disappeared, except the nephrostomes which, although slightly altered, continue to function, passing coelomic fluid into the anterior cardinal vein. The pronephric duct usually persists in the undifferentiated region behind the pronephros, and can often be traced to the cardiac region in the adult.

The changes which occur with age, and the death and resorption of the cells of the pronephros, are shown to have several points of similarity with changes which occur with increasing age and pathological conditions in mammalian kidneys.

The only interspecific difference found between pronephroi of the three species studied occurs in the time of onset and the rate of tubular degeneration. In L.planeri, atrophy begins earlier and is completed sooner than in P.marinus; onset and completion of tubular degeneration in L.fluviatilis appears to be intermediate to the other two species. The possible reasons for this difference are discussed in relation to the life history of lampreys, and it is concluded that earlier and faster degeneration of the tubules is related to the trend towards neoteny in L.planeri.

Primary experimental evidence shows that the pronephros is capable of responding to changes in the osmotic pressure of the environment by adapting certain features of the tubular structure, principally the degree of lateral and basal interdigitation and the

size of the intercellular spaces, to facilitate increased or decreased water resorption.

The ultrastructure of the pronephros is discussed in relation to the ultrastructure of other kidneys, and pronephric function is considered, both by reference to the experimental results and by extrapolation from ultrastructural and functional studies of the kidneys of higher vertebrates.

This investigation shows that although there are minor differences the essential ultrastructural similarity between the pronephros and the opisthonephros, mesonephros and metanephros suggests that the former should not be regarded as the ancestral excretory organ of vertebrates. On the contrary, the evidence presented here indicates that the regions of the vertebrate excretory system are serially homologous. Support is thus given to the holonephric theory of the evolution of the kidney in vertebrates.

Acknowledgements

It is a pleasure to acknowledge the assistance and co-operation of many individuals during the course of this study. Miss Wendy Dear and Mrs. Elizabeth Williams assisted with histological work and Mr. James McCrae gave invaluable assistance with the electron microscopy. The study was carried out in the laboratories of the Department of Zoology at Oxford University, and thanks is due to Professor J.W.S. Pringle, F.R.S., and to many members of the staff of the Department for the provision of excellent facilities for teaching and research.

Several people advised me in the preparation of the study and in the collection of specimens in Britain: Dr. Quentin Bone, Marine Biological Laboratory, Plymouth; Mrs. R.P. Edwards, Department of Human Anatomy, Oxford; Mr. M.W. Hardisty, Bristol College of Science and Technology; Dr. Ronald Morris, University of Nottingham; Dr. Savile Bradbury of the Department of Human Anatomy, Oxford, made several helpful suggestions about the procedure of the study, electron microscopical techniques and the preparation of part of the manuscript.

I should like to thank Mr. John Youson of the University of Western Ontario for his many discussions about his studies of the lamprey opisthonephros. His research supervisor, and my former teacher, Dr. D.B. McMillan, provided me with research facilities for part of the study and gave invaluable advice and encouragement.

I am also indebted to my parents and to my uncle, Mr. C.S. Campbell, Q.C., for their encouragement and financial assistance.

INTRODUCTION

Miss Christine Court assisted with the preparations of the text-figures, which were photographed by Mr. John Haywood.

Finally, it is a pleasure to acknowledge the guidance of my supervisor, Dr. John R. Baker, F.R.S., for his advice, criticism and unflagging enthusiasm throughout the course of this investigation.

Introduction

At the turn of the century, two conflicting views had been proposed to account for the phylogenetic origin of the vertebrate kidney. One of these held that the vertebrates had developed three separate distinct adult kidneys, the pronephros, mesonephros and metanephros, and that this evolutionary history was recapitulated in the embryonic development of the amniotes. The second theory proposed that the pro-, meso- and metanephroi were regional specializations of an ancestral vertebrate. This latter came to be known as the archinephric (Lankester, 1877) or holonephric (Price, 1897) theory. The degree of serial homology in the vertebrate excretory system was obviously a basic question which would help to decide which theory was the more plausible, and with the accumulation of embryological and anatomical evidence showing similarities between the three kidneys, it became increasingly evident that the holonephric theory of vertebrate kidney evolution was the more reasonable explanation. However, most of the early evidence supporting the holonephric theory showed similarities between the meso- and metanephros and, as Goodrich pointed out in 1930, the relationship between the pro- and mesonephros was still uncertain. More recent evidence, mostly from experimental embryology (summarized by Fraser, 1950 and Fox, 1963) emphasizes the essential unity of nephrogenic material, indicating that the pronephric zone has basically the same potential as that which lies posterior to it.

In the past 15 years, as the electron microscope has become an increasingly common tool in studies of development, structure and

function, further support for the holonephric theory has come from studies which have shown that the cellular components of kidneys from a wide variety of vertebrates are essentially the same as those of the mammalian metanephros, by far the most-studied kidney. Although there are obvious inadequacies in generalizations based on the observations of a few widely-scattered types of kidneys, there is nonetheless a fairly accurate picture available of the origin and structure of the vertebrate excretory system, with two notable exceptions: first, there are still many wide gaps in our knowledge, particularly among the lower classes of vertebrates such as the cyclostomes; second, the pronephros has been almost completely ignored in recent years, there having been only one ultrastructural study published, a study of the pronephros of Ambystoma, (Christensen, 1964).

The nephron, the basic unit of the vertebrate kidney is found, in one form or another, in all vertebrates, from fossil imprints of ostracoderms to living mammals. The closest living relatives of the ostracoderms are the agnathans, and they, especially the larval petromyzonts, are the most generalized and simplest living vertebrates, in spite of their specialized and degenerate features. In view of this, and of the close evolutionary relationship between the kidneys of the lower and higher vertebrates (Fraser, 1950; Torrey 1965), it is surprising that so little attention has been paid to the excretory organs of the cyclostomes, and that no ultrastructural studies have been published.

The pronephros of the lamprey was first described by Rathke in 1827. In the latter half of the 19th century, there were numerous studies of the excretory system of lampreys, mostly by German workers. These have been reviewed by Wheeler (1899), whose work on the urogenital system of the lamprey is still the major source of information, and is the standard textbook reference for descriptions of the vertebrate pronephros. However, his work, along with those that preceded it, and that of Hatta (1900) is primarily an embryological study, with scant attention paid to the functional pronephros of the ammocoete. Two other studies have been made of the lamprey pronephros since that time. The first (Inukai, 1930) is a histological study of the pronephros of the young ammocoete, but which contains little worthwhile information. The second is a brief functional study (Torrey, 1938), which presents data on some pronephric activities and includes some incidental morphological information. It was hoped that a further study of the pronephros of the lamprey, during the post-embryonic larval period, would both present a more complete record of the life history of the organ and also clarify some of the more obscure and contradictory points raised by the studies of earlier workers. At the same time, an ultrastructural study should indicate whether or not the components of the pronephros reflected the assumptions of the holonephric theory.

This study deals with three species of lamprey commonly found in Britain: Lampetra planeri, Lampetra fluviatilis and Petromyzon marinus. The two species of the genus Lampetra form a pair of

sibling species (Hardisty, 1963), one of which (planeri) attains sexual maturity within a few months of metamorphosis, having eliminated the adult migratory and feeding phases from its life cycle, and spends its entire life in fresh water, while the other (fluviatilis) has a lengthy marine parasitic phase. P. marinus is also an anadromous lamprey, although certain landlocked populations have established an entirely fresh water life cycle, whose parasitic phase may be somewhat shorter than that of the marine parasites; the landlocked lampreys especially those of North America, are often referred to as dwarf P. marinus. All three species have a similar life history in most respects: sexually mature adults spawn in fresh water, in spring or early summer, and the larvae spend up to 5½ years before metamorphosis in the streams in which they were spawned. The pronephros appears at an early stage of embryonic development (Wheeler, 1899; Hatta, 1900), and persists at least until metamorphosis (Müller, 1875; Furbinger, 1878, Bujor, 1891). On hatching, the ammocoete is approximately 3mm. in length (Hatta, 1900); the first appearance of the definitive adult kidney occurs at 12-17mm. in length (Wheeler, 1899; Inukai, 1930). Following this period, the pronephros begins to atrophy slowly. Thus, for the purposes of this study, the life history of the pronephros can be divided into two stages: the functional stage, during which it is the sole nephric excretory organ, and the degenerative stage, during which the organ may continue to perform some functions, but is undergoing resorption.

The pronephros is composed of two separate functional units, the glomus and the tubules. In the following chapters their components, in the functional pronephros and in the degenerative phase, are described separately. In the final chapter, functional considerations of the pronephros are discussed, and their structural and functional aspects considered in terms of evolutionary biology.

Lepomis gibbosus

River Tame, Ludlow, Shropshire.

River Tamar, near Looe, Cornwall.

River Windrush, near Woodstock, Oxfordshire.

Lepomis fluviatilis

River Kenn, near Exeter, Devonshire.

Exocoetis nasutus

River Tame, near Exeter, Devonshire.

Unidentified specimens

Stange Creek, Fort Stevens, Nevada (June 1900).

Trick's Creek, near Dayton, Nevada (June 1900).

These specimens provided no material, when kept for the purpose of the examination of the pronephros, but were used for the examination of the pronephros. The pronephros of the pronephros was found to be composed of two separate functional units, the glomus and the tubules. In the following chapters their components, in the functional pronephros and in the degenerative phase, are described separately. In the final chapter, functional considerations of the pronephros are discussed, and their structural and functional aspects considered in terms of evolutionary biology.

Some specimens of *Lepomis fluviatilis* and *Lepomis gibbosus* were examined for the purpose of the examination of the pronephros. The pronephros of the pronephros was found to be composed of two separate functional units, the glomus and the tubules. In the following chapters their components, in the functional pronephros and in the degenerative phase, are described separately. In the final chapter, functional considerations of the pronephros are discussed, and their structural and functional aspects considered in terms of evolutionary biology.

Methods and Materials

Collection of Specimens

The animals used in this study were collected either with the use of an electric shocker (Tibbles, 1961) or by digging. After capture, the live ammocoetes were transported to the laboratory, where some were fixed immediately and other held in aquaria until needed.

Lampetra planeri:

River Teme, Ludlow, Shropshire.

River Tamar, near Launceston, Cornwall.

River Windrush, near Woodstock, Oxfordshire.

Lampetra fluviatilis:

River Kenn, near Exeter, Devonshire.

Petromyzon marinus:

River Kenn, near Exeter, Devonshire.

Landlocked specimens:

Youngs Creek, Port Revere, Ontario (Lake Erie).

Trick's Creek, near Bayfield, Ontario (Lake Huron).

Some specimens preserved in formalin, taken from the collection of the Department of Zoology, Oxford, were used for dissection, principally for tracing the course of the pronephric duct in larger specimens of L. planeri and P. marinus. The source of these specimens is unknown.

Adult specimens of L. fluviatilis and P. marinus were obtained from pre-spawning and spawning runs on the River Severn with the

assistance of Mr. Philip Gaskin.

Optical Microscopy

The larvae were anaesthetized in 5% urethane, measured for total body length from the tip of the oral hood to the tip of the tail, and fixed in toto. Following fixation and dehydration, specimens were embedded in paraffin and sectioned serially. Fixatives and stains used are noted in Appendix 1a.

Electron Microscopy

Animals were not anaesthetized, but decapitated, measured and fixed immediately. Very small animals were fixed in toto, others, from 15-25mm, were cut into thin sections with a razor blade before fixation. Large animals were dissected in cold physiological saline following decapitation, the pronephroi removed and placed in cold fixative. Following fixation and dehydration, the specimens were embedded in Araldite (Luft, 1961). Thin sections, silver or silver-grey, were cut on either a Huxley ultramicrotome or an LKB Ultratome, mounted on uncoated copper or nickel grids, stained with heavy metals and examined with an Akashi Transcope (50 kV) and an AEI EM6B (60 kV). Fixatives and stains are described in Appendix 1b and discussed in Appendix 1c.

Reconstructions

In order to understand more thoroughly the arrangement of the pronephric tubules, reconstructions (Pusey, 1939) were made from serial sections of L.planeri and P.marinus larvae of 15-25mm. Measurements of tubular diameters and lengths were made from these

The Glomus of the Functional Pronephros

Introduction

The mammalian metanephric glomerulus, its differentiation, development and structure in health and disease, have been the subjects of many recent optical and electron microscopical studies. There are several reviews and summaries of this work (Mueller, 1958; Kurtz and McManus, 1959; Rhodin, 1963; Kurtz, 1964; Jørgensen, 1966). The ultra-structure of the mammalian mesonephros has also been investigated (Leeson, 1960; De Martino and Zamboni, 1966), but non-mammalian glomeruli have, with a few exceptions, been almost entirely ignored (Pak Poy and Robertson, 1957; Pak Poy, 1957a, b, 1958a, 1959; Anderson, 1960; Christensen, 1964).

The pronephric glomus,¹ in vertebrates other than the lamprey, has been studied by several authors (Mibayashi, 1928; Strüer, 1932; Holmgren, 1950; Christensen, 1964; Dornesco and Marco, 1964) but there has been only one study which has used the electron microscope (Christensen, 1964). The pronephric glomus of the lamprey, particularly its development, has been described briefly in the two principal studies of the pronephros (Wheeler, 1899; Hatta, 1900). Inukai (1930), in the only major non-embryological study of this organ, presents a brief histological description

1. Several terms used in this study, such as glomus, have not been carefully defined by other authors, or are used interchangeably incorrectly (such as glomus and glomerulus), often causing considerable confusion. Terms which might be unfamiliar, or whose use is frequently ambiguous, have been defined and discussed in Appendix 4.

of the glomus, as does Bargmann (1933) in a comparative study of vertebrate glomeruli. In most respects, the glomus of the lamprey pronephros has not been described in detail; thus its degree of similarity to other vertebrate glomeruli is not known.

Results.

No differences, histological or cytological, were detected between the glomera of the species studied. In the description which follows, therefore, the different species are not distinguished.

There are two glomera, left and right, located on the ventro-medial surface of the anterior cardinal vein, between the second and third pairs of pronephric nephrostomes. The glomer blood supply is via an arteriole which branches from the dorsal aorta; blood from the glomus passes through capillaries into the anterior cardinal vein, with no intervening venule apparent. The general topography is shown in Figs. 1a and b and in Text-Fig. 1. The glomus is a lobed organ, of capillary loops and anastomoses, covered by visceral epithelium (Figs. 2, 3). There is no evidence of any juxtaglomerular apparatus in the arteriole supplying the glomus. The ultrastructure of the components of the glomus is described below.

Capillary endothelial cells. The most prominent feature of these cells is the large cell body, containing the nucleus, which projects into the capillary lumen on the axial side of the lumen (Fig. 4). The nucleus is large, notched and nucleolated, although no nucleolus is shown in

Text-Figure 1. A diagrammatic saggital section through the pronephric region of a first year ammocoete, illustrating the anatomy of the cardiac region.

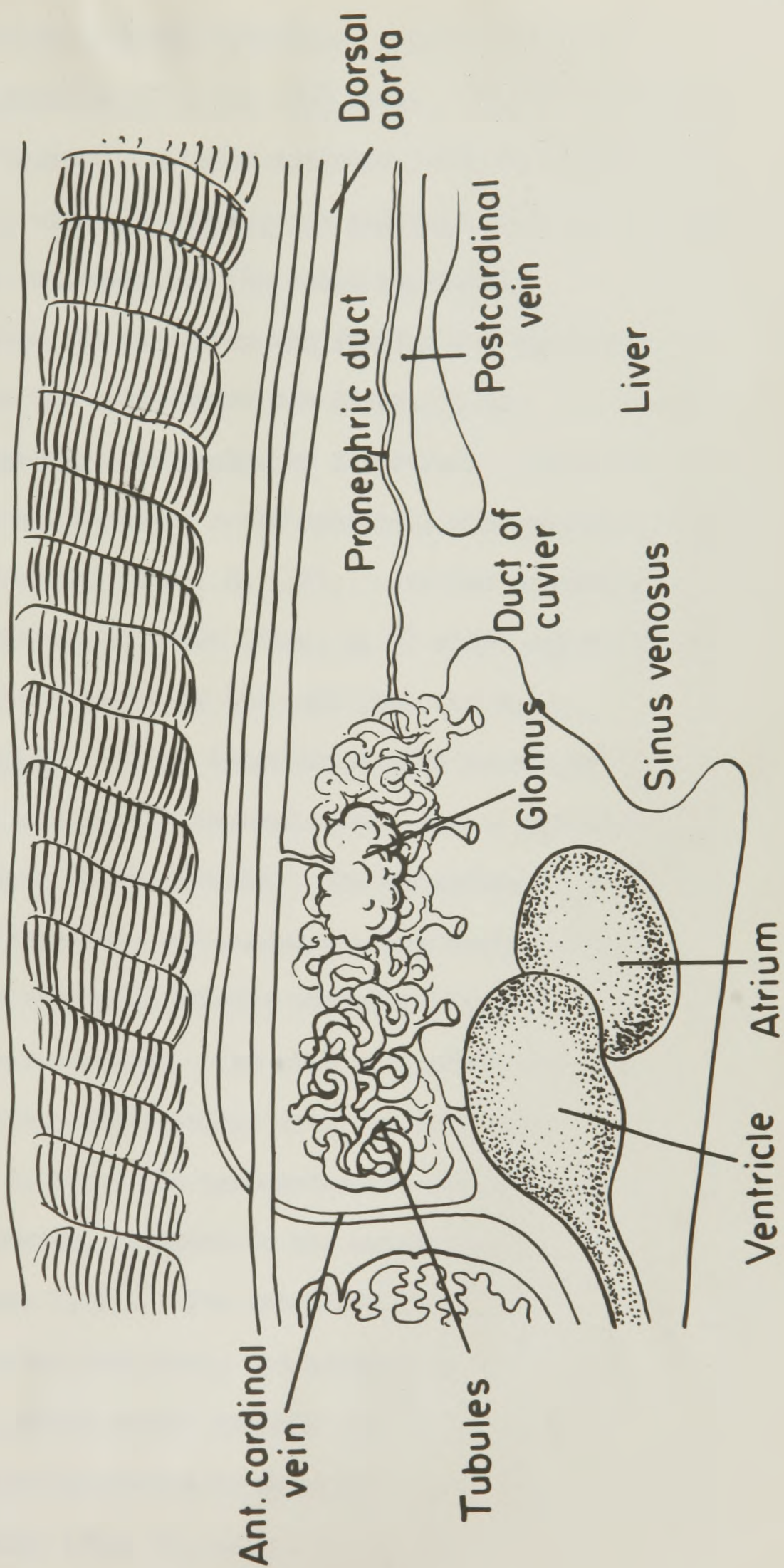


Fig. 4, and is bounded by a double membrane. Small spherical mitochondria are sometimes seen in the cell body. Other organelles, occasional circular vesicles and small vesicles, oval to tubular in shape, are bounded by a single membrane, are possibly part of the ergastoplasm (endoplasmic reticulum) or the Golgi apparatus.

