Morphogenesis and growth of the definitive opisthonephros during metamorphosis of anadromous sea lamprey, *Petromyzon marinus* L.

By E. C. OOI and J. H. YOUSON

From the Department of Zoology, University of Toronto

SUMMARY

The definitive opisthonephros of the adult lamprey, *Petromyzon marinus* L., develops during metamorphosis from the nephrogenic cord confined within a nephric fold and extending from the posterior tip of the larval opisthonephros to the cloaca. This development is initiated prior to the first signs of external metamorphosis and begins with the simultaneous appearance of clusters of cells scattered along the entire length of the cord. Proliferation of these cell clusters and their elongation to connect to the closely associated archinephric duct results in the formation of rudimentary nephron units. Subsequent development involves the formation of tubular lumina, branching of the tubules, and the participation of the proximal ends of the newly formed tubules in the formation of the single renal corpuscle. Growth in size of the kidney is the result of lengthening of the existing tubules through cell proliferation rather than through the addition of new nephrons. This growth appears to be at the expense of adipose tissue within the nephric fold. During later stages of metamorphosis, cell proliferation is more prevalent in the ventral part of the nephric fold where a parallel system of tubules develops.

The development of the definitive opisthonephros during metamorphosis of lamprey may prove to be a useful model for further studies of tissue differentiation and interaction during kidney development in vertebrates.

INTRODUCTION

The parasitic adults of the anadromous form of the sea lamprey, *Petromyzon marinus*, have the ability to live in both fresh water and salt water (Potter & Beamish, 1977), yet during the filter-feeding larval stage (Beamish & Potter, 1975; Ooi & Youson, 1976) the animals reside in fresh water only. The larval (ammocoete) stage of at least 5 years is terminated by a phase of metamorphosis when the external and internal features of the adults are introduced (Youson, Wright & Ooi, 1977). Morphological transformation and biochemical changes that take place during metamorphosis (Hardisty & Potter, 1971) probably

1 Author's address: Department of Zoology, University of Toronto, Scarborough College West Hill, Ontario, M1C 1A4, Canada.
provide the adults with the mechanics necessary for osmoregulation in both environments (Morris, 1972).

The opisthonephric kidneys of the adults are thought to be one of the principal organs of osmoregulation (Morris, 1972; Youson, 1975). The kidneys are paired, strap-shaped structures each consisting of one elongated, dorsally located renal corpuscle, numerous tubules, and an archinephric duct. The tubular nephrons extend from the renal corpuscle and each is differentiated into a neck, proximal, intermediate, and distal segment before connecting with a collecting duct. The latter empty into the archinephric duct located along the ventral border of each kidney (Youson & McMillan, 1970a, b).

There are fundamental differences between the kidneys of the larvae and adults (for review see Youson & McMillan, 1970a, b; Morris, 1972). Although Wheeler (1899) suggested that the mesonephros (opisthonephros) of the larval lamprey is not the same organ as the opisthonephros of the adult, he was not certain whether the ammocoete opisthonephros becomes part of the adult opisthonephros. More recent preliminary studies indicated that the larval opisthonephros does not contribute at all to the formation of the definitive opisthonephros in the adult lamprey (Youson, 1970) and there are no signs of the adult opisthonephros prior to metamorphosis (Ooi & Youson, 1976). However, there is little information on the mode of development of the definitive opisthonephric kidney during metamorphosis of lamprey. Indeed, the development of the lower vertebrate kidney during late stages of ontogenesis (Balinsky, 1975) has so far only been studied in amphibians (Hall, 1904; Gray, 1930, 1932).

We have therefore undertaken a study of the morphogenesis and pattern of growth in the definitive opisthonephros of anadromous P. marinus during metamorphosis, using light microscopy, autoradiography, and quantitative techniques.