Extending from the cell body and forming the periphery of the capillary lumen is the cytoplasmic rim (Figs. 3, 4). It varies in thickness and is frequently interrupted by fenestrae. Like the cell body, the cytoplasmic rim contains a few spherical mitochondria, vacuoles and smaller tubular vesicles (Figs. 3, 4, 7). Another occasional feature is the presence of cytoplasmic blebs (Figs. 4, 7) which appear to be small bits of cytoplasm extruded by the cell into the lumen.

Visceral epithelial cells. These large cells with prominent, often indented, nucleolated nuclei with characteristic double membranes, form the outer (coelomic) limit of the glomerulus. The cytoplasm in the region of the nucleus is not abundant, and contains a few small, spherical mitochondria, vacuoles and small tubular vesicles (Fig. 3). Cytoplasmic blebs are also seen occasionally. The cell body gives rise to long processes, the trabeculae, which extend from the cell body towards the capillaries (Figs. 3, 5, 6). The trabeculae are usually subdivided into short, broad foot processes, attached to the outer layer of the capillary basement membrane (Figs. 7, 8). The cytoplasm of the trabeculae contains the same organelles as the cell body, and contains as well bundles of fine fibrils (Fig. 6), which do not exhibit any transverse periodicity. The cytoplasm of the foot processes is vacuolated and contains the smaller vesicles noted previously (Fig. 7), and may also contain fine fibrils.

The foot processes appear to interdigitate with the foot processes of the adjacent trabeculae (Fig. 6). The spaces between the foot processes, the filtration slits, are bridged by one to several membranes, 7 μ thick, the filtration slit membranes (Figs. 5-8). The cytoplasmic membranes, and perhaps the cytoplasm, of the foot processes is more dense in the region of the attachment of the filtration slit membranes (Figs. 7, 8). In some regions, the trabeculae are not subdivided into foot processes but are directly attached to the basement membrane (Figs. 3, 5, 6). Occasionally the cell bodies exhibit a similar attachment (Fig. 3).

Basement membrane. The basement membrane lies in the subendothelial space, between the endothelial cytoplasmic rim of the capillary and the point of attachment of the epithelial foot processes (Figs. 3, 5, 7, 8). It is a continuous, uniformly electron dense structure (the lamina densa of Hall, 1955), with an average thickness of 2,75 μ . On either side it is bounded by less electron-dense layers (the lamina rara interna and lamina rara externa of Yamada, 1955), which mark the attachment of the adjacent endothelial and epithelial cells to the dense component. In some areas, large spaces are seen between the basement membrane and the endothelial cytoplasmic rim (Fig. 9), possibly pericapillary spaces or perhaps the result of mechanical separation of the tissues during processing. There is no evidence, at high magnification, of any fine fibrillar structure of the basement membrane.

In thin sections stained by the PAS technique (McManus and Henry, 1960), the basement membrane is shown to be continuous with the basement membrane of the glomerular arteriole (Fig. 2) and with the basement

membrane of the anterior cardinal vein (Fig. 10). Electron microscopical evidence of the former is lacking, but Fig. 11 shows the continuity in the latter case.

Mesangial cells (Intercapillary cells). These cells, which are not numerous, are located on the axial sides of the capillaries, near their points of apposition (Figs. 2, 3). Where they are not in contact with the capillaries, they border on the coelom and are covered by the epithelium. Their nuclei are large, irregular in shape, nucleolated and bounded by a double membrane (Fig. 12). The cytoplasm, which is not abundant, contains a few small spherical mitochondria, vesicles of varying size and a prominent Golgi apparatus, located in an indentation of the nucleus. The basement membrane, which covers part of the mesangial cell, is continuous with the capillary basement membrane, and is interposed between the mesangial cell and its epithelial covering. It is not present in those regions in which the mesangial cell is adjacent to epithelial cells. In these regions, the two cells types are separated by a space containing an amorphous substance of low electron density, similar to the internal pale layer of the basement membrane or to the mesangial matrix. The matrix is most evident in those regions in which it separates the mesangial cell from the basement membrane. The mesangial matrix is a pale, amorphous substance which surrounds the mesangial cell body. The cell gives rise to pseudopodal extensions (Fig. 12) which are directed towards the capillaries and may penetrate the subendothelial space, separating the endothelium from the basement membrane. Smaller, shorter processes also extend from the cell body which in section appear to be independent

the basement membrane seems to be such were applied to the section planes. Most of this complexity is due to the presence of numerous

islands of cytoplasm in the mesangial matrix.

Pericapillary space. Evidence for the presence of pericapillary spaces in the glomus is not conclusive. It is possible that the space shown in Fig. 9 is a pericapillary space, but it could also be a mechanical artifact. Such spaces are rare in the glomus; if they are true pericapillary spaces, they contain no cytoplasmic material.

Discussion.

In most respects the pronephric glomus is remarkably similar to the glomeruli of mesonephric and metanephric kidneys (Pak Poy and Robertson, 1957; Pak Poy, 1957a, b, 1958a, 1959; Anderson, 1960; Rhodin, 1963; Christensen, 1964; Jørgensen, 1966). The principal differences, although minor ones, are these: there is no evidence of any juxtaglomerular apparatus; the internal organization of the glomer cells appear to be less complex, the organelles less well-defined; the mesangium is less well-developed; the foot processes are less specialized in their attachment to the basement membrane, and the slits between them are often bridged by multiple rather than by single membranes; there are no fenestral membranes in the endothelium. These dissimilarities, and possible reasons for them, are discussed below, but the most important feature of the glomus is its similarity to the glomeruli of higher vertebrates.

In the only electron microscopical study of a pronephros that has been published, Christensen (1964) presents two electron micrographs of the glomus of Ambystoma. Its general organization appears to be the same as that of the lamprey, although the composition of the region of the basement membrane seems to be much more complex in the amphibian glomus. Most of this complexity is due to the presence of numerous

pericapillary processes (probably the mesangial processes of this study), although Christensen makes no reference to the presence of mesangial cells. The basement membrane appears more tortuous and the endothelium less discrete; the foot processes are not clear, and it is not certain that filtration slit membranes are present. However, it seems likely that a more detailed investigation of the glomus of Ambystoma would reveal a much greater similarity to the lamprey pronephros than is afforded by Christensen's figures.

Juxtaglomerular apparatus. The absence of any detectable structure corresponding to the mammalian juxtaglomerular apparatus is not surprising, in the light of other investigations. It has been reported that it is not present in the glomeruli of teleosts, amphibians, reptiles, birds or marsupials (Pak Poy and Robertson, 1957; Pak Poy, 1957a, b, 1968a, 1959; Anderson, 1960), nor does it appear in the human mesonephros (De Martino and Zamboni, 1966). It seems possible that this absence, at least in some cases, is due to incomplete investigation, as Smith (1966) reports the presence of a juxtaglomerular apparatus in the chicken and the pigeon. The circulatory system of the lamprey probably operates at a low pressure, as it does in other lower vertebrates (Woodbury and Hamilton, 1937), and this may preclude the need to have intimate control of the filtration pressure. The arteriole which supplies the pronephric glomus of the carp is reported to have a valve which controls the entry of blood from the dorsal aorta (Dornesco and Marco, 1964), but there is none in the lamprey glomerular arteriole. Young (1950) maintains that the absence of a valve in renal arteries allows the maintenance of a high filtration pressure.

Endothelial cells. The organelles in these cells are more widely distributed and less well-defined than those in glomeruli. Mitochondria occur in the cytoplasmic rim, apparently unique to the glomus; the Golgi bodies and ergastoplasm which occur in the cell bodies of mammalian glomerular endothelium have not been seen in the glomus. The small tubular vesicles, which are not localized but occur throughout the cytoplasm, may be substitutes for these organelles. Vacuolated cytoplasm seems to be characteristic of endothelia (Bloom and Fawcett, 1962; Rhodin, 1962, 1963); fenestrae occur in all vertebrate glomerular endothelia (Pak Poy and Robertson, 1957; Pak Poy, 1957a, b, 1958a, 1959; Rhodin, 1963), and occur in other endothelia as well (Bloom and Fawcett, 1962). The fenestral membrane of the mammalian glomerulus (Rhodin, 1962, 1963) seems not to occur in the glomus; it is not clear from the published electron micrographs of other authors (Pak Poy and Robertson, 1957; Pak Poy, 1957a, b, 1958a, 1959; Christensen, 1964) whether the membranes occur in other non-mammalian vertebrates. Occasionally there appears to be a diffuse structure bridging a fenestra (Fig. 8), but it is likely that this is a junction between two endothelial cells rather than a fenestral membrane (Farquhar et al., 1961).

Epithelial cells. These cells appear to be the most similar to their glomerular counterparts. There are only three minor differences. First, the high degree of organization reported in mammalian glomerular epithelia (Farquhar et al., 1961; Rhodin, 1963; Kurtz, 1964) is lacking in the glomus. The only obvious organelles are a few small mitochondria, distributed throughout the cell body and trabeculae, and vesicles of

two sizes. The larger vesicles, which appear to originate in the regions apposed to the basement membrane, are presumably pinocytotic vacuoles which correspond to those reported in mammals (Farquhar et al, 1961; Elias et al, 1965). The other, smaller vesicles, which occur throughout the cytoplasm, may represent in the glomus the well-developed Golgi apparatus and ergastoplasm occurring in glomeruli (Farquhar et al, 1961). Although the cytoplasmic fibrils in the glomus do not appear to be as numerous or as widely distributed as fibrils in the mammalian glomerular epithelium (Kurtz, 1964), the occurrence of fibrillar bundles (Fig. 6) seems to be exclusively glomar. While foot processes of the mammalian epithelium are broad-based at their point of attachment, tapering toward the trabeculae, the glomar foot processes are of uniform shape, perhaps a less-specialized mode of attachment.

Second, the filtration slit membrane (Yamada, 1955), which appears to be present in all vertebrate glomeruli (Pak Poy and Robertson, 1957; Pak Poy, 1957a, b, 1958a, 1959; Rhodin, 1963; Christensen, 1964), is commonly multiple in the glomus, while in glomeruli it has always been reported as single. The glomar filtration slit membranes, taken singly, are the same as those described in mammals (Farquhar et al, 1961; Rhodin, 1963; Kurtz, 1964), except that the central knob, thought to represent a second membrane at a right angle to the filtration slit membrane (Farquhar et al, 1961), has not been seen in the glomus.

Third, Elias et al (1965), in studying their own electron micrographs and those published by other workers, have concluded that the epithelium itself forms an almost complete barrier to the passage

of filtrate, in addition to the barriers imposed by the basement membrane and the filtration slit membranes. Although their 'sub-podocytic lacunae' have been seen in the glomus (Fig. 6), there is no evidence that they form a complex system, similar to that which they suggest occurs in mammalian glomeruli.

Basement membrane. The basement membrane of the glomus shows no striking differences from the glomerular basement membranes described by other workers (Hall, 1955; Bergstrand and Bucht, 1958; Rhodin, 1963; Kurtz, 1964). The relative thickness of the various layers seems to vary specifically, so no particular significance attaches to the thickness of the layers in the glomus, on the basis of available information. The apparent absence of any fine structure in the glomerular basement membrane resembling that reported in mammalian glomeruli (Sitte, 1959; Farquhar *et al.*, 1961; Kurtz, 1964) may be due to methods of tissue preparation rather than actual homogeneity.

The origin of the glomerular basement membrane, and especially of the lamina densa, has been a subject of long controversy. Evidence for an endothelial-epithelial origin (Rhodin, 1963), for at least a partial endothelial origin (Farquhar *et al.*, 1961; Pierce and Widgeley, 1963), and for an entirely epithelial origin (Kurtz and Feldman, 1962) of the lamina densa has been presented. It is tempting to suppose that the fibrils seen in the glomerular epithelium represent a basement membrane precursor, but since that basement membrane has not been shown to be fibrillar, this is a risky assumption. Pak Poy (1958b) has suggested that there is a less-close relationship between the membrane and the endothelium than between the membrane and the epithelium, based on the evidence for the existence of pericapillary spaces. In the glomus, the non-capillary

portion of the basement membrane lies between the mesangial cells and the epithelium, but not between the mesangium and the endothelium. Jørgensen (1966) reports that the basement membrane occurs in both regions of the human glomerulus, although it is not always present in the mesangial-endothelial area. The presence of the basement membrane only in the mesangial-epithelial regions of the lamprey glomus supports the suggestion that the basement membrane is at least partly, if not wholly, of epithelial origin.

The continuity of the glomerular basement membrane with that of the afferent and efferent arterioles has also been the subject of much discussion (Mueller, 1958; Kurtz and McManus, 1959). They are said to be continuous in the human glomerulus (Jørgensen, 1966). In the glomus, the basement membrane of the arteriole, glomerular capillaries and anterior cardinal vein are continuous, as might be suspected logically.

Mesangial cells. First described by Zimmermann (1933), early electron microscopists disagreed as to their existence. Suzuki et al (1963) have reviewed this discussion and concluded that the evidence supports their existence. In the glomus, they are not as abundant as in some glomeruli, nor are they as complex (Pak Poy and Robertson, 1957; Pak Poy, 1957b, 1958; Suzuki, 1959; Jørgensen, 1966). Their structure supports the contention that they are primitive cells, retaining mesenchymal characteristics which allow them to differentiate into other cell types (Suzuki, 1959; Suzuki et al, 1963). The large Golgi apparatus is probably evidence that these cells elaborate the mesangial matrix. The 'pale' and 'dark' processes described in the mammalian glomerulus (Kurtz, 1964) have not been seen

in the glomus, although processes do occur. Their function is not apparent, although it may be that they are the means by which the cells absorb metabolites from the capillaries, via the endothelial cytoplasmic rim. There is no evidence that these processes at any time penetrate the capillary lumen, as has been reported to occur in mammals (Zimmermann, 1933; McManus, 1948; Yamada, 1955; Jørgensen, 1966). Other inclusions in the mesangial cytoplasm of mammals (RNP granules, fibrils) have not been seen in the mesangium of the normal glomus.

Pericapillary spaces. These spaces have been seen only rarely in the glomus, and it is possible that they are sectioning artifacts. If they are not artificial, they are certainly not as elaborate as those described by others (Pak Poy, 1958a, 1959; Sakaguchi and Suzuki, 1960; Farquhar and Palade, 1962). Capillary outpouchings, external to the basement membrane, have been seen occasionally in the glomus, but they are extremely small and are obscure in their origin and function. They have been seen in other published electron micrographs (Pak Poy, 1958a, 1959), but little comment has been made about them. A possible third type of pericapillary space occurs between the endothelial cells and the adjacent regions of mesangial cytoplasm or mesangial matrix. The cytoplasmic material which is said to occur in the pericapillary spaces of glomeruli (Pak Poy, 1958a, 1959; Suzuki et al, 1963) does not appear in any of these three possibilities.

Introduction.

The previous section has dealt with the structure of the pronephric glomus at the time when the pronephros is the sole nephric excretory organ in the ammocoete. In the following section, changes in the structure of the glomus are described which occur as the opisthonephros (Appendix 4) takes over as the principal excretory organ.

Results.

No differences were found to exist between the glomera of the three species studied. Thus in the following description, the species are not distinguished.

Optical microscopy. The total length of the glomus in a 10mm larva is approximately 175 μ . This increases to a length of about 400 μ in a 40mm larva, beyond which there is no further increase in the length of the glomus, although the body length increases to a maximum of 175mm at the time of metamorphosis. However, the diameter of the glomus continues to increase until early in the fourth year of larval life. In PAS preparations (McManus and Mowry, 1960), it can be seen that this increase is due mainly to the proliferation of the axial (central) portion of the glomus (Fig. 13), most of which seems to be due to a proliferation of the mesangial cells, although endothelial cells are probably involved as well (see below). Fig. 13 also shows that the basement membrane has thickened during the same period (compare with Figs. 2, 10), an increase which makes the basement membrane visible in routine histological preparations.

It is possible that the number of capillary loops decreases with age, but this is not certain. However, the numbers of blood corpuscles seen within the capillaries are certainly fewer in older animals, although the arteriole supplying the glomus appears to undergo little change throughout the life of the ammocoete, and is almost always filled with blood corpuscles. Electron microscopy, however, shows that at least some of the capillaries are filled with a dense material not seen with the optical microscope, which prevents the passage of blood (see below).

One further change in the glomus noted with the optical microscope is that certain lobules, in the second and third years, exhibit focal proliferation of cells. Following this, the glomus loses much of its lobular appearance.

Electron microscopy. General features. Figures 14, 15 and 16 are low-power electron micrographs of the glomus, illustrating respectively its structure during the latter part of the first year, during the third year and during the fifth year, immediately before metamorphosis. In general, the changes which begin in the first year become more pronounced with age, until the time of metamorphosis when more rapid changes are noted. The principal changes noted are:

1. Some capillaries become filled with an electron dense material, sometimes containing fibrous material. Other capillary lumens become narrower, and some of these are eventually obliterated, by the proliferation of the basement membrane and endothelial cytoplasmic rim.
2. The epithelial and mesangial cells become more fibrous.
3. The basement membrane, especially the dense component, becomes more tortuous, and the lamina rara interna greatly thickened.

4. Epithelial cells tend to lose their foot processes.
5. The mesangial cells and the mesangial matrix become more prominent.

These changes are described in greater detail below.

Endothelial cells. In the latter part of the first year, the cell body, projecting into the lumen, consists of a large nucleus with several electron dense patches in sparse cytoplasm, which contains few organelles (Fig. 14). The cytoplasmic rim is greatly attenuated, containing few organelles, and interrupted by wide fenestrae with no fenestral membranes (Fig. 17). Late in the second year, or early in the third, the cytoplasmic rim becomes thicker, with many vacuoles of varying sizes and occasional mitochondria. Fenestrae are almost entirely absent. The cytoplasmic rim becomes even more prominent in the fifth year, prior to metamorphosis (Fig. 16), containing lysosomes, mitochondria and vesicular bodies of various sizes. The nucleoplasm is almost uniformly dense, and the cytoplasm surrounding the nucleus is crowded with organelles similar to those seen in the rim. The rim is focally thickened, in some capillaries almost obliterating the lumen. Fenestrae are no longer present.

Epithelial cells. The most striking change in these cells is the partial loss of foot processes and the proliferation of fibrils, presumably reticular, since they show no transverse periodicity. The nuclei are large and have indentations that are more pronounced in later years. Fibrils are numerous, and occur singly, forming a meshwork, or in bundles and whorls (Fig. 18). The trabeculae arising from the cell body occasionally give rise to foot processes, but more often are attached directly to

the basement membrane. Where foot processes do occur, the gaps between them are not always bridged by filtration slit membranes. Organelles, rare in the first year, include occasional mitochondria, dense bodies and numerous vesicles of various sizes. In the period just before metamorphosis, certain epithelial cells lose all their foot processes, become directly attached to the basement membrane, and form attachments with adjacent cells (Fig. 19).

Mesangial cells. In the early years, these cells are rare, but become larger and more abundant in the later years (Fig. 16). The most prominent features of these cells are the long cytoplasmic processes which extend toward the capillaries (Fig. 20), and the large areas of mesangial matrix. The matrix is extremely fibrous, and becomes increasingly more abundant. The fibres are of two types: small, presumably reticular, occurring singly and forming a meshwork, and coarse, electron dense fibres. The transverse periodicity of the latter suggests that they may be collagenous. The coarse fibres are most prominent first in the matrix surrounding the cell bodies, but are later seen in abundance in the subendothelial space, beneath the basement membrane (Fig. 21). The cytoplasmic processes penetrate the subendothelial space, separating the cytoplasmic rim from the basement membrane (Fig. 20). The cell bodies have large, lumpy nuclei; their cytoplasm is vacuolated and contains mitochondria and fibrils. The cytoplasm of the processes is similarly vacuolated and fibrous, with a few mitochondria.

Basement membrane. The lamina densa remains almost constant in thickness throughout the larval period, although in the final year it becomes

thicker and somewhat fibrous, and its course more tortuous (Fig. 22). Occasionally it may be focally diffuse. The adjacent, less-dense portions of the basement membrane become more altered with age.

The lamina rara externa remains nearly constant in thickness. In some regions it appears to be thickened, but this is probably due to its fusion with the flattened foot processes, with the virtual disappearance of the intervening cytoplasmic membrane.

The lamina rara interna shows the greatest change of all. It is highly variable in thickness, often contains vesicular bodies of various sizes, and is generally fibrillar, the fibres being either collagenous, reticular, or a mixture of both (Fig. 23). At first, the collagen fibres are few, and both they and the reticular fibrils are irregularly arranged. By the fifth year there are many more collagenous fibres, and both collagenous and reticular fibres are arranged parallel to the lamina densa. It might be thought that the variations in thickness were due to the differences in the direction of section, but this cannot be so, because great variations are seen in this layer though the lamina densa and lamina rara externa are of their ordinary thickness.

Breakdown of the glomus. In the final year of larval life, especially in the period just before metamorphosis, there are definite indications that the glomus is beginning to be resorbed. The typical optical microscopic appearance is shown in Fig. 24. It is impossible to tell whether the processes seen with the electron microscope occur in any particular order. The dense material occluding some of the lumens begins to disappear, and the reticular and collagenous fibrous coat, continuous with the lamina rara interna, surrounding the capillaries, begins to collapse.

The cytoplasmic rim is foamy and loses its plasma membrane. Large vesicles, sometimes bounded by a double membrane, appear in the mesangial processes in the subendothelial space (Figs. 25, 26), as well as lysosome-like bodies (Fig. 22), and small vesicles and dense bodies which may be the remains of mitochondria. Eventually the capillaries almost entirely disappear (Fig. 27), leaving remnants only faintly resembling the previous structure. Other regions of dense fibrous material surrounded by traces of the outer layers of the basement membrane, principally the lamina densa, and of the epithelium, may represent areas from which all traces of the capillaries have disappeared.

Discussion.

In comparing the structure of the glomus at the stage of pronephric excretion with the glomer structure described above, it is clear that after the opisthonephros begins to function, the glomus begins to undergo changes which presumably affect its function as a filtering apparatus and result in the eventual debilitation and destruction of the organ. The principal differences between early and later glomera are:

1. Occlusion of at least some capillaries, either by an amorphous substance containing fibrils, or by proliferation of the endothelium, lamina rara interna and mesangial processes.
2. A proliferation of the mesangium, and especially of the mesangial matrix, with an associated deposition of reticular and collagenous fibrils.
3. A progressive fibrillation of the epithelium, and a loss of the characteristic structure of these cells.

The occlusion of some capillaries, regardless of the method involved, indicates that less filtering probably occurs in the later years.

The origin of the amorphous substance in the lumens is not clear from the present study, but it may be either mesangial or endothelial in origin, or perhaps a combination of the two. The occlusion of capillaries by the proliferation of the endothelium may be caused by swelling of the cytoplasm or by an increase in the number and/or volume of cells. There is no positive evidence to indicate which is happening, although it seems that the number of cell bodies seen in a given section is greater in older larvae than in very young ones.

Endothelial proliferation, thickening of the basement membrane and changes in the structure of the epithelium are all observed in several types of renal pathological lesions. These have been investigated and discussed elsewhere (Farquhar et al., 1957; Vernier et al., 1959; Farquhar and Palade, 1960; Vernier et al., 1961; Vernier and Birch, 1962; Unanue et al., 1967), but are important in the present study as they represent the only investigations comparable with this study. There is no indication that the structures observed in the aging lamprey glomus are in any way pathological, but it is noteworthy that they are generally similar to those noted in aging human glomeruli. Also, the human kidney continues to function, although exhibiting proteinuria, under various pathological conditions (Farquhar, 1959; Farquhar and Palade, 1960, 1962) which are structurally altered in a strikingly similar way to the alterations in the aging lamprey glomus. Thus it seems reasonable to assume that the glomus is at least partly functional, possibly until breakdown begins, although it cannot be said that the changes observed are a cause of proteinuria and a damaged basement membrane, as is thought to be the case in mammals.

It is difficult to say whether the assumption that the basement membrane thickens with age is correct, since the origin of the basement membrane, in both the glomus and in glomeruli, is obscure (Farquhar et al, 1961; Kurtz and Feldman, 1962; Kurtz, 1964). However, it would appear that the material which is added to the lamina rara interna is structurally identical with the layer present in young animals. The presence of large numbers of fibrils in the additional material seems to point to its origin being the mesangial processes which invade the subendothelial space, since all fibrils within the basement membrane seem to be associated with the mesangium rather than with the endothelium. However, referring to the present study, it is impossible to exclude the possibility that the endothelium is at least in part responsible for the deposition of material which is added to the lamina rara interna. The possible addition of material derived from the epithelium to the lamina rara externa has been seen, but it seems more likely that this represents the flattening of trabeculae and foot processes against the basement membrane rather than an actual fusion of the layers.

There can be little doubt that the glomus is undergoing a gradual necrosis in the period immediately before metamorphosis. Other authors (Fürbringer, 1878; Wheeler, 1899) have reported that the glomus persists in the adult lamprey, but examination of adult lampreys during this study has not revealed any structure which could be said to be a remnant of the glomus. It seems most likely that the glomus disappears entirely at, or perhaps immediately after, metamorphosis.

The Tubules of the Functional Pronephros

Introduction.

The second functional unit of the pronephros is the tubular portion which, by means of nephrostomes, collects coelomic fluid and transports it to the pronephric duct. Brief descriptions of tubular histology (Wheeler, 1899; Hatta, 1901; Inukai, 1930) and function (Torrey, 1938) have been published, but there has been no detailed or ultra-structural study of this organ. In the following section, an anatomical and cytological description of the pronephric tubules is presented.

Results.

Anatomy and Histology.

The paired masses of tubules which constitute this part of the pronephros are located in the dorso-lateral quadrants of the coelom, in the cardiac region (not, as most texts state, in the pericardial cavity; see Appendix 4). They extend from the anterior limit of the coelom, immediately posterior to the last gill pouch, to the anterior end of the liver (Figs. 1a,b; Text-fig. 1).

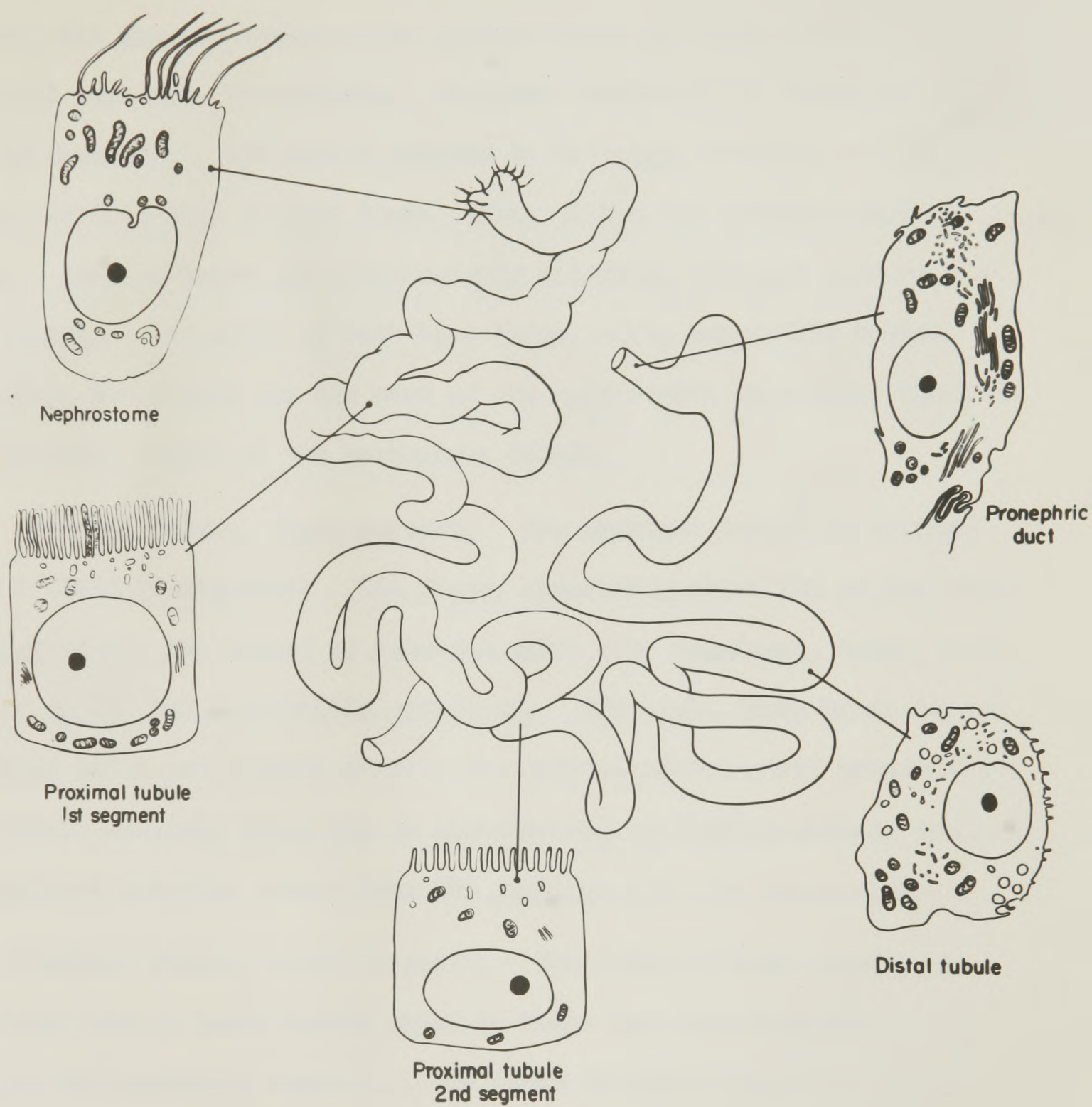
The group of pronephric tubules on each side is covered by the epithelium of the anterior cardinal vein. The special relationship of the tubules with the circulatory system is discussed in Appendix 4. Only the anterior end of each tubule, a prominent, ciliated nephrostome, protrudes from this vein. The posterior end of each tubule is connected to a common pronephric duct which drains each pronephros. The pronephric duct passes backwards along the ventral edge of the mid-dorsal nephrogenic ridge and opens into the cloaca.

A reconstructed pronephric tubule is shown in Text-figure 2. It was 3.2mm in length, from the mouth of the nephrostome to the beginning of the pronephric duct. It is the second anterior tubule of the right pronephros of a 20mm larva of P. marinus, and is representative of those tubules which were reconstructed. The cell types which are illustrated with it are diagrammatic composites taken from a series of electron micrographs. In this figure, a second, posterior tubule is shown joining with the complete tubule; this does not happen frequently in the pronephros, but is common enough to warrant its illustration.

The tubules of P. marinus were found to be slightly longer than those of L. planeri, the former varying from 3.0 to 3.6mm, the latter 3.0 to 3.4mm. These measurements do not include incomplete tubules such as the more posterior one shown in Text-figure 2, which was composed of nephrostomal and proximal segments only, and measured 1.1mm in length, as opposed to a mean of 1.6mm in complete tubules for nephrostome+proximal tubule.

In animals of all ages in which there was a continuous, unbroken series through the pronephros, it was found that neither the right nor the left organ had the greater number of nephrostomes, and that the number of animals in which there were more funnels on one side than on the other was greater (18) than those in which there was an equal number on each side (11). The only interspecific difference found in the pronephric tubules was that the average number of nephrostomes in each pronephros (and thus the number of pronephric tubules) of L. planeri is 4, while in P. marinus the average is 5. Sufficient series of L. fluviatilis were not available to allow an accurate estimate to be made of the number of nephrostomes.

Text-Figure 2. A single pronephric tubule of a first year ammocoete. Diagrammatic representations of the various cell types, and their positions along the length of the tubule, are shown. In the lower left of the figure, the tubule immediately behind the one illustrated anastomoses with the longer tubule.



The pronephric tubule can be divided into 5 distinct segments (Text-Fig. 2):

1. Nephrostome. This is made up of two portions. The first is the funnel, the flared, trumpet-like portion which protrudes from the anterior cardinal vein into the coelom. In cross section it is round and measures 75 μ in diameter. The second portion is the neck, tapering to 35 μ in cross section, with an oval lumen, lying within the anterior cardinal vein. Both portions are conspicuously ciliated, the neck less so than the funnel. The cells of both have large, oval, basophilic nuclei. Together, the funnel and the neck of the nephrostome constitute about 6% of the total length of the pronephric tubule.

2. Proximal Tubule, first segment. The proximal tubule is divided into 2 distinct segments. The first, comprising about 22% of the total tubular length, is formed of cuboidal cells with prominent, round, basophilic nuclei and acidophilic cytoplasm. The high, dense brush border of these cells can be seen readily in routine histological preparations. The tubule measures about 50 μ in diameter and is tightly coiled, following an indirect anterior course from its junction with the nephrostome.

3. Proximal Tubule, second segment. The cells of this segment are cuboidal, with a brush border which is lower and less conspicuous than that of the preceding segment. The lumen is wider than that of the first segment, although the overall diameter of the tubule averages about 45 μ . This portion of the tubule is more loosely coiled and follows an indirect posterior course. It is the longest single segment of the pronephric tubule, accounting for some 50% of the total length.

4. Intermediate segment. This short segment connects the proximal tubule to the distal tubule, and its cells reflect the transition from one cell type to the other. The cells are lower, with slightly basophilic cytoplasm, and the brush border is indistinct or absent. The lumen is wider than that of the proximal tubule. It does not account for more than 2% of the total tubular length.

5. Distal Tubule. This is the narrowest segment, about 40 μ in diameter, with cuboidal cells, small, apical, basophilic nuclei and strongly acidophilic cytoplasm. The lumen of the distal tubule is the widest of all segments. The tubule is arranged in long, flat coils and follows a posterior course. Together with the short intermediate segment, it makes up about 22% of the total tubular length.

The change in cell type from the nephrostome to the proximal tubule is an abrupt one, but the changes between other segments of the tubule are more gradual, especially the change from proximal to distal in the intermediate segment.

The pronephric duct is not generally considered to be a part of the tubular system, as it is separately derived embryologically (Wheeler, 1899; Hatta, 1900), but for the purposes of this study, its description will be included. Its cells are remarkably similar to those of the distal tubule, except for the slight acidophilia of their cytoplasm. The duct measures approximately 30 μ in cross section and has a narrow lumen.

2. Ultrastructure.

The more detailed cytological observations of the cells of the pronephric tubules are presented overleaf. Certain features of the tubular segments are common to all, and are described first.

Basement membrane. All cells rest on a continuous basement membrane which is smooth, except in the region of the nephrostome. Underlying it is a layer of connective tissue of reticular and collagenous fibres embedded in an amorphous matrix. Frequently cell bodies and cytoplasmic processes are seen in this layer. The basement membrane is continuous with that of the anterior cardinal vein at the nephrostome, and also continuous with that of the pronephric duct at the posterior end of the tubule.