Since there is an inconsistent usage of terms to designate the kidneys of lower vertebrates, it seems necessary to explain the use of the term opisthonephros in the present study. The term 'opisthonephros', originally proposed by Kerr (1919), includes the whole series of tubules behind the pronephros in anamniotes (Goodrich, 1930). According to Hyman (1949), the opisthonephros 'has used up the mesomere tissue from which in amniotes both mesonephros and metanephros come. It therefore is not exactly equivalent to the amniote mesonephros but topographically represents both mesonephros and metanephros.' The term 'larval opisthonephros' is used for the ammocoete kidney in preference to 'mesonephros' because the mesonephros in amniotes is an embryonic structure and is usually incorporated within the male reproductive duct system during ontogeny (Torrey, 1971). No reproductive duct system occurs in lampreys (Gerard, 1954). The 'definitive' opisthonephros is exclusively an adult organ.
MATERIALS AND METHODS

Collection and maintenance of animals

Metamorphosing sea lampreys, *Petromyzon marinus*, were collected by means of an electric-shocking device from Dennis Stream near St Stephen, New Brunswick, between 27 August and 4 September 1973, between 13 and 22 August 1974, and between 7 and 26 July 1975. Metamorphosing animals were classified as to a 'prometamorphic stage' (Youson *et al.* 1977) and five stages (I-V) of metamorphosis based on external criteria (Manion & Stauffer, 1970). The most advanced stage (V) was a juvenile, non-feeding adult (macrophthalmia) stage (Manion & Stauffer, 1970; Beamish & Potter, 1972). A total of 115 prometamorphic and metamorphosing lampreys, ranging in length from 113 to 161 mm and in weight from 2·4 to 6·7 g, were used in the present investigation. In addition, 20 large ammocoetes ranging in length from 112 to 141 mm were also used.

Some animals were used immediately after their collection and transport to the Huntsman Marine Laboratory at St Andrews, New Brunswick. The remainder were transported to the laboratory at Scarborough College and were maintained at 20 ± 1 °C in fibreglass or glass tanks containing aerated, dechlorinated tap water and 10 cm of river silt. The water temperature roughly paralleled that of the stream from which the animals were obtained. Metamorphosing animals which were captured in early stages were permitted to reach more advanced stages before they were used. Several animals at the macrophthalmia stage were allowed to feed on carp, *Cyprinus carpio* L., kept at 10 °C from 2 to 8 weeks.

Before killing, several groups of animals received injections of [3H]thymidine (New England Nuclear, specific activity 46·4 Ci/mmmole). The animals were anaesthetized, measured, killed 6 h later, fixed *in toto* in Bouin’s fluid for at least 24 h, and stored in 70 % ethanol for variable periods. The nephric fold from the posterior end of the larval opisthonephros to the cloaca was then excised in the ammocoetes while in metamorphosing animals portions of the body were taken which contained either an anterior or a posterior region of the definitive opisthonephroi. The tissue samples were then dehydrated in a graded series of ethanol, cleared in terpineol, and embedded in Tissue-Prep (m.p. 56·5 °C, Fisher Scientific). Serial transverse or longitudinal sections were cut at 7 μm and mounted on precleaned glass slides (Baserga & Malamud, 1969).

The slides from animals which had received injections of [3H]thymidine were oxidized with periodic acid according to Sawicki & Rowinski (1969), washed in distilled water, air-dried, and then dip-coated with Kodak NTB-2 Nuclear Track Emulsion according to the method of Kopriwa & Leblond (1962). After exposure for 14 days, the autoradiographs were developed in Kodak D-19 developer and fixed in Kodak fixer. They were eventually post-stained with
Schiff's reagent (prepared according to Lillie, 1951), counterstained with Mayer's acid haemalum (modified by Lillie, 1942), and mounted in Permount. Sections of kidney tissue from lampreys which had not received injections of an isotope were occasionally included in the procedure above to serve as controls.

**Routine light microscopy**

The kidneys of a number of uninjected ammocoetes and transforming lampreys were removed and prepared for routine light microscopy as described above, except that sections were cut at 10 μm.