Epithelial layers. Beneath the basement membrane and its associated connective tissue layer there is sometimes an epithelial layer, the parietal peritoneum, which separates the tubules from the coelom. This feature is often not clearly seen because of another layer, the wall of the anterior cardinal vein which, during embryonic development, has surrounded the pronephric tubules. In its engulfing process, the anterior cardinal vein tends to surround groups of tubules, so that an individual tubule may have one or two layers of epithelium beneath its basement membrane, or both may be absent. Both epithelial layers form bridges of tissue between adjacent tubules, which thus subdivide the lumen of the vein. These bridges are easily recognized by the conspicuous pigmented cells found in both layers (Fig. 29).

Nuclei. All nuclei are bounded by a porous double membrane, enclosing nucleoplasm which is more electron dense than the cytoplasm and which often contains electron dense patches.

Golgi apparatus. (Figs. 39, 40c). The Golgi bodies of the pronephric tubules are inconspicuous, occurring only in the cells of the proximal and distal tubules. They are perinuclear, and do not appear to be connected anatomically with other organelles.

Nephrostome. The cells of the nephrostome form a simple, ciliated columnar epithelium resting on an undulating basement membrane (Figs. 30, 31). The tall columnar cells of the funnel gradually taper to low columnar in the neck. A large, oval basophilic nucleus occupies the major portion of each cell. It is nucleolated and often has prominent indentations in its membrane. The most abundant organelles are the mitochondria, located above and below the nucleus. They are generally rod shaped, with their long axes oriented in the long axis of the cell. A few mitochondria are branched. Other organelles are clear vacuoles and dense bodies, located primarily in the apical cytoplasm.

The most conspicuous feature of the nephrostomal cells is the long, prominent cilia which originate in the apical cytoplasm (Fig. 32). Internally they exhibit the usual $[9 \times 2] + 2$ filamentous pattern. Each of the nine peripheral structures is composed of a pair of filaments. One has an electron dense core and a pair of sidearms, while the other has a pale central region; at the point at which the two join, they appear to share common material (Fig. 32b). The central tubules are less closely apposed than the peripheral pairs, and each has a pale core; fine fibrils extend from the central tubules toward the periphery. The limiting membrane of each cilium is continuous with the apical plasma membrane of the cell (Fig. 32c). The ciliary fibrils are anchored in a basal plate, but the peripheral fibrils pass through this plate to terminate in a basal body, from which arise striated rootlets (Fig. 32a).

interconnected by a meshwork of reticular fibrils. The cilia are most numerous in the funnel, becoming fewer and perhaps shorter in the neck region. In the neck, the apical plasma membrane often bulges out into the lumen of the nephrostome. Short, blunt microvilli are also present in the nephrostome. They appear to have an internal skeleton of longitudinal fibrils (Fig. 32d). In the lumens of material fixed for both optical and electron microscopy, the cilia appear to be grouped into units; this phenomenon is also seen in freshly killed animals in which the cilia are still beating. Each ciliary unit appears to be made up of cilia which arise from a single cell (Fig. 33).

Adjacent cells of the nephrostome are joined together by one to several subapical intermediate junctions, by desmosomes and by interdigitation of the lateral cell membranes (Fig. 34). The terminology of cell attachment zones is discussed in Appendix 4.

Proximal Tubule. Although this segment is divided into 2 distinct portions, many features of the structure are common to both. They consist of a cuboidal epithelium with large, round, nucleolated nuclei resting on a smooth, continuous basement membrane. The apical surface is notable for its prominent brush border (Fig. 35), which is PAS-positive (Fig. 10). The microvilli are longer and more numerous in segment I than in segment II; in the former they are tightly packed and arise separately from the apical surface of the cell; in the latter, they are more widely spaced, and often 2 or 3 arise from a common base. Adjacent microvilli, especially in segment I, are often seen to be fused, or perhaps branched, at one or more points along their lengths (Fig. 36). In the same regions

as the fusions, there are often large, pale, distended bodies among the microvilli, causing local displacement. They are limited by a single membrane and often contain electron dense bodies which have the appearance of microvillar and membranous fragments (Fig. 37).

Within the cytoplasm of the microvilli of segment I, there is sometimes a microtubule which forms a spiral core in the centre of the microvillus. The microtubules are limited by a single membrane and can be seen in both longitudinal and cross sections. In longitudinal section (Fig. 38) they rarely appear straight, but sometimes are seen fragmented, suggesting a sectioning artifact produced by their spiral configuration.

The number, size, shape and amount of included material of the various vesicular structures in the cytoplasm of the cells of the proximal tubule vary greatly from cell to cell, although a few cells have been seen to contain all of the vesicular structures that can be described. Small, clear vesicles are often seen near the apical membrane, while ones of equal size, but much more electron dense, are seen deeper in the cell. Larger vacuoles are quite common, but vary greatly in electron density from pale, almost clear structures to ones which are opaque. The larger vesicles often contain material which may be amorphous, membranous or vesicular. Most are bounded by a single membrane. A description of all the cytoplasmic vesicles found in the cells of the proximal tubule is presented below and illustrated in Fig. 39; the numbers below refer to the numbers on the figure.

1. Spherical to oval in shape, bounded by a double membrane, containing a few dark and/or light vesicles. Moderately electron dense

interior, generally located near the lateral cell membrane, in the basal portion of the cell.

2. Spherical, bounded by a single membrane, containing numerous small, dense particles. Interior approximately as electron dense as the cytoplasm, generally located near the lateral membrane, in the middle of the cell.

3. Spherical, bounded by a single membrane, very electron dense, the largest vesicular inclusion. Located in clusters in the central, basal portion of the cell.

4. Clusters of small, dense bodies, usually located close to the mitochondria. The smallest of the cytoplasmic vesicles.

5a. A generally large, irregular body, bounded by a single membrane, containing occasional vesicular inclusions, and having a pale, amorphous interior.

5b. Small, irregular bodies, bounded by a single membrane, with an electron dense halo.

5a and 5b are probably sections cut in different directions through the same type of inclusion.

6. Irregular in shape, bounded by a thin, single membrane, similar electron density to the cytoplasm, containing many small vesicular inclusions.

7. Apical in location, generally small, bounded by a thick, electron dense, single membrane.

8. Apical in location, amorphous, with no visible limiting membrane, very electron dense.

9. Elongate in shape, bounded by a single membrane, with a clear, pale interior, located in the middle or basal cytoplasm.

10. Microtubular structures, bounded by a single membrane, more electron dense than the cytoplasm, principally apical or basal in location. These structures are dealt with in greater detail immediately below.

Microtubules are common cytoplasmic organelles, usually localized in their distribution within the cell in either the apical or basal regions. They are smaller in diameter, and more electron dense, than the microtubules of the microvilli, and appear to form a dense meshwork. Neither apical nor basal tubules have any particular orientation. In some cells they are found adjacent to the lateral and basal membranes of the cell; when seen in this location, they appear less tubular, more irregular, and with less electron dense interiors (Fig. 40b). Microtubules are often absent from cells in segment II (Fig. 40c). In some cells, they seem to form attachments to the basal plasma membrane (Fig. 41).

Numerous dense, elongate mitochondria are a prominent feature of the cells of the proximal tubule. They tend to be concentrated in the basal cytoplasm. Occasional branched or circular mitochondria are also seen. They do not appear to have any particular orientation.

Ergastoplasm is not abundant in the cells of the proximal tubule but is seen in some cells in the cytoplasm which surrounds the nucleus. (Fig. 35b). For the most part, it appears to be the rough type, the external surfaces covered with small dense bodies which are presumably ribosomes; no free ribosomes have been seen.

There is often a space between the cells of the proximal tubule, bridged by a variety of attachment zones (Fig. 42). Generally, the apical junction is an intermediate one; in some cells, a second, longer

subapical intermediate junction is also present. Apical tight junctions, desmosomes, and basal interdigitations are also seen occasionally. In the mid-cell region, there are a few 'press-stud' interdigitations in some cells. Attachment zones are less variable in segment II, in which there are usually subapical tight junctions and an occasional apical tight junction.

The differences between the two segments of the proximal tubule may be summarized as follows:

1. Differences in structure, size, arrangement and distribution of microvilli and attachment zones, as detailed above.
2. The cells of the second segment are lower, and the lumens are wider; the overall diameter of segment II is reduced about 5 μ .
3. Segment I is tightly coiled, and anteriorly directed, while segment II is more loosely coiled, and posteriorly directed.
4. The vesicular inclusions in segment II are less varied than those of segment I, and fewer in number.

Intermediate Segment. There is some difficulty in accurately delimiting and defining this segment. It is a zone of transition from the proximal tubule to the distal tubule, in which the cells of the two segments tend to be mixed together or to be neither one type nor the other (Figs. 43a,b). The cells lose their brush borders, typical of the proximal tubule, and become low cuboidal, sometimes slightly flattened, with spherical or slightly oval nucleolated nuclei, which occasionally bulge out into the lumen of the tubule. Rarely, single cilia project from the apical surface of a cell of the intermediate segment (Fig. 43a). The cells rest on a smooth basement membrane; the underlying connective tissue and epithelial layers may be very thin or quite thick.

Distal Tubule. The cells are low cuboidal with spherical or slightly flattened nuclei which bulge out into the lumen of the tubule (Figs. 44a,b).

The apical surface of these cells is smooth except for the occasional short blunt microvillus, which has no discernible internal structure. The organelle content of the apical cytoplasm is variable, at times a great concentration of small vesicles, dense bodies and microtubules, in other cells sometimes only a few, or none, of these being present (Fig. 45). In these cells, devoid of apical organelles, there is a concentration of such structures, especially microtubules, near the basal membrane. In some cells, the microtubules are concentrated near the lateral cell membrane, and appear to connect with it and to interconnect with the microtubules of the adjacent cell (Fig. 45). The intercellular spaces are wide, bridged by a variety of attachment zones (Fig. 46). There is usually an apical tight junction accompanied by a subapical intermediate junction which may extend over half the height of the cell. In the absence of the tight junction, the intermediate junction occurs alone. In the basal region of the cell there are frequently intricate, extensive interdigitations of the lateral cell membranes of adjacent cells (Fig. 47). Microtubules in these regions appear to connect with the cell membranes.

There are numerous small, mainly spherical mitochondria in the cells of the distal tubule, their extreme electron density often obscuring any internal structure. In some distal tubule cells from certain individuals, small dark spherical inclusions are seen within the mitochondria (Fig. 48). Mitochondria which contain these granules are frequently less electron dense than non-granular mitochondria.

Patches of rough or smooth ergastoplasm are sometimes seen, but are not abundant. In the cytoplasm of some of the distal tubule cells large accumulations of small, electron dense particles are found between the mitochondria and the microtubules, which may be glycogen particles (Fig. 49).

Vesicles are, for the most part, concentrated in the apical cytoplasm of the cells. Some of them may arise from pinocytotic activities, but are fewer and smaller than those of the proximal tubule, less varied in form and rarely contain inclusions. Large, opaque, amorphous granules, irregular and lacking any external membrane, sometimes occur in some cells. In some cells, there does not appear to be any basal plasma membrane, but only a thick, fibrous layer separating the cytoplasm from the basement membrane; microtubules and vesicles open directly on to this layer (Fig. 49).

The cytoplasm of these cells is the most electron-dense of any of the cells of the pronephric tubule. In routine histological preparations it stains brightly acidophilic.

In certain regions, the cells of the distal tubule are flattened, almost to the point of being squamous, especially in sections through the non-nuclear portion of the cell (Fig. 50). These very flat regions have fewer organelles, which are principally mitochondria and microtubules, and are characterized not only by their shape but also by the high degree of interdigitation of their lateral cell membranes.

Pronephric Duct. Ultrastructurally, the cells of the pronephric duct are almost identical with the cells of the distal tubule, the only marked difference being that the nuclei of the duct cells are more basal in location, and thus less protruding, than those of the distal tubule cells (Figs. 51a,b).

Organelles include pale vesicles, dense bodies and prominent clusters of microtubules. The latter tend to be arranged in parallel groupings beneath the nucleus, close to the basal membrane. They appear to make intercellular connections and to connect with the basal and lateral cell membranes as well (Fig. 52). The apical surfaces of the cells are smooth except for a few short, blunt microvilli with no internal structure. The apical cytoplasm is generally empty of organelles except for the small, round, electron-dense mitochondria, which are scattered throughout the cytoplasm. The nuclei are large and spherical; they are not nucleolated.

The large spaces between the cells are bridged by apical tight junctions by elaborate interdigitations of the lateral cell membranes and by occasional desmosomes. (Fig. 53).

Discussion.

Although the anatomy of the lamprey pronephros is unique in several ways, the ultrastructure of the tubular portion, like that of the glomus, bears a remarkable similarity to its homologues in the higher vertebrates. Few ultrastructural studies have been made of the kidneys of non-mammalian vertebrates but it is evident that the basic structure of the proximal and distal tubules in all classes of vertebrates is essentially the same, and that the differences that occur, for the most part slight ones, are due to a more highly developed and specialized tubular system, especially in the higher (amniote) vertebrates.

The only notable difference found between the pronephroi of the L. planeri and P. marinus, the species collected in large enough numbers for accurate comparisons to be made, was in the number of pronephric

funnels: 4 in L. planeri and 5 in P. marinus. It is evident from the reconstruction in Text-figure 2 that the number of nephrostomes does not necessarily indicate the number of complete pronephric tubules, but it is probably indicative of the number of segments in which tubules have developed and persisted, at least to some degree. Wheeler (1899) states that there is a tendency during later development for the more posterior tubules to fuse with ones anterior to them, and Torrey (1938) maintains that the pronephric tubules branch and anastomose, but gives no evidence to support this contention. It must be emphasized that this anastomosing is not a common occurrence - in this study, it was found in about 15% of the pronephroi, usually only one anastomosis in any one animal. When anastomosis occurred it was always found that the posterior tubule was attached to the one immediately anterior to it.

That the pronephros is segmental in origin does not seem to be in doubt, although Wheeler (1899), in supporting this view, cites previous authors who disagree. Both Wheeler and Hatta (1900) agree that the pronephros develops in each of the 6 post-branchial segments, although they are not in accord as to which ones atrophy before hatching. Wheeler (1899) believes that he has found evidence of pronephric rudiments in some branchial segments, before the appearance of the definitive gill pouches, as Price (1904) believes that he has found crainial pronephric rudiments in Edellostoma. From the present study, it would appear that in some cases at least, more than 6 tubules begin development in at least one of the pronephroi of an individual, some of which may atrophy, or that perhaps more than 6 may begin development and that none or a few atrophy before hatching.

Wheeler (1899) and Hatta (1900) agree that in both L. planeri and P. marinus 6 pairs of tubules begin development, but before hatching, 2 (Wheeler, L. planeri) or 3 (Hatta, P. marinus) atrophy. From the studies of post-embryonic animals in this study, it seems that there is no set pattern in either species. Most authors who have studied ammocoete pronephroi agree that 4 or 5 pairs of tubules is the usual number, regardless of the species under investigation (Müller, 1875; Fürbringer, 1879; Scott, 1881; Shipley, 1887; Goette, 1890; Wheeler, 1899; Torrey, 1938). Inukai (1930) maintains that there are predominantly 4 pairs in L. fluviatilis and 5 in L. planeri, but his data do not support this conclusion. However, all these investigators and others (Bujor, 1891; Hatta, 1900) have noted the variation observed in this study, and have stated that the variation may be from 1 pair to 6 pairs, although in the present study, fewer than 3 were not noted in any pronephros, and there were as many as 8 pairs in one specimen. This variation in number does not appear to be linked to any specific factor such as age or species. That this variation extends to each pronephros of a pair has not been generally noted except by Inukai (1930) and Torrey (1938). Again, this variation is not a consistent one, in that there are more nephrostomes in one pronephros of a pair than in the other, although the right pronephros is generally shorter than the left, due to the asymmetrical development of the liver.

So it would seem that this variation in the number of nephrostomes is basically an individual one, not related to any specific factor. It is possible that it reflects a general tendency towards a reduction in the size of the pronephros, as atrophy begins before hatching, and animals with few nephrostomes are more common than those with many.

Inter- and intraspecific differences in the length of kidney tubules have been noted in mammals (DeRouffignac and Morel, 1967), but the inter- and intraspecific differences noted in the lamprey pronephric tubule length will have to be studied in a much larger sample than the present one before any definite conclusions can be drawn.

There is no special blood supply to the pronephric tubules. Their close association with the anterior cardinal vein is presumably sufficient; this association with part of the venous system is a common feature of many pronephroi. In Myxine (Holmgren, 1950) it is associated with an anastomosis of the anterior and posterior cardinal veins; in Bdellostoma, there is a specialized pronephric vein (Price, 1904); the pronephric tubules of Ambystoma are associated with the posterior cardinal vein (Christensen, 1964). There is a small arterial blood supply entering the anterior cardinal vein in the region of the tubules, from the glomus, but it is likely that this is a small amount compared with the volume of blood in the vein. Thus the blood supply to the tubules is essentially venous, as is the case in many other vertebrate kidneys which have a renal portal system.

The most extensively studied kidneys have been those of mammals, primarily rats and mice, and although there are differences between various species of mammals, both in micro-anatomy and in ultrastructure (De Rouffignac and Morel, 1967; Latta et al., 1967), the overall structure and arrangement of tubules is remarkably similar. The greatest differences between the kidney tubules of mammals and non-mammals seems to be that in the latter, several segments found in the mammalian kidney may be lacking. This is evident in the lamprey pronephros which has, at the most, 5 distinct segments, while the mammalian kidney may have

as many as 12 (Latta et al, 1967).