Measurements were made of the cross-sectional areas of the kidney tissues within the nephric folds of metamorphosing lampreys from the slides prepared for routine light microscopy and for autoradiography. Tissues from three lampreys at each of the various stages of metamorphosis and the prometamorphic stage were measured. Ten sections chosen at random from each of the middle segments of these kidney samples were photographed in the light microscope with an ×4 objective lens. Negatives were enlarged onto photographic paper to reveal in detail the developing definitive opisthonephroi. The cross-sectional areas of both the developing opisthonephroi and the entire nephric folds were determined using a compensating polar planimeter (Keuffel & Esser); each of 10 sections from each animal was measured twice and an average was taken. The cross-sectional area of the definitive kidney was expressed as a percentage of the whole cross-sectional area of the nephric fold to compensate for the difference in size of the animals within each stage of metamorphosis.

**RESULTS**

The nephrogenic cords, which would give rise to the definitive opisthonephroi during metamorphosis, were located within the portion of the nephric folds which extended from near each terminus of the larval opisthonephros to the cloaca. Each cord of nephrogenic tissue consisted of two parallel rows of cell

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**Figures 1-4**

Fig. 1. Autoradiograph of a transverse section of a portion of the nephrogenic cord (arrow) and the archinephric duct (A) within the posterior section of the nephric fold of an ammocoete. There is no evidence of [³H]thymidine incorporation. × 600.

Fig. 2. Transverse section of the posterior section of the nephric folds of a prometamorphic animal revealing the earliest sign of development of the definitive episthonephroi (arrows). Note the renal artery (R). × 80.

Fig. 3. Longitudinal section near the ventral tip of a nephric fold from a prometamorphic animal showing the archinephric duct (A) and the intermittent arrangement of rudimentary nephron units (arrows). × 130.

Fig. 4. Transverse section showing the connection between a Y-shaped rudimentary nephron unit (R) and the archinephric duct (A) in an early prometamorphic animal. × 600.
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1

2

3

4

A

R

15:2
condensations which were derived from the peritoneum on the medial walls of the nephric folds (Fig. 1). The cords ran parallel with and a little above the archinephric duct which were located along the ventral edge of the nephric folds (Fig. 1). The tissue of the nephrogenic cords appeared to be uniform and continuous, for there was no apparent periodic thickening throughout its length. No \([^3]H\)thymidine was incorporated into the cells of this cord prior to metamorphosis (Fig. 1).

The differentiation of the nephrogenic tissue began in animals at the 'prometamorphic stage' when external signs of metamorphosis were not evident (Youson et al. 1977). At this point, degeneration of the larval opisthonephros could not be observed in the light microscope. There was no sharp demarcation between the two kidneys at their point of junction. The first sign of differentiation was the simultaneous increase in the number of cells in the nephrogenic tissue in both kidneys (Fig. 2). This occurred at intervals along the entire length of the cord (Fig. 3), and resulted in the appearance of a parallel series of equally spaced, rod-shaped cell clusters. Two opposite rods of cell clusters elongated distally and united to become an inverted Y-shaped structure (Fig. 4). The distal end of this structure eventually extended to the archinephric duct and finally established a connexion with the latter, thus producing a rudimentary nephron unit (Fig. 4). Autoradiographic examination revealed that many of the cells within the rudimentary nephrons at this early stage were synthesizing DNA. [\(^3\)H]Thymidine was also incorporated into cells in the archinephric ducts in both the larval and in the developing definitive opisthonephroi, as well as in the renal corpuscles and haemopoietic tissue of the larval opisthonephroi.

At later stages of prometamorphosis, the rudimentary nephron units simultaneously first acquired lumina (Fig. 3) and this was followed by branching

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**Figures 5-8**

**Fig. 5.** An autoradiograph of a transverse section of the developing kidney in a prometamorphic animal revealing further branching of a rudimentary nephron unit at its proximal end and connexion of its distal end to the archinephric duct. Labelled cells occur in the rudimentary nephron unit (small arrows), the mesenchyme (M), and the archinephric duct (large arrow). \(\times 450\).