Basement Membrane. There are no features of the basement membrane which distinguish it from the basement membranes which have been described in ultrastructural studies by numerous authors in a variety of organs and animals. As pointed out on p. 20 , there has been considerable discussion as to the continuity of the basement membrane in mammalian nephrons. Perhaps as could logically be expected, the basement membranes of all parts of the pronephros, from the glomerular arteriole through the capillaries to the cardinal vein and the tubules, is a continuous structure, and may indicate, as has been suggested, that the basement membrane forms a continuous structure throughout the body.

The connective tissue layer beneath the basement membrane is presumably supportive and helps to bind the basement membrane to the enveloping layer of parietal peritoneum; it may also be concerned with the maintenance of the tubular lumens. Cytoplasmic processes which are encountered in the connective tissue originate from the peritoneal cells and other cell bodies, which are probably fibroblasts and/or wandering macrophages; Torrey (1938) believes that the reticulo-endothelial system surrounds all the tubules. The connective tissue and peritoneal layers are thickest, and the cytoplasmic processes most frequent, in the regions below the nephrostomes, which presumably require the greatest support and stabilization.

Nuclei. The nuclei of the tubules are similar to other vertebrate nuclei and the differences seen in the nucleoplasm of various cells of the proximal and distal tubules may reflect different functional states, or probably variation in the action of different fixatives (Appendix 1c).

Golgi. The Golgi apparatus is not large in any cells in which it is found, and is seen only in the proximal and distal tubule cells. Its size and perinuclear location seems to be common in all vertebrates, except that in some mammals it may be quite prominent (Latta et al, 1967).

Mitochondria. Differences between the mitochondria of the proximal and distal tubules were apparent in this study, those of the proximal tubule being elongate, sometimes branched, and usually basal in location, while those of the distal tubule were small, spherical and located throughout the cell. There were also instances in which variations in mitochondrial structure in different cells of the same segment were seen, and it is possible that a closer study of these organelles would reveal structural differences reflecting different functional states and/or seasonal variations, as has been reported in some amphibian kidney cells (Himmelhoch and Karnovsky, 1961; Karnovsky, 1963). The mitochondria are also peculiar in that they are very electron dense, in many cases obscuring the cristae.

Microtubules. The organelles which, in this study, have been called microtubules are not exactly equivalent to the microtubules described by other authors in non-nephric cells (Behrke, 1964; Porter et al, 1964; Jensen and Bajer, 1969; Kruger and Maxwell, 1969), yet are similar enough to warrant closer investigation and comparison. Morales and Duncan (1966) described microtubules in cat cerebellum which closely resemble those of the lamprey pronephros; these authors felt that the microtubules were specializations of the ergastoplasm, but that they were too complex and refined to be without special purpose or function, and because of their unusual nature were unlikely to be carrying out purely ergastoplasmic

functions. Similar organelles have been seen in other nephric cells; they have been called tubular invaginations (Christensen, 1964), tubular profiles of smooth ergastoplasm (Latta et al, 1967), or have appeared in electron micrographs but have not been specially commented upon (Anderson, 1960; Himmelhoch and Karnovsky, 1961; Karnovsky, 1963). Similar, but not identical, organelles appear in some descriptions of mammalian kidney cells (Boler and Arhelger, 1966; Threadgold, 1967), but these are more accurately described as tubular invaginations. The microtubules which have been described by other authors and which are the most similar to those of this study are found most commonly in non-mammalian vertebrates (teleost larvae and adults: Revel et al, 1963; Bulger and Trump, 1965a,b; Threadgold, 1967; amphibia: Himmelhoch and Karnovsky, 1961; Revel et al, 1963; reptiles: Anderson, 1960; possibly Schmidt-Nielsen and Davis, 1968).

It should also be clear that the microtubules of all portions of the pronephros are not identical, but differ qualitatively and quantitatively in various segments, and these differences may reflect functional differences. The microtubules of the proximal tubule are the smallest, most electron dense, and fewest. They do not appear to be extensively branched or interconnected with other cell organelles. The microtubules of the distal tubule are the most numerous, are most widely distributed throughout the cytoplasm although this distribution tends, in some cells, to be somewhat localized, and form the most extensively interconnected system. The microtubules are largest in the pronephric duct, are almost always subnuclear and are frequently arranged in parallel rows.

Similar structures are found in the chloride cells of Fundulus heteroclitus and in the gastric mucosa of Rana pipiens (Revel et al, 1963).

Behnke (1964) states that microtubules are of very wide occurrence, in intestinal, tracheal and pancreatic epithelial cells, skeletal and smooth muscle cells of the rat; these microtubules are unbranched and are not connected to the ergastoplasm.

Threadgold (1967) has published an electron micrograph and an explanatory tridimensional diagram of the cytoplasmic microtubular network found in the chloride-secreting cells in the gills of a larval teleost, Sardina. This irregular lattice bears the closest resemblance of any microtubular network to that seen in the distal tubule of the ammocoete pronephros. Threadgold believes that this system is part of the ergastoplasm, and shows it to be interconnected with the plasma membrane. He suggests that it is associated with ion transport and secretion in the chloride cells. The tubules curve, branch and anastomose, and in some areas are grouped together in parallel formations. This description and possible functional activity fits in with much of what is seen in the lamprey pronephros, especially in the distal tubule.

The tubular nature of these structures of the pronephros is undeniable. Their function is uncertain but is probably connected with resorption and perhaps with secretion as well. It is possible that the microtubules form a dynamic system, which would explain why their locale varies in different cells. Near the basal plasma membrane of the proximal tubule they appear to lose some of their tubular nature, which may indicate a change in or a cessation of function. They may form an intercellular pathway in the regions where they interconnect with microtubules from adjacent cells.

Nephrostome. The nephrostome, with its prominent cilia, is probably characteristic of all pronephric kidneys (Fraser, 1950). It has been described in the pronephros of Bdellostoma (Price, 1910; Conel, 1917), of Myxine (Holmgren, 1950), of the carp (Dornesco and Marco, 1964), of Sphenodon (Tribe and Fisk, 1941), and in all amphibian pronephroi that have been studied (reviewed by Fox, 1963; Christensen, 1964). There is also a marked similarity between pronephric nephrostomes and the opisthonephric nephrostomes of elasmobranchs and teleosts (Weichert, 1958) and amphibians (Chase, 1923; Peyrot and Massimello, 1961) and the opisthonephric neck segments of elasmobranchs (Kempton, 1943) and teleosts (Chase, 1923; Peyrot and Massimello, 1961). The nephrostomes of lamprey opisthonephros appear to be identical with those of the pronephros (Youson, 1968, personal communication; Bowen, unpublished observations). The pronephric nephrostomes of the lamprey are larger and more protruding than any similar structures described previously.

The nephrostomal cilia present no obvious differences from those described in other chordates and in invertebrates (Hookes et al, 1967); they do not have any characteristics which distinguish them as being particularly cyclostome, vertebrate or nephric. Secondary longitudinal fibres, thought by some to be uncertain (Hookes et al, 1967) are not seen, but radial fibrils are seen in cross sections. Each member of each pair of peripheral fibrils is of the same size as its partner, unlike some others described (Hookes et al, 1967). The striated rootlets arising from the basal body are presumably anchoring devices, further reinforced by the fine meshwork of reticular fibrils, similar to the pattern seen in Xenopus (Bargmann et al, 1955).

Proximal Tubule. To attempt a review of the studies on mammalian kidney cells is beyond the scope of this paper. The masses of data which have been accumulated have been thoroughly summarized by Rhodin (1958; 1962; 1963) Kurtz (1964) and Latta, Maunsbach and Osvaldo (1967).

The brush border is characteristic of all vertebrate proximal tubule cells, and has also been described in several invertebrate excretory systems (annelid metanephridia: Benham, 1891; Edwards and Schnitter, 1933; crustacea: Dahlgren and Kepner, 1908; Beams et al., 1956; Andrew, 1959; insect Malpighian tubules: Wigglesworth, 1930; Smith and Littau, 1960; Tsubo and Brandt, 1962; Threadgold, 1967; molluscan kidney: Dahlgren and Kepner, 1908; Bouillon, 1960). There is some variation in the structure of the brush border among the animals in which it has been described, but that seen in the lamprey pronephros is of the more common, dense type, as found in mammals (Latta et al., 1967). It is somewhat reduced in the second segment of the proximal tubule, but not as much as the relatively sparse brush border of some amphibian kidneys (Bargmann et al., 1955; Himmelhoch and Karnovsky, 1961; Christensen, 1964) and reptilian kidneys (Anderson, 1960). The amorphous layer which overlies the brush border, most prominently near the bases of the microvilli, is of common occurrence in mammals (Latta et al., 1967).

Vacuoles and tubular cores in microvilli have been reported by other authors (Sampaio et al., 1958), sometimes under special fixation conditions (Hanssen and Herman, 1962; Maunsbach, 1964; Osvaldo and Latta, 1966), but they are not common, and have been interpreted by some as preparation artifacts (Latta et al., 1967). Similar structures have been

reported in teleost opisthonephros (Bulger and Trump, 1965b) and amphibian pronephros (Christensen, 1964), and have been seen but not described in other opisthonephroi (Karnovsky, 1963). In this study, they have occurred with such frequency, in tubules fixed with a variety of fluids, that the conclusion that they are artificial seems unlikely; this view is supported by Christensen (1964). However, it is impossible to say with certainty what their function might be. Crane (1966), studying isolated microvilli, has postulated a fibrous skeleton which supports the microvillus, and at least some of the structures in the microvilli in this study may be performing a supportive function, although they bear no resemblance to the structures described by Crane. It seems more likely that these spiral cores and vacuolar-like inclusions, which are probably sectional views of the spiral cores, have some function related to the resorptive or secretory functions of the proximal tubule. Christensen (1964) believes them to be a part of the ergastoplasm.

Microvillar fusion and compression has been described by Rhodin (1963), and electron micrographs of microvilli similar to those of the pronephros have been published but without further comment (Bargmann, et al 1955, amphibian opisthonephros; Anderson, 1960, reptilian opisthonephros). They are not found in the lamprey opisthonephros, in spite of the similarity of the two nephric organs of the lamprey (Youson, 1968, personal communication). It does not seem unreasonable, from a study of Figs. 36-38, to suppose that these phenomena are related to the pale, distended bodies which occur among the microvilli, most commonly in segment I. These contain debris which looks like membranous, or tubular, fragments, and they may well be secretion granules. Secretion of various

materials by proximal tubule cells is thought to be of common occurrence among the lower vertebrates (Marshall and Smith, 1930; Bulger and Trum, 1967) and in mammals (Gersch, 1937; Sjöstrand and Rhodin, 1953; Berliner, 1963). In the horned toad, Phrynosoma cornutum, (Anderson, 1960), similar structures are thought to be secretion granules containing uric acid, a phenomenon first noted in a variety of reptiles by Cordier (1928). The same phenomenon is said to occur in the Malpighian tubules of Rhodnius (Wigglesworth, 1930) and in the green gland of Cambarus (Anderson and Beams, 1956).

The infolded basal membrane of proximal tubule cells of mammals is absent in the cells of the lamprey pronephros, as it is in the amphibian pronephros (Christensen, 1964).

Some of the vesicles (5a, b. Fig.39) of the apical cytoplasm, seen in formation at the bases of the microvilli, are identical with those described in mammals (Rhodin, 1962; Farquhar and Palade, 1963) and are characteristic of pinocytotic cells (Roth and Porter, 1962). In mammals, these pinocytotic vesicles are associated with the microtubular structures in the apical cytoplasm; Christensen (1964) feels that the tubular invaginations are concerned solely with uptake of large molecules in a functional association with the pinocytotic vesicles. In the lamprey pronephros, the microtubules have not been found to be connected to the pinocytotic vesicles, so that it is possible that they may have some separate function.

In the pronephros, ergastoplasm is present in small quantities, mostly in the form of rough ergastoplasm. Free ribosomes have not been positively identified. The microtubular network may be a form of smooth

ergastoplasm, especially in the basal portion of the proximal cells where it seems to lose much of its tubular structure and may be in a functionally modified state. Christensen (1964) believes that the microtubular structures which he has described in the amphibian pronephros are fragments of smooth ergastoplasm which extend into the microvilli. Smooth ergastoplasm is present in considerable amounts in mammalian proximal tubule cells (Latta et al, 1967) and is abundant in amphibian proximal tubule cells (Maunsbach, 1964); it may also be localized in its distribution within the cytoplasm (Ericsson et al, 1965; Bulger, 1964). Thus it is not unreasonable to assume that the microtubules of the pronephric proximal cells may be related in some way to smooth ergastoplasm, but their unique structure suggests that they may be concerned with carrying out more than purely ergastoplasmic functions.

Emulsified fat droplets, common in the amphibian pronephros (Christensen, 1964) and large lipid inclusions in close association with mitochondria seen in reptiles (Anderson, 1960) have not been seen in the pronephric proximal tubule cells.

It is a simple task to describe the cytoplasmic vesicles of the proximal tubule, but it is impossible to assign a definite function to many of them. Various vesicles almost certainly form members of a series which, in time, may change their size, shape, density, inclusions and location within the cytoplasm. From studying electron micrographs of similar cells in different species, it is obvious that there is a great deal of inter- and intraspecific variation in what may be functionally similar organelles. Many terms have been proposed for the various types

without the other, at least in some reptiles (Anderson, 1960; Anderson and Davis, 1963). In mammals, this series of

of vesicular structures, the best of which imply neither function nor origin unless, as is the case with pinocytotic vesicles, this has been definitely established. Maunsbach (1966) has proposed a classification based on size and thickness of limiting membranes, but this system breaks down, at least when applied to the lamprey pronephros. These structures require a great deal more investigation, especially at the cytochemical level, before definite origins and functions can be ascribed.

A good example of this problem of nomenclature is the lysosome (de Duve et al, 1955; Novikoff et al, 1956; de Duve, 1959). This term is essentially a biochemical definition, and has not been used in the above description as cytochemical studies have not been performed to show which bodies are definitely lysosomes, the essential feature being the presence of acid phosphatase. It has been shown to occur in a variety of cytoplasmic bodies in proximal tubule cells (Novikoff, 1959, 1963; Miller, 1962; Ericsson and Trump, 1966), indicating the many morphologically dissimilar bodies may resemble lysosomes biochemically.

The mitochondria of the lamprey proximal tubule are smaller and less numerous than their mammalian counterparts and, like those of the amphibian pronephros (Christensen, 1964) and reptilian opisthonephros (Anderson, 1960), do not show the typical mammalian orientation, perpendicular to the basement membrane, contained within compartments of the infolded basal plasma membrane (Sjöstrand and Rhodin, 1953; Rhodin, 1954). Infolding and compartmentalization of mitochondria are closely related phenomena in proximal tubule cells of vertebrates and in the kidneys of invertebrates (Beams et al, 1956; Todd, 1964), although the one can occur without the other, at least in some reptiles (Anderson, 1960; Schmidt-Nielsen and Davis, 1968). In mammals, this association is thought to be

an aid to rapid transfer across the basal and basement membranes (Latta et al., 1967), and its absence in the lamprey pronephros may indicate a much less rapid rate of transfer.

The above discussion has dealt primarily with the cells of the first segment of the proximal tubule. On the whole, the cells of the second segment are similar enough to warrant the same comparisons being made, as they are doubtless proximal tubule cells, but their differences from the cells of segment I can be summarized in the word reduction. This is a reduction in size, frequency and distribution of cell organelles, of height and density of brush border, of tubular diameter and coiling, and all this may indicate a reduced, or slightly different, function from that of the cells of segment I. For example, the reduced height and number of microvilli, fewer pinocytotic vesicles and less variety of cytoplasmic vesicles may indicate a reduced resorption in segment II, or perhaps even reduced capacity for resorption.

Segmentation of the proximal tubule is a common occurrence in many vertebrates (elasmobranchs: Kempton, 1943, 1962; Edwards and Schnitter, 1933; Guyton, 1935; Bulger and Trump, 1965a, 1967; amphibians: Peyrot and Massimello, 1961; reptiles: Cordier, 1928; Edwards and Schnitter, 1933; birds: Longley and Fisher, 1954; mammals: Suzuki, 1912; Sjöstrand, 1944; Rhodin, 1954; Sakaguchi and Suzuki, 1958; Thoenes, 1961; Caulfield and Trump, 1962). These differences are anatomical (DeRouffignac and Morel, 1967, cytological (Maunsbach, 1964), cytochemical (Longley and Fisher, 1954) and functional (Edwards and Schnitter, 1933). Thus the segmentation of the proximal tubule of the lamprey pronephros is not surprising, and may reflect a functional difference between the two segments.

Intermediate Segment. This segment is thought to be present in all vertebrate kidneys (Edwards and Schnitter, 1933), readily distinguished by the presence of cilia in the amphibian pronephros (Christensen, 1964) and opisthonephros (Chase, 1923; Bargmann et al, 1955; Himmelhoch and Karnovsky, 1961) and in the opisthonephros of teleosts and amphibians (Cordier, 1928; Edwards and Schnitter, 1933; Guyton, 1935, Anderson, 1960; Peyrot and Massimello, 1961). It is believed that the intermediate segment of opisthonephroi and reptilian metanephroi has become modified in birds and mammals to form the thin segment and loop of Henle respectively (Huber, 1932; Scheer, 1948). The cilia found in some intermediate segments are for additional propulsion of the tubular fluid (Edwards, 1933; Marshall, 1934). In the lamprey pronephros there are no characteristics which separate sharply the intermediate segment from those preceding and following it. There are few cilia present, and there is no abrupt transition to a different cell type. This is similar to the situation in the lamprey opisthonephros (Youson, 1968, personal communication) and in Hippocampus (Gabe, 1957). In the pronephros, the intermediate segment is probably in an undifferentiated state, and the gradual transition from one cell type to the other and the gradual narrowing of the tubule probably reflects a lack of functional distinction.

Distal Tubule and Pronephric Duct. Although it has been stated that these two segments are separately derived in the developing embryo (Wheeler, 1899; Hatta, 1900), the remarkable structural similarities between them casts doubt on this observation. A parallel similarity has been noted in the human mesonephros (DeMartino and Zamboni, 1966), in which the cells of the distal tubule are structurally almost identical with those of the

metanephric collecting tubule. Further, Armstrong (1932) has noted that both the distal tubule and the major portion of the duct are secretory in the pronephros of Fundulus and several other teleosts, indicating a functional similarity which would possibly be reflected by cytological similarities.