**Fig. 6.** Transverse section through a portion of a nephric fold of a stage I animal demonstrating numerous tubules radiating in a semi-circle from the former position of the nephrogenic cord. The proximal end of one of the tubules is dilated (arrow) and the distal ends of several tubules unite with a common collecting tubule (C) before it connects with the archinephric duct (A). \(\times 400\).

**Fig. 7.** Transverse section of a portion of the nephric fold of an early stage II animal revealing the renal corpuscle (R) and the convoluted appearance of the proximal portion (arrows) of the tubules. \(\times 370\).

**Fig. 8.** Transverse section of the nephric fold of a late stage II animal demonstrating the large renal corpuscle (R) and the further growth of the tubules (T). The majority of the nephric fold is composed of adipose tissue (AT). \(\times 120\).
of the primary rudimentary nephrons, giving rise to secondary units of rudimentary nephrons (Fig. 5). This lengthening and branching was due to an intense proliferative activity of the cells of the rudimentary nephrons, as evidenced by the presence of mitotic figures and by the labelling of cells with [³H]thymidine (Fig. 5). Mesenchyme cells in the interstitium between the rudimentary nephrons were also undergoing DNA synthesis (Fig. 5).

By stage I of metamorphosis, tertiary units of rudimentary nephrons resulted from further branching of the proximal portion of the primary rudimentary nephrons (Fig. 6). An examination of serial sections revealed that there were 7–9 tubules draining into one collecting tubule which in turn opened into the archinephric duct. Autoradiography indicated that regional differential growth of the tubules began to occur during stage I of metamorphosis. In animals of this stage, the distribution of cells synthesizing DNA appeared mainly in the proximal portions of the developing tubules. Mitotic figures were also most prevalent in this region. The ciliated neck and the proximal segments of each developing nephron appeared to increase in length rather rapidly during stage I of metamorphosis. As the tubules increased in length through active proliferation of their cells, they became more convoluted in appearance (Fig. 7). The proximal ends of the tubules which remained associated with the peritoneum became dilated (Fig. 6) and were arranged in a particular pattern such that in every transverse section five or six tubules appeared to radiate in a semicircle from the position of the original undifferentiated cord (Fig. 6). The renal arteries appeared early in development (Fig. 2) and, after the subsequent appearance of the afferent and efferent arterioles and the capillaries, the compound renal corpuscle began to be formed (Figs. 6, 7). As metamorphosis proceeded, the glomus (compound glomerulus) became increasingly more elaborate upon the development of the renal vasculature and the nephric capsules (Fig. 7). In animals of late stage II of metamorphosis, the renal

Figures 9–12

Fig. 9. An autoradiograph of the ventral portion of the kidney of a late stage II animal showing numerous labelled cells in distal portions of the tubular nephrons (small arrows). Cells of the archinephric duct (large arrows) are also labelled. × 265.

Fig. 10. An autoradiograph of the kidney of a stage III animal revealing labelled cells mainly in the ventral portion (small arrow). Cells of the archinephric duct are labelled (large arrow). × 110.

Fig. 11. An autoradiograph of the ventral portion of a kidney from a stage IV animal demonstrating the presence of labelled cells in the archinephric duct (large arrow) and in straight parallel tubules (small arrows). × 275.

Fig. 12. A transverse section of a kidney from a feeding, newly metamorphosed adult indicating the small amount of dorsally located adipose tissue (AT), the large renal corpuscle (R), and the parallel tubules (arrow) in the ventral part of the kidney. Compare with the kidney in Fig. 8. × 110.
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corpuscle was large and appeared to be complete (Fig. 8). Many labelled cells were found in the region around and within the renal corpuscle, but more labelled cells now became localized more distally (Fig. 9) in the position of the distal and collecting tubules of the mature adult opisthonephros (Youson & McMillan, 1971). A greater frequency of labelling was also observed in the epithelium of the archinephric duct (Fig. 9) than at earlier stages. Thus, there was a tendency for a change in the pattern of cell proliferation toward more distal portions of the tubules. In animals of stage II and the later stages of metamorphosis, the pattern of DNA synthesis suggested that no new nephron units were added. Instead, increase in size of the definitive opisthonephros appeared to be the result of growth in length of the existing tubules through cell proliferation.