Wheeler (1899) assumed that the long flat coils of the distal tubule ("collecting tubule") were functionally distinct from the so-called glandular portion of the tubule (proximal and intermediate segments), acting only as a connection between them and the pronephric duct. Edwards and Schnitter (1933) concluded that the cytology of the opisthonephric distal tubule is essentially the same in all vertebrates in which it occurs, except marine teleosts, in which it may be completely absent (also Gabe, 1957). However, the distal tubule in different vertebrates shows different staining properties: in some it is acidophilic (fresh water teleosts, Edwards and Schnitter, 1933; mammals, Bloom and Fawcett, 1962), while in others it is basophilic (marine teleosts and reptiles, Edwards and Schnitter, 1933). In all cases studied, the staining reaction of the duct leading from the distal tubule is the same as that of the distal tubule, and although this has not been commented upon nor studied further, it may indicate differences in function in various distal tubules, in spite of their cytological similarities. In the lamprey pronephros the distal tubule appears to be more than a simple connecting segment, as supposed by Wheeler (1899), and it is almost certain that at least some of its functions are being performed by the pronephric duct as well.

Unlike mammals and some other vertebrates, the distal tubule of the lamprey is not morphologically subdivided. Ultrastructurally, the

cells resemble those of the higher vertebrates in having an abundance of mitochondria and short blunt microvilli, but the mitochondria do not exhibit any of the more typical orientation perpendicular to the basal membrane, nor are they enclosed in elaborate basal infoldings of the basal membrane, as is the case in mammals (Rhodin, 1963; Kurtz, 1964). Similar structures are also absent in the human mesonephros (DeMartino and Zamboni, 1966).

The intramitochondrial granules of the distal tubule, first described by Porter and Hogeboom, in mammalian cells (1946, unpublished results), have been reported to occur in a variety of cells of different animals (Fawcett, 1966). They occur in many organs of widely differing groups of animals (Bargmann et al., 1955; Revel et al., 1963; Smith, 1963; Peachey, 1964; Fawcett, 1966), and under certain experimental or cyclic conditions (Karnovsky, 1963; Peachey, 1964; Williamson, 1964). They have been associated with cytochrome oxidase activity in the proximal tubule of the frog nephron (Karnovsky, 1963), with the accumulation of divalent cations in isolated mitochondria from rat kidney cells and toad urinary bladder (Peachey, 1964) and are thought to be particularly abundant in tissues which transport large amounts of ions or water (Jollie and Jollie, 1967). Their purpose and function in the lamprey pronephros is uncertain; as they are not found in all cells, it may be that they are involved in a cyclical or unusual activity.

The reduction in number, or complete absence of organelles other than mitochondria and microtubules from the cells of the distal tubule and pronephric duct suggests a somewhat limited function for these cells. In mammals, there is evidence of sodium concentration in the cells of the distal tubule (Spater et al., 1958; Goldfischer et al., 1964) and of

fluid and salt control (Stone et al, 1961; Latta et al, 1962a, b), and the microtubules may be associated with these activities. In mammals, acidification of the urine is thought to take place in the distal tubule (Rhodin, 1963), by the formation of H^+ and OH^- ions, with the excretion of the former, and this may account, at least partially, for the acidophilia of the cytoplasm of the distal tubule and duct. In the horned toad (Anderson, 1960) and other vertebrates, the distal tubule is said to be concerned primarily with water resorption, but this is obviously unlikely in the lamprey.

The cells of the distal tubule and the duct differ markedly from mammalian cells in two other respects: their relatively electron dense cytoplasm and the often elaborate lateral interdigitations. The latter are most notable in the flattened portions of the distal tubule, which resemble the cells of Henle's loop in mammalian kidneys.

Because it is impossible to maintain particular stages of degeneration with specific ages of lampreys, as well, it is impossible to distinguish between the degeneration which results in the loss of the proximal tubules and that which occurs in the distal tubules. Since degeneration begins later in the latter, but follows a different course in each respect,

perhaps, the process is a single one, in lampryde tubes. The lampryde tubes are filled with cellular material, which is gradually, but rapidly, destroyed, and are finally destroyed by cellular phagocytes. The precise point at which degeneration begins is difficult to determine, but seems to coincide with the loss of cellular

The Degeneration of the Tubules

Introduction

The degeneration of the pronephric tubules has been noted by other authors (Wheeler, 1899; Hatta, 1900; Inukai, 1930; Torrey, 1938) but it has not been examined in detail. Ultrastructural studies of tissue degeneration are rare, and none have been made of nephric tissue, apart from studies of pathological processes in mammals.

Results

Although there are distinct stages in the degeneration of the pronephric tubules, it is not a uniform process, as the anterior tubules begin to deteriorate, and may be completely resorbed, before there is any sign of breakdown in the posterior tubules. For this reason, it is impossible to associate particular stages of degeneration with specific ages of ammocoetes. As well, it is necessary to distinguish between the degeneration which results in the destruction of the proximal tubules with that which occurs in the distal tubules, since degeneration begins later, in the latter, and follows a different course in some respects.

Basically, the process is a simple one, in descriptive terms. The tubules become occluded with cellular material, dilate considerably, then rapidly collapse, and are finally destroyed by invading phagocytes. The precise point at which degeneration begins is difficult to determine, but seems to coincide with, or follow shortly

after, the onset of function in the opisthonephros, during the second six months after hatching.

It is in the degenerative process that the only interspecific difference is seen between the pronephroi of the species studied. The greatest difference is between L.planeri and P.marinus. In the former, degeneration begins early, in the first year, and is usually completed by the beginning of the fourth year, a total of 2½-3 years. In landlocked P.marinus, the first breakdown begins in the third year, although there may be earlier signs, and tubular remnants persist until metamorphosis, so that degeneration is not completed till three or four years later. It was not possible to collect a large enough sample of L.fluviatilis to allow accurate estimates to be made of the degenerative process. However, it appeared that although the process was essentially the same in both species of Lampræta, in L.fluviatilis it began later, at about the beginning of the second year, and lasted longer, tubular remnants still being present at the beginning of the fifth year. As well as temporal differences, there were also a few minor differences in the actual pattern of degeneration between the three species studied. The following description is an outline of the atrophy of the pronephric tubules of L.planeri, with comments on and comparisons with the condition found in P.marinus in each age group.

The pronephric tubules of L.planeri begin to atrophy in the second half of the first year. The first sign, seen only with the electron microscope, is the appearance of large, pale, irregular

cytoplasmic bodies in the cells of the proximal tubule (Fig.57). These vesicles contain various dense bodies and membranous fragments. Soon after, cells begin to invade the tubular epithelium from the anterior cardinal vein, passing between the epithelial cells and entering the lumens (Fig.54). This passage seems to cause some of the nephric cells to rupture, and the debris and the invading cells accumulate in the lumens, eventually blocking the tubules (Fig.56). By late in the first year or early in the second, this blockage has caused the tubules to dilate, and the cells of the tubules subsequently flatten due to stretching (Fig.56a). There is an increase in the incidence of mitotic figures (Fig.57) in the tubular epithelium at this stage, but the new cells thus formed do not begin to cope with the stretching that has occurred. The nuclei of the nephric cells become more basophilic and crenated.

The most notable ultrastructural changes occur in the cells of the proximal tubule. The microvilli are reduced in height and number, and their internal spiral cores disappear (Fig.58). The number of vacuoles in the apical cytoplasm is reduced, and numerous small, dense bodies are seen in the same region; pinocytosis at the bases of the microvilli is also greatly reduced. Apical microtubules are fewer and have lost much of their tubular appearance. Mitochondria are unaffected at this stage. In the cytoplasm surrounding the nucleus, fibrous patches are sometimes seen, especially in the region above the nucleus. The nuclei are now basal in location, with more dark patches in the nucleoplasm and crenated margins.

Large, pale vesicles containing membranous and vesicular inclusions are common in the middle of these cells. When the tubules collapse following dilation, some cells rupture, extruding their cytoplasm into the lumens; the cytoplasm is foamy and vacuolated, and contains few organelles. Of the intact cells, some seem to have a much more electron-dense cytoplasm than others, so that two types of cells, dark and light, are found (Fig.59). Some tubules at this stage are completely surrounded by the invading cells, presumably macrophages, which push their way into the lumens, passing through the inter-cellular spaces in those areas in which the lateral cell membranes are still intact. Some tubules have been invaded to such a degree, and their cells so altered in appearance, that it is impossible to tell in electron micrographs whether proximal or distal tubules are being examined (Fig.60). From serial sections made for optical microscopy, it appears that only the proximal tubules are affected in the manner described above at this stage.

The distal tubules do not begin to regress at the same time as the proximal tubules, and when atrophy does begin, it takes a different course. There is no phagocytic invasion at first, although some tubules become dilated, (Fig.61a), due to blockage or collapse of the pronephric duct. In the dilated tubules, the cells are again considerably flattened. The cytoplasm is more vacuolated and less acidophilic than that of the healthy pronephros, (Fig.61b), and the nuclei are more basophilic and somewhat crenated. Ultra-structurally, the number of organelles is greatly reduced, the nuclei

are shrunk, and large areas of the cytoplasm are either extremely vacuolated or empty, apart from pale amorphous cytoplasm containing some dense granules (Fig.62).

The pronephric duct is unaffected at this stage, excepting occasional blockage by debris or by local collapse.

The nephrostomes are similarly unaffected, (Fig.63), excepting a possible slight shrinkage and an increase in the number of cytoplasmic vacuoles. The cilia continue to beat regularly in freshly-killed specimens. The basement membrane beneath the cells of the nephrostomes is slightly more undulating, and the underlying connective tissue layer has thickened.

In the anterior cardinal vein, there are numerous leucocytes adjacent to the tubules, associated with the connective tissue and epithelial layers which surround the tubules.

In the latter part of the second year, or in the beginning of the third, the anterior tubules, which were dilated, have collapsed, and their lumens are completely occluded with phagocytes and cellular debris (Fig.67). Some of the more posterior tubules, or portions of them, are completely unaffected as yet, except for the presence of intracellular organelles containing dense bodies and membranous fragments, seen only with the electron microscope. By this stage, the ammocoetes have approximately doubled in length and trebled in girth, compared with their size at the stage when the first signs of pronephric atrophy was noted, but the size and numbers of the tubules has not increased. Tubular collapse and shrinkage

makes them seem much smaller and less conspicuous than they were in the first-year animals. At the beginning of the third year, the spaces between the tubules have increased in size, and are filled with leucocytes; rapid phagocytic invasion of the distal tubules now begins, and in some cases it is impossible to identify individual tubules as proximal or distal, even with the optical microscope. Ultrastructurally, the situation is best described as a confused mess. Most cells are filled with large, irregular vesicles, although a few may be more or less intact. The lumens are completely occluded, and the few identifiable cells of the proximal tubules have ceased pinocytosis. The mitochondria have become rounded, and have either lost their internal structure or have become so electron-dense that it cannot be seen.

After the tubules have collapsed, it can be seen that during the period of dilation, the basement membrane and the connective tissue beneath it have proliferated greatly (Fig.68). The basement membrane has not thickened, but there is much more of it; on collapse, it is thrown into prominent folds. The connective tissue is greatly thickened following collapse; most of the increase appears to be due to a proliferation of collagen fibres.

In L.fluviatilis, atrophy follows the pattern outlined above, but at a time lag of approximately six months.

It has already been mentioned that in P.marinus, the first signs of tubular degeneration are seen early in the third year. Previous to this, there have been no indications of atrophy, although

PAS preparations indicate a reduced activity in the brush borders of the proximal tubule cells of second year animals (Fig.65). Possible increased vacuolation of the cytoplasm is sometimes seen, but the only sign of incipient disaster is the build-up of nests of leucocytes in the anterior cardinal vein, immediately adjacent to the tubules (Fig.66). Phagocytic invasion of the anterior proximal tubules begins early in the third year; the distal tubules of these, and the tubules of the more posterior segments, are not affected at this stage. In P.marinus, the tubules do not dilate as extensively as they do in Lampetra, nor does the dilation appear to persist for as long a period, for soon after the first signs of invasion are seen, the collapsed, shrunken tubules appear, blocked by an amorphous coagulum which does not include much cellular material. Only after collapse do the phagocytes appear in quantity.

By the beginning of the fourth year, all tubules and tubular remnants have disappeared from some specimens of L.planeri. In the remaining specimens of the same age, the anterior tubules have disappeared, as have the proximal tubules of the posterior segments, with the remaining distal tubules in the final stages of regression (Figs. 67, 68). The latter cells have few or no organelles, except for small, dense bodies in a clear, amorphous cytoplasm. The cell membranes are irregular and incomplete. Occasional mitochondria are still seen, but do not show any internal structure. The connective tissue beneath the basement membrane has proliferated further. The nephrostomes are still present, and their cilia are still functioning.

In many specimens, the section of the anterior cardinal vein which has contained the pronephros has become longitudinally compressed, so that the nephrostomes are crowded together. They open directly into the anterior cardinal vein (Fig.69). The only traces of the pronephric tubules are the cords of venous wall and peritoneum which still subdivide the vein. The anterior end of the pronephric duct has closed or collapsed, but the duct itself is still present, although slightly reduced in diameter, and can be traced to the opisthonephric region. It may have collapsed or broken in a few places.

In the fourth year, the pronephric tubules of P.marinus are still present, but they have shrunk, and in some cases, masses of leucocytes completely obscure the tubules. By the fifth year, most of the tubules in some specimens have disappeared, although remnants of posterior tubules, consisting of a thin, almost squamous epithelium and lumens containing an acidophilic coagulum, are still seen in a few specimens (Fig.70). The nephrostomes, opening to the anterior cardinal vein, and the pronephric duct are still intact as in Lampetra.

A preliminary investigation of the degeneration of the anterior end of the opisthonephros, which begins in all three species at some time in the second year, indicates that the process is almost identical to that seen in the pronephros, although there are a few points of dissimilarity. In the opisthonephros, there is little tubular dilation, and the atrophy is complete: neck segments (equivalent to the pronephric nephrostomes), glomus and archinephric (pronephric)

duct are resorbed, although in a few cases the duct may persist.

Discussion

As was the case in the degeneration of the glomus, the atrophy of the tubules is a process which is similar in some respects to tubular degeneration which occurs in various pathological conditions, and to changes which occur with age, in mammalian kidneys. These include reduced histochemical reactivity in the brush border of the proximal tubules (Wilmer, 1943; Wachstein, 1955), probably due to a reduction in the number of microvilli (Novikoff, 1959), dilation of tubules and extrusion of cytoplasm, with the appearance of large numbers of lysosomes (Novikoff, 1959), which are morphologically similar to the vesicles described above, and a reduction in the number of tubules (Kennedy, 1958). Many features of the electron micrographs published with these studies of pathological changes (Novikoff, 1959; Jackson et al, 1962) are comparable with those which were seen in this study.

Tissue breakdown is a common occurrence in embryonic and larval systems, but there have been no ultrastructural studies of similar processes for comparison. However, the degeneration described in this study is unlike embryonic and larval breakdown, and similar to pathological degeneration, in two ways: first, it is an extremely slow process, and second, not all of the organ is resorbed, and the remaining structures are altered somewhat. As Saunders (1966) has pointed out, cellular death is a common feature of development, and

can take several forms. Pronephric degeneration is a sort of phylogenetic death (Glücksmann, 1951), but is much slower in the lamprey than in other animals in which atrophy, both nephric (Jaffee, 1954; Fox, 1962a,b; Krichinskaya, 1965) and non-nephric systems (Wigglesworth, 1955; Weber, 1962; Saunders, 1966), has been studied. In most animals, regression is rapid, often occurring in a matter of hours or days, but in the lamprey pronephros, regression takes at least 3 years, and may only be completed fully with the drastic reorganization which occurs at metamorphosis. The reason for this leisurely atrophy is not immediately apparent, but is probably that the opisthonephric tubules are assuming the functions of the pronephros and there is no hurry. The process of degeneration must be genetically controlled, but the influence of hormonal and tissue-environment controls, the other factors which are thought to operate to regulate degenerative processes (Saunders, 1966) are not understood in this instance, and may be responsible for the lengthy resorption observed here.

Degeneration of pro- and mesonephroi has been studied as a natural process (Fox, 1962a; Krichinskaya, 1965) and as part of experimental processes (Fox, 1962b). The resorption of the lamprey pronephros has several points of similarity with the atrophy described by these authors, principally occlusion of tubules accompanied, or perhaps initiated, by phagocytosis. The cause of the onset of this atrophy is not clear, nor is it clear in other tissue-degenerative processes, but probably the first evidence is the appearance of

large lysosomes, the so-called "suicide bags" of Novikoff (1959), thought to release autolytic enzymes into the cytoplasm. Saunders (1966) maintains that the appearance of lysosomes in a tissue is a manifestation, rather than a cause, of death, contrary to the more widely accepted view that lysosomes are organelles adapted to destruction, under genetic control.

There is some disagreement in the literature about pronephric function in the lamprey. Wheeler (1899) maintains that the pronephros of L. planeri is fully functional in the fourth year, whereas Inukai (1930) states that the height of pronephric activity occurs at 12-14mm. in the first year. Torrey (1938) implies that the tubules are functional in Ichthyomyzon until at least the fourth year, indicated by the ability of the nephrostomes to take up material from the coelom and pass it into the tubules. This study illustrates several new points about pronephric function, particularly regarding the period during which it functions, when function begins to cease, and the results of degeneration. There can be little doubt that the pronephros, for at least six months, is the sole nephric excretory organ; later, its action is at first duplicated and finally completely assumed by the opisthonephros. The precise onset of opisthonephric function is beyond the scope of this investigation, but from incidental evidence, it seems to occur some time during the second six months, probably just preceding, or perhaps coinciding with the first evidence of pronephric atrophy. It is equally difficult to determine the cessation of function in the pronephros.