The pattern of differential distribution of cells synthesizing DNA in the tubules seemed to be more conspicuous during stage III of metamorphosis (Fig. 10), and the tubules in the distal region near the archinephric duct tended
Table 1. Cross-sectional areas of kidney tissue regions and nephric folds of the definitive opisthonephroi in lampreys at various stages of metamorphosis

<table>
<thead>
<tr>
<th>Stage of metamorphosis</th>
<th>No. of animals observed</th>
<th>Body length (range, mm)</th>
<th>Cross-sectional area of nephric fold, mm$^2$ (mean ± 1 S.E.)</th>
<th>Cross-sectional area of kidney region (mm$^2$) (mean ± 1 S.E.)</th>
<th>Kidney area as percentage of whole area of nephric fold (mean % ± 1 S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prometamorphic</td>
<td>3</td>
<td>118–139</td>
<td>0·561 ± 0·032</td>
<td>0·023 ± 0·010</td>
<td>4·2 ± 0·1</td>
</tr>
<tr>
<td>I</td>
<td>3</td>
<td>123–135</td>
<td>0·514 ± 0·019</td>
<td>0·062 ± 0·004</td>
<td>12·1 ± 0·6</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>119–128</td>
<td>0·536 ± 0·010</td>
<td>0·103 ± 0·012</td>
<td>20·3 ± 0·4</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>114–125</td>
<td>0·601 ± 0·023</td>
<td>0·193 ± 0·011</td>
<td>32·1 ± 1·2</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>117–126</td>
<td>0·411 ± 0·017</td>
<td>0·191 ± 0·014</td>
<td>46·5 ± 0·7</td>
</tr>
<tr>
<td>Macrophthalmia-</td>
<td>3</td>
<td>127–134</td>
<td>0·555 ± 0·031</td>
<td>0·414 ± 0·029</td>
<td>74·6 ± 1·1</td>
</tr>
<tr>
<td>non-feeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophthalmia-</td>
<td>3</td>
<td>121–145</td>
<td>0·837 ± 0·032</td>
<td>0·694 ± 0·024</td>
<td>82·9 ± 0·2</td>
</tr>
<tr>
<td>feeding</td>
<td></td>
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To grow in parallel pattern (Fig. 10). In addition labelled cells continued to be observed in the archinephric duct (Fig. 10). A similar pattern of distribution of cells labelled with $[^3]$Hthymidine continued into stage IV of metamorphosis. However, there appeared to be an overall decrease in the number of labelled cells in the definitive opisthonephros of the lampreys at this stage. Perhaps as a result of the differential growth rate and rapid increase in length of the distal and collecting segments of the tubules, a distal region of parallel tubules appeared to be becoming more prominent in the developing kidney (Fig. 11). The archinephric duct also appeared larger, presumably as a result of the rapid proliferation of the cells in stage III.

The opisthonephros continued to grow slowly during the macrophthalmia stage (newly transformed, non-feeding adult stage), as evidenced by the presence of fewer labelled cells. Nevertheless, once the newly metamorphosed adult began to feed on a host fish, the definitive opisthonephros seemed to increase in size very rapidly, replacing most of the adipose tissue of the nephric fold with kidney tubules (Fig. 12).

In ammocoetes the whole nephric fold of the prospective definitive opisthonephros, except the archinephric duct and the nephron rudiment, was composed of adipose tissue (Ooi & Youson, 1976). After the onset of metamorphosis (Fig