The nephrostomes continue to function until metamorphosis; the cilia continue to beat regularly long after the disappearance of the tubules, passing coelomic fluid into the anterior cardinal vein. Tubular function ceases much earlier: pinocytosis in the cells of the proximal tubules is greatly reduced, or perhaps even ceases, soon after the first signs of degeneration in L. planeri, and in P. marinus, brush border activity is reduced in the second year, although morphological evidence of atrophy cannot be found at this stage. According to Torrey (1938), the first sign of atrophy is the disappearance of the pronephric duct in the region immediately posterior to the pronephros, but this has not been seen in this study. In a few specimens, the duct has been found to be fully or partly collapsed in places and there is evidence of some severance and resorption of portions of the duct, but in most cases, the duct has persisted intact during early tubular degeneration. In later stages, it may shrink in diameter, but persists after metamorphosis in many adult specimens of P. marinus and L. fluviatilis.

Following tubular degeneration, the nephrostomes persist, although often reduced in size, and continue to function. It has been suggested (Torrey, 1938) that at this stage they are acting, in conjunction with the reticulo-endothelial system, as a filter for the coelomic fluid, similar to the opisthonephric nephrostomes of elasmobranchs and teleosts (Weichert, 1958) and amphibians (Fox, 1963). It is unlikely that the glomus is functional at this age; coelomic fluid is therefore probably derived solely from the cells of

the peritoneum, and the nephrostomes are the means by which the fluid can be drained from the coelom, filtered, and returned to the circulatory system. A similar pattern has been shown in the pronephric remnants of the adult Myxine (Holmgren, 1950), the nephrostomes forming a direct connection between the coelom and the venous system, which Holmgren regards as a filtration and excretion mechanism. Persistent pronephroi, or pronephric rudiments, are also found in several adult teleosts (Hyman, 1942; Weichert, 1958), but it has not been shown that they are functional excretory organs; in Acipenser, degeneration of the pronephros is accompanied by the formation of accessory peritoneal funnels (Fraser, 1927), which may perform a similar filtration function. In adult animals in which the pronephros, or pronephric remnant, is found, it has usually been assumed that it functions as a lymph gland, having lymphopoietic/haemopoietic functions (Holmgren, 1950; Willmer, 1960), but these assumptions have not been demonstrated. Certainly, the histological arrangement seen at times in the pronephros, especially when the tubules are collapsed and obscured by leucocytes, is reminiscent of mammalian Hassal's corpuscles, and the clusters of leucocytes are similar to clusters of glandular lymphocytes of other vertebrates. Their location, at the point at which the nephrostomes open into the anterior cardinal vein, certainly suggests a filtering mechanism. Further, in the author's experience, haemopoiesis is almost universal in organs of the lower vertebrates, especially elasmobranchs and teleosts, and is known to occur in the kidneys of some teleosts

(Leenheer, 1968; personal communication) and perhaps reptiles (Larsen, 1967; personal communication), and appears to be occurring in the lamprey opisthonephros, especially at the anterior end following degeneration (Torrey, 1938; Bowen, unpublished observation). Torrey (1938) also maintains that there is haemopoietic tissue in the intertubular spaces of the lamprey opisthonephros. Thus it is possible that there is similar activity in the lamprey pronephros, but such a function remains to be demonstrated conclusively.

Holmgren (1950) has described cyclical variation in the pronephric kidney of Myxine, previously noted by other authors (Kirkaldy, 1894; Conel, 1917), in which the organ has an excretory/phagocytic activity alternating with a haemopoietic function. This variation is probably controlled hormonally (Fänge, 1963), but supporting evidence is lacking. No similar activity has been seen in this study. Possible endocrine activity has been attributed to the lamprey pronephros (Gerard, 1933) and to pronephroi in Myxine (Holmgren, 1950; Euler and Fänge, 1961) and in some teleosts (Nandi, 1962), but cytochemical evidence is necessary to confirm this contention, especially in the lamprey.

It has been suggested that the number of pronephric nephrostomes may vary with age. Torrey (1938) believes that there may be a decrease with age, while in the present study, there is a suggestion that the numbers may increase with age, although sufficiently large samples, especially of older animals, have not been made to confirm

this suggestion statistically. However, it is certain that the number does not decrease with age, nor is there any evidence of confluence of nephrostomes in older animals, as has been observed in amphibian pronephroi (Jaffee, 1954; Fox, 1963), although they do become crowded together. An increase in numbers of nephrostomes with increasing age would indirectly support the holonephric theory, and in this study there is some suggestion that this may occur, as the greatest numbers of nephrostomes were found in older specimens of P. marinus. Anterior-posterior development (Wheeler, 1899) and anterior-posterior atrophy of the lamprey pronephros and opisthonephros, a feature seen in many other vertebrate kidneys (Tribe and Fisk, 1941; Hyman, 1942; review by Fraser, 1950; Leeson and Baxter, 1957), is further support of this theory.

It has been pointed out elsewhere that individual growth rates in the various species of lamprey are approximately the same (Appendix 3), so it is difficult to explain the different rates of pronephric resorption. However, several seemingly unrelated factors may have some bearing on this problem, and may help to explain this observation.

Hardisty (1961a) has shown that the growth rate of L. planeri in the first year is greater than that of parasitic species, and that sex differentiation and the appearance of oocytes is retarded in P. marinus, compared with gonadal development in L. planeri (1960). Further, he has published a graph (1965) comparing the pattern of gonadogenesis in planeri and marinus, which shows that the onset of

rapid gonadal growth occurs at approximately the same time as pronephric degeneration observed in this study. Saunders (1966) has posed several questions concerning the fate of the products of cellular death, and although the situation is still unclear, he has shown that phagocytosed material is transported by macrophages from the site of embryonic death, although its ultimate fate is still unknown. He feels that it is possible that cellular components released at death may be broken down into diffusible molecules which may be incorporated into other cells or added to pools of metabolites, coenzymes and other components. If this is the case, and there is some recent evidence to support this contention in embryonic systems (reviewed by Saunders, 1966), then the degenerating pronephros may be yielding material which could be incorporated by the rapidly developing gonads. The coincidental timing of pronephric breakdown and gonadal development suggests a possible relationship. In any case, as Hardisty (1963) has suggested, the evolution of L. planeri has been accompanied by a general acceleration of gonadal development consistent with the trend towards neoteny; this trend is probably reflected in the comparatively early onset and more rapid completion of pronephric degeneration in L. planeri.

Functional Considerations

It is important to emphasize once again the most important aspect of this study: the marked similarity of the cells of the pronephros to the kidney cells of higher vertebrates. The various cell types of the pronephros exhibit the same striking specializations that have been described in the corresponding cell types in the opistho- meso- and metanephroi of many species.

Although this study has been morphological rather than functional, it is essential to remember that the structures which have been described are part of a dynamic system. They have been pictured as static of necessity, but they become fully meaningful only in a functional context. There have been few studies which attempt to relate ultrastructure to function and, with the exception of the studies of Schmidt-Nielsen and Davis (see below), all such work has dealt with mammals. Although the lamprey pronephros is not an ideal organ for experimental studies of kidney function, some preliminary work has been done, and is summarized briefly below.

The cells of the proximal and distal tubules of the lamprey pronephros differ from their counterparts in the kidneys of most vertebrates in lacking basal infoldings, which are actually interdigitations of adjacent cells (Latta et al., 1967). Many reptilian kidneys lack these infoldings, but have well-developed lateral interdigitations, more complex but similar to those described in the distal tubule of the lamprey pronephros. It has been suggested (Davis and Schmidt-Nielsen, 1967) that the lateral interdigitations

of reptilian kidneys are similar to the basal infoldings of mammalian kidneys, and also to the lateral interdigitations of the mammalian gall bladder (Diamond and Tormey, 1966; Kaye et al, 1966), and that they, along with the inter-cellular spaces, are implicated in fluid transport across the tubular epithelium. Schmidt-Nielsen and Davis (1968) have shown that the inter-cellular spaces and the degree of interdigitation are variable in reptilian kidneys which have been experimentally salt-loaded, water-loaded and poisoned.

In the present investigation, young ammocoetes, thought to be at the pronephric stage of excretion, were placed in various fluids which were expected to cause osmotic stress. These were distilled water, and 2.5%, 3.5% and 5% aqueous solutions of NaCl. The period of immersion varied from a few minutes, in 5% NaCl, to 72 hours, in distilled water. Upon removal from the test solution, the animals were decapitated, measured, and fixed for electron microscopy. Examination of the tubules in the electron microscope revealed many changes in their structure.

Ammocoetes in NaCl solution.

In the cells of the proximal tubule, pinocytotic activity and the number of apical cytoplasmic vesicles appeared normal. Straight internal cores were present in some microvilli, and other microvilli had terminal bulbous swellings. The most striking changes in these cells was the appearance of prominent, elaborate basal and lateral channels, which may be interdigitations (Fig.71); no such structures were seen in the normal pronephros. Intercellular spaces were often

wider, and some apical junctional complexes were broken. In the cells of the distal tubule (Fig.72), there was a similar increase in the degree of interdigitation, although not to the extent seen in the proximal tubule. Intercellular spaces were often very wide and junctional complexes often missing or broken. In the cytoplasm, the microtubules were usually found in close apposition to the mitochondria, which were longer, less electron dense and showed some degree of apical-basal orientation. Apical microvilli were longer and more numerous.

Ammocoetes in distilled water.

In the cells of the proximal tubule, the number and/or variety of vesicles was usually reduced, and pinocytotic activity appeared to be reduced as well (Fig.73). The lateral cell membranes were closely apposed, although channels which probably connect with the intercellular space were not uncommon. Microtubules were absent. The apical cell membrane frequently protruded into the lumen and in many cases the microvilli had disappeared from the apical surface. The remaining microvilli had no discernible internal structure. The cytoplasm was usually less electron dense than that of normal cells. In the cells of the distal tubule, the number of mitochondria was greatly reduced, and there were no microtubules. The number and variety of cytoplasmic vesicles was increased, but apical microvilli had almost completely disappeared. The nucleus was not apical, but located in the middle to basal region of the cell (Fig.74). The lateral cell membranes were closely apposed, and interdigitations were

usually absent.

Although the significance of many of these changes in structure is still obscure, two important conclusions can be made about the function of the pronephros, based on the results presented above. First, pronephric tubules respond to changes in the osmotic pressure of the environment, and the changes which occur are often rapid and extensive. The cells shown in Figs. 71 and 72 have undergone alteration in less than 10 minutes, involving considerable reorganisation to form the elaborate and complex interdigitations not found in cells of the normal pronephros. Second, as has been suggested by other authors (Windhager et al., 1966; Davis and Schmidt-Nielsen, 1967; Hoshi and Sakai, 1967), transport of material from the lumen of kidney tubules does not always follow intracellular pathways, and that extracellular shunts are probably brought into action if necessary. Wide intercellular spaces and elaborate interdigitations similar to those seen in this study have been reported to occur in salt-loaded kidneys of several reptiles, and have been interpreted as mechanisms facilitating extracellular movement of fluids (Schmidt-Nielsen and Davis, 1968). A similar system is thought to operate in some amphibian kidneys (Hoshi and Sakai, 1967) in which the proximal tubules are known to be leaky.

Turning to the normal pronephros, it is possible to explain some of the observations in a functional way by comparing the ultrastructure of the pronephros with the ultrastructure of the mammalian kidney, about whose function much more is known, by assuming that

structural similarities probably reflect functional similarities.

There cannot be much doubt about the function of the nephrostome: to collect coelomic fluid, which includes the glomerular filtrate and drive it towards the tubules by ciliary action. This activity has been observed in freshly killed animals in this study and concluded, on the basis of experimental evidence, by Torrey (1938).

The ammocoete excretes a hypo-osmotic urine (Morris, 1960), as do most fresh-water fish, so there must be an efficient ion resorption mechanism in the kidneys of such fish. Evidence which supports this contention has been presented by Wikgren (1953), and backed by Morris (1960), the latter with specific reference to the lamprey. It has also been established by Hardisty (1956) that the opisthonephric kidney is capable of almost complete resorption of Cl^- ions. In the very young ammocoete, moreover, Cl^- content is increased four-fold in the first 35 days after hatching (Hardisty, 1957) and it seems likely that most of this increase is due to the action of the pronephric tubules. The site of ionic resorption in fresh water teleosts is believed to be the distal tubule (Grafflin, 1937a, b), and it is possible that the distal tubule of the pronephros acts in a like manner. The ultrastructural similarity of the pronephric duct to the distal tubule indicates that it is probably carrying out at least some of the same functions as the distal tubule. The highly distinctive structure of the microtubules of these segments, and particularly their similarity to the microtubules of the chloride-secreting cells of some marine teleosts, supports the contention that

they are sites of ion resorption. Microtubules are also more abundant in tissues fixed in media which are rich in divalent cations, particularly Ca^{++} (Boler and Arhelger, 1966), which not only indicates that microtubules are probably concerned with salt movements, but also suggests that they are a dynamic organelle. The changes in microtubular number and arrangement under normal and experimental conditions is evidence which tends to confirm this, although it is impossible to be certain about the mode of action involved. The connection of microtubules to the lateral and basal cell membranes may be evidence of movement of material into or out of the microtubules.

It has been suggested (Rhodin, 1963) that the apical vacuoles of the mammalian distal tubule are involved in the elaboration of H^+ and OH^- ions. In mammals, acidification of the urine takes place in the distal tubule, and if a similar process is going on in the pronephric distal tubule, this may account for the marked eosinophilia of the cytoplasm of this segment.

The absence of any distinct cell type or organelle in the intermediate segment is almost certainly a reflection of a lack of any distinct function in this portion of the tubule beyond acting as a junction between tubes having different functions.

The complexity of cytoplasmic vesicles in the cells of the proximal tubule is probably indicative of an extremely complicated and diverse series of functions. The strong PAS-reaction in the brush border probably indicates the resorption of glucose, as is

thought to be the case in other animals (Brown et al., 1950). In mammals, this process is mediated by alkaline phosphatase concentrated in the brush border (Longley and Fisher, 1954; Wachstein, 1955), probably in association with the plasma membrane (Möllbert et al., 1960), an action which is probably characteristic of all vertebrate proximal tubules. The other principal activity of the apical region of these cells is pinocytosis, discussed previously, which facilitates the resorption of larger molecules, principally proteins, which cannot enter the cell by diffusion (Miller, 1960). The numerous and varied cytoplasmic vesicles are probably concerned with the transport, digestion and modification of the resorbed materials, as has been shown to be the case in mammalian proximal tubules under experimental conditions (Miller, 1960; Ericsson and Dallner, 1962; Farquhar and Palade, 1963; Maunsbach, 1963; Ericsson, 1965a, b).

Mammalian proximal tubule cells are also important in the active transport of Na^+ (Spater et al., 1958; Goldfisher et al., 1964; Wachstein and Besen, 1964), thought to be facilitated and accelerated by the infoldings of the basal plasma membrane and the contained mitochondria. If lateral and basal interdigitations are analogous to basal infoldings (Davis and Schmidt-Nielsen, 1967; Schmidt-Nielsen and Davis, 1968), then this would explain the appearance of interdigitations in the proximal tubule under artificially high salinity.

The large pale bodies seen among the microvilli have been regarded as possible secretion droplets, as it is known that the

proximal tubules of many vertebrates are secretory. The contents of these droplets is less certain. They may contain debris from the destruction of cell organelles, waste products of cellular metabolism, secretion products which have been absorbed from the anterior cardinal vein, or remnants of resorbed material. Their size suggests that they may contain a large amount of fluid, possibly water which is taken up during pinocytotic resorption but for which the animal has no use and thus excretes back into the lumen.

The tubular cores of the microvilli may be involved in the absorption and/or secretion activities of the proximal tubule. That they are absent from the microvilli of animals kept in distilled water, which also results in a reduction of pinocytosis, but are present in the tubules of salt-loaded animals, suggests that they are involved in resorptive activities. They lose their identity as microvilli begin to fuse, and if this fusion leads to the formation of the large secretion droplets, then the membranous remnants that the droplets contain may be microtubular remains. If the microtubules are ergastoplasmic, as has been suggested by some writers, then it is difficult to determine what special function they could be performing in the microvilli.

The wide variety of cell attachment zones, with no definite pattern in any segment, suggests that the arrangement of attachment zones may be variable, perhaps fluctuating in response to the activities of the tubules. In the experimental animals in NaCl solution, the attachment zones in both the proximal and distal tubules

were altered or broken, the intercellular spaces widened and lateral and basal interdigitations increased. These alterations, along with the intracellular reorganization of microtubules and mitochondria in the distal tubule, are probably indicative of an attempt to resorb greater quantities of water from the hypo-osmotic urine. The actual mechanisms are not clear, but these results indicate that the structure of the pronephric tubules is variable, and that a dynamic system is operating which facilitates fluid transport, possibly in a manner similar to that which is thought to operate in mammalian kidneys (Suzuki, 1958; Spater et al 1958).

Concerning the functional aspects of the glomus, there is little evidence at this time to indicate that the glomus is capable of rapid and extensive structural changes under experimental conditions of osmotic stress. There is some suggestion that the barrier across which the filtrate must pass is capable of some alteration by variation of the thickness and fenestration of the endothelium and the attachment of the epithelium, but more detailed study is necessary to confirm this. However, it is possible, by comparison with the mammalian glomerulus, to make several probable assumptions about the glomerular function.

In the normal glomus, the variable thickness of the cytoplasmic rim suggests a constantly changing structure; the regions in which the rim is only as thick as two apposed cytoplasmic membranes may indicate a pre- or post-fenestral condition. Pinocytotic vesicles in the foot processes indicate that the material which has probably

passed through the basement membrane is being absorbed by the epithelium, which the presence of filtration slit membrane between the foot processes indicates the probable functioning of a monitoring system, as suggested by Farquhar et al (1961).

The significance of the multiple filtration slit membranes in the glomus is obscure. It is possible that the basement membrane is not as selective as that of glomeruli, and thus is it necessary to slow down the passage of filtrate to allow a more lengthy monitoring. Farquhar et al (1961) suggest that filtration slit membranes, which bear a close resemblance to desmosomes (Odland, 1958) and to adhesion plates (Fawcett, 1958), may be supportive as well as playing a part in the filtering system. If this is the case, it is not obvious why there should be the need for more support in the glomus than is necessary in glomeruli.

Certain features of epithelial cells suggest that they may be more active in the filtering system than simply functioning as a monitor. Their attachment to the basement membrane, in some places by numerous foot processes and in other places by the attachment of trabeculae and cell bodies, may indicate a method of controlling the rate of filtration. In the former instance, the rate of filtration would be maximal, but in the latter, reduced to a minimum by the imposition of a cytoplasmic barrier. Intermediate stages in such an alteration of structure may be partly shown by the multiple filtration slit membranes. They occur where adjacent foot processes are most closely apposed, and may indicate a partial adhesion of

recently-formed foot processes. Such an alteration would call for a re-assessment of the concept of interdigitation of foot processes, which may not be as complex as some authors have suggested, for it is difficult to imagine filtration slit membranes arising between pre-arranged foot processes. It has been suggested (De Martino and Zamboni, 1966) that the fibrils in the epithelial cells may be contractile; such contractility would permit at least some degree of re-arrangement of trabeculae and foot processes.

However, such re-arrangement would seem purposeless if, as some authors contend (Elias et al., 1965) almost all the filtrate is actively transported through the epithelial cytoplasm. Although there is almost certainly some active transport occurring in the glomer epithelium, the pinocytotic vesicles and subpodocytic lacunae are much smaller and fewer in the glomus than in glomeruli. If the degree of active transport is in some way dependent upon the filtration pressure, which is much lower in primitive vertebrates (Woodbury and Hamilton, 1937), then the glomus may be a less-active organ than the glomerulus, producing less filtrate per unit of surface area.

In summary, it is evident that the pronephros of the lamprey, although different in some specialized features from the kidneys of higher vertebrates, is basically an intricate structure which is remarkably similar in most respects to the most highly evolved kidneys of vertebrates. The findings of this study deny the generally supposed primitive nature of the pronephros and indicate, as others

have suggested, that nephrogenic material is basically the same throughout its length. The ultrastructural differences between the pronephros of the lamprey and the kidneys of other vertebrates are not great, and the marked similarities, both structural and functional, are further corroboration of the holonephric theory.

several changes of the structure, suggested by the evidence, cleared in vertical and horizontal sections. The structure was very similar to that of the lamprey, but the structure was different in some details. The structure was similar to that of the lamprey, but the structure was different in some details. The structure was similar to that of the lamprey, but the structure was different in some details.

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Appendix 1a

Techniques for optical microscopy

Unless otherwise noted in the captions accompanying the photomicrographs, ammocoetes were fixed in Bouin's fixative,¹ washed in several changes of 70% ethanol, dehydrated to 95% ethanol, cleared in terpineol and embedded in paraffin. Sections were cut serially at 8-10 μ , stained with alum haematoxylin (Ehrlich, 1886) and stained with Bouie's eosin.²

Other fixative's used were:

Zenker's (McManus and Mowry, 1960).

Formaldehyde-saline (Baker, 1965).

Other stains used were:

Haematal 8 and Biebrich scarlet (Baker, 1965)

Periodic acid-Schiff reaction (McManus and Mowry, 1960).

Iron haematoxylin (McManus and Mowry, 1960).

-
1. 75 vols. saturated picric acid; 25 vols. formalin; 5 vols. glacial acetic acid.
 2. 1% eosin Y in distilled water; 1% alcoholic eosin in 80% ethanol; 1% eosin B in 30% ethanol; 3 solutions mixed in equal volumes, diluted to 50% with 70% ethanol and acidulated immediately before use.

Appendix 1b

Techniques for electron microscopy

Most material in this investigation was fixed in a cold, 1% aqueous solution of osmium tetroxide for 1-1½ hours. Latterly, however, 1% buffered osmium tetroxide was used on a few occasions (Youson, personal communication). The differences between the two fixatives are discussed in Appendix 1c.

Other fixatives were used unsuccessfully, and are also discussed in Appendix 1c. They were:

Formaldehyde-sucrose (Baker, 1965).

Buffered glutaraldehyde, with post-fixation in osmium tetroxide (Sabatini et al., 1964).

All sections were stained with a saturated solution of uranyl acetate in 50% ethanol. In some cases, other stains were used in conjunction with uranyl acetate. They were:

Lead-ammonium acetate (Björkman and Hellström, 1965)

Lead citrate (Reynolds, 1963)

Phosphotungstic acid (Huxley, 1957).

Animals which were dissected before fixation were kept in a cold solution of physiological saline, modified for poikilotherms (Gatenby and Beams, 1950).

Appendix 1c

Notes on fixation and staining.

It was found that fixatives for electron microscopy that were not based on osmium tetroxide did not give satisfactory results. Buffered glutaraldehyde produced fixation artifacts such as crenated nuclear membranes, folded basement membranes, distorted cilia and microvilli and extruded cytoplasm. Formaldehyde-sucrose virtually destroyed all organelles beyond recognition, although cilia and basement membranes appeared to be normal. In all probability, these fixatives could have been modified in such a way as to render them useful, but as this study was not intended to be one of cytological technique, these solutions were abandoned in favour of those which produced results which were comparable with previously published studies.

Buffered osmium tetroxide produced slightly different results from non-buffered osmium solutions. These differences did not concern the quality of fixation, but made more clear some cellular details, although seemingly at the expense of others. It was felt that the two fixatives tended to complement each other: aqueous osmium tetroxide gave cleared details of microvilli and microtubular structures, and a greater variety of cytoplasmic vesicles. Buffered osmium tetroxide produced more details of ergastoplasm and of chromatin content of nuclei.

Maunsbach (1964) has shown that fixation techniques can affect the state of mammalian kidney tubules, which tend to collapse when

blood pressure is interrupted. Such effects were not noted in this study: tubular collapse was rare, and was not associated with any particular method of tissue handling (fixation in toto, in situ or after dissection).

That fixation techniques can markedly affect ultrastructure is a well-documented phenomenon (Bulger and Trump, 1965a,b), especially in the fixation of the proximal tubules of mammals (Maunsbach, 1966), and this may be at least part of the explanation for the difficulties experienced with some fixatives in this study. Other workers have experienced similar difficulties with non-mammalian nephric tissues. Leenheer (1968, personal communication) reported difficulties with glutaraldehyde fixatives on teleost kidneys, particularly with regard to membrane distention. Larsen (1967, personal communication) studying snake kidney principally by optical microscopic methods, has reported both fixation and staining problems with techniques which prove satisfactory with mammalian tissue. Youson (1968, personal communication), has not had any such difficulties in his studies of lamprey opisthonephros, although he has used osmium tetroxide fixatives exclusively. Glauert (1965) reports the necessity of slight alterations in techniques when working with non-mammalian and invertebrate tissues.

In this study, difficulties were also encountered in histochemical techniques used to investigate mitochondria and Golgi bodies. In some cases these were found to be adequate, but in others completely useless, although control sections of suitable mammalian tissues were

always satisfactory. Again, it is possible that slight modifications of these techniques is necessary for satisfactory results with non-mammalian tissues.

The first of the two methods described above is that of the use of a small amount of tissue, which is usually obtained from a single animal. This method is particularly useful for the study of the effects of a single treatment on a single animal. The second method is that of the use of a large amount of tissue, which is usually obtained from a group of animals. This method is particularly useful for the study of the effects of a treatment on a group of animals. Both methods have their own advantages and disadvantages, and the choice of method will depend on the nature of the study and the resources available.

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Identification of Ammocoetes

There is some confusion about the identification of larval lamprey, principally due to a lack of distinguishing characteristics in very young ammocoetes. Many species of lamprey occur in what appear to be closely related species pairs (Hardisty, 1963), both species having a fresh water larval period, but only one of the two having a marine, parasitic phase. Artificially bred hybrids do occur (Inukai, 1930), in at least one of these pairs (L. planeri and fluviatilis), so presumably the species are kept separate by morphological and ecological means, rather than genetic incompatibility. This close relationship is reflected in the high degree of similarity shown by L. planeri and fluviatilis, and is further complicated by the fact that the two species occur sympatrically throughout a large part of their ranges.

Petromyzon marinus is one of the few species of lamprey which does not appear to have a closely related non-parasitic "twin", and is readily separated from other sympatric species by its grey to black dorsal coloration. In the collecting areas listed on P. P. marinus occurred sympatrically with only two other species, L. fluviatilis, in Britain, and E. lamottenii in Canada, from which it was easily distinguished (Vladykov, 1950, 1960; MacDonald, 1959b).

Separation of the two species of Lampetra was found to be much more difficult. The key provided by MacDonald (1959b) was not found to be accurate when applied to very young larvae. Other authors

(Weissenberg, 1927; Hardisty, 1964) found that the only valid means of separation was by oocyte counts, but this too breaks down when applied to young animals with underdeveloped or undifferentiated gonads (Lewis and McMillan, 1965; Hardisty, 1965), and is obviously inapplicable to males.

In the areas in which collections were made for this study, there was no evidence of overlap of the two species of Lampetra (although L. fluviatilis occurred sympatrically with P. marinus). In any case, subsequent study of the pronephros and interspecific comparisons led me to believe that any specific differences were not reflected in the nephric system. The only interspecific difference was in the rate of pronephric regression, and specimens whose identification was in any way doubtful were omitted from this part of the study.

Appendix 3

Age-Length Relationships of Lampreys

In describing the sequential events in the development and degeneration of the pronephros, reference must be made to the ages of the ammocoetes in relation to their lengths, so that the pronephric cycle can be related to the life history of the animal.

Many studies have been made of ammocoetes in an attempt to establish a firm-age-length relationship. These studies have concentrated on two species: Petromyzon marinus, principally the North American landlocked variety (Gage, 1928; Applegate, 1950, 1961; Stauffer, 1956, 1962; Wigley, 1959; Erkkila, 1962), and European populations of Lampetra planeri (Ivanova-Berg, 1931; Knowles, 1941; Zanandrea, 1951, 1954; Hardisty, 1961d, 1965). The overall conclusion drawn from these studies is that, due to the variability of individual growth rates, even amongst members of the same population, there is considerable overlapping of modes representing year classes, making it impossible to assign well-defined limits to the length ranges of particular age groups. It is generally believed (Hardisty, 1961; Thomas, 1962; Lewis and McMillan, 1965) that the larval period lasts for more than 5 years, metamorphosis occurring in the sixth year, although some individuals may metamorphose one year earlier or later, males tending to be earlier than females (Hardisty, 1961). However, it is probable that the onset of metamorphosis is dependent on age rather than length, because of the variation of the length of metamorphosing animals (Hardisty, 1961a,c;

Thomas, 1962).

Growth is most marked in the spring, and there may be a slight recession in length during the winter (Hardisty, 1965). In both L. planeri and P. marinus, the annual increments are about 20mm, except in the first year, in which L. planeri may grow as much as 35mm., and the last year, when the increase may be somewhat greater than 20mm (Hardisty, 1961b, 1965; Thomas, 1962; Lewis and McMillan, 1965).

The ages of the ammocoetes used in this study were determined as follows:

The ages of the landlocked specimens of P. marinus were estimated from the length-frequency histograms of Lewis and McMillan (1965), as many of the specimens used in this study came originally from these samples, and the remaining specimens were collected from the same sources. Most specimens of L. planeri were collected from a single location (River Teme). Length frequency histograms were constructed and the ages of ammocoetes estimated, using the studies of Hardisty (1961^a, 1965) as guides. Specimens of P. marinus from Britain and of L. fluviatilis were not collected in sufficient quantities to allow accurate length-frequency diagrams to be prepared; ages of these specimens were estimated from the studies of Hardisty (1961^a) and MacDonald (1959a, b).

Appendix 4

Certain terms used in this study which may be unfamiliar or which have been used ambiguously by other authors are defined below. The definitions may not be universally accepted, but they convey the meaning of the terms as they are used in this study.

Anterior Cardinal Vein

Many authors (Wheeler, 1899; Goodrich, 1946; Weichert, 1958) describe the pronephros as being located within the cardinal vein, while other authors (Young, 1950; Romer, 1962) fail to point out the special relationship between the pronephros and the venous system. The anatomy of the cardiac region of the ammocoete, illustrated in Text-figure 2, indicates that the pronephros is closely associated with the anterior cardinal vein. In some specimens, the pronephric tubules extend into the ducts of Cuvier (noted also by Wheeler, 1899), and in some instances are intermingled with cords of liver tissue which have extended into the Cuvierian ducts via the hepatic vein. This situation occurs most frequently in the right duct, possibly related to the fact that the left duct of Cuvier either degenerates or fuses with the one on the right at metamorphosis (Weichert, 1958).

At the 3-4mm. prehatching stage of development, the pronephric tubules protrude prominently into the coelom (Wheeler, 1899), covered by the peritoneum which is continuous with the epithelium of the tubules at the nephrostomes, the tubules themselves being retroperitoneal in position. Shortly thereafter, the cardinal veins begin

to develop, and at stage 11 (Wheeler, 1899) just before hatching, the anterior cardinal vein begins to branch and anastomose, growing forward and completely surrounding the pronephric tubules. In this process, it penetrates and envelops the peritoneum separating the tubules from the coelom so that at hatching, the pronephric tubules are surrounded in most places by a single layer, the wall of the anterior cardinal vein, but in a few places by the wall of the vein and by the coelomic peritoneum. The pronephric tubules are thus enclosed by, but outside the anterior cardinal vein, in a close relationship with the circulatory system analagous to that found in the higher vertebrates (Weichert, 1958; Latta et al, 1967).

Glomus

The filtering apparatus of the lamprey pronephros was first called a glomus by Wilhelm Müller in 1875, but he did not define the term. Early anatomists studying the pronephros used the terms glomus and glomerulus interchangeably. Kingsley (1912) distinguished the two, defining the glomus as the organ resulting from the union of two or more segmental glomeruli, projecting into the coelom and covered by visceral epithelium. This is the exact situation in the lamprey: Hatta (1900) showed that the glomus was formed by the union of two segmental glomeruli; Kingsley's anatomical description is that shown in Fig.1 and Text-Fig.1. For these reasons, the pronephric filtering apparatus of the lamprey is properly called a glomus.

Junctional Complexes

Sjöstrand and Elfvin (1962) have suggested that there are 3 types of cell attachment zones, based on a study of the mouse pancreas. The first type is a 7-layered structure, formed by the apposition of 2 triple layered membranes separated by a pale space. The adjacent cytoplasm on each side of the junction shows an increased electron density. The second type is similar to the first, formed by the apposition of 2 triple-layered membranes which have fused to form a 5-layered attachment zone. Again, the neighbouring cytoplasm shows an increased electron density. The third type is one which has a widened space between the adjacent cell membranes, but this space is filled with opaque material, and the cytoplasm surrounding the junction is extremely electron dense.

Farquhar and Palade (1963) have proposed the terminology which is used in this study:

- i. 5-layered: tight junction (zonula occludens)
- ii. 7-layered: intermediate junction (zonula adhaerens)
- iii. desmosome (macula adhaerens).

They suggest that in ultrastructural studies, the name terminal bar should not be used, as it refers to both tight and intermediate junctions.

Generally the desmosome is basal to both tight and intermediate junctions, and tends to be localized, whereas the other two may be quite extensive.

Mesonephros and Opisthonephros

The term mesonephros is commonly used to refer to the adult kidney of anamniotes and to the second, transitory stage in the development of the amniote kidney (Fraser, 1950; Christensen, 1964). To avoid the confusion that sometimes arises from this double usage, some comparative anatomists (Hyman, 1942; Weichert, 1958; Romer, 1962) prefer to use opisthonephros (Kerr, 1919) to refer to the adult kidney of anamniotes. There are several good reasons, especially in this study, for making this distinction.

The mesonephros and opisthonephros appear to be histologically similar, but in the anamniotes, the adult kidney arises from mesomere tissue which, in the amniotes, gives rise to both meso- and metanephros. Thus the kidney of adult anamniotes represents, topographically, both meso- and metanephros. Further, the opisthonephros shows a concentration of tubules at its posterior end, while the anterior end may degenerate and may, in the male, become associated with the reproductive tract (Weichert, 1958 and others).

The structure of the kidney of the adult lamprey is outside the scope of this investigation, but in the present context it is worth noting that although it shows many features which are similar to the kidneys of other adult anamniotes, it is probably more similar to the lamprey pronephros than to any other vertebrate kidney. It has segmental glomeruli which unite to form a single, continuous glomus, ciliated funnels which appear to be identical with the pronephric nephrostomes, and which are not separated from the glomus by a Bowman's

capsule, and its anterior tubules undergo a degeneration almost identical to that seen in the pronephros. Youson (1968, personal communication) who has investigated in some detail the kidney of Petromyzon marinus, is inclined to agree with Wheeler (1899) in believing that the primordial opisthonephros differs greatly from the latter, more posterior kidney, and speculates that there may be a mesonephros as well as an opisthonephros. This point obviously needs some more clarification, but preliminary evidence indicates that the situation may not be as simple as is generally supposed. For these reasons, the non-pronephric kidney of the lamprey and of other anamniotes is called an opisthonephros in this study.

'Pericardial Cavity'

Other authors, in referring to the pronephros of the lamprey, usually indicate its position as being within the pericardial cavity (Wheeler, 1899; Goodrich, 1946; Young, 1950 and others). However, from studying the serial sections made during the course of this investigation, it appears that there is no subdivision of the coelom in the ammocoete; the cavity is continuous. Weichert (1958) states that there is a subdivision in the adult, formed by the sinus venosus and the ducts of Cuvier with their associated mesenteries, forming a true pericardial cavity; but as this division is not present in the larva, it is not strictly correct to refer to the presence of a pericardial cavity in the ammocoete.

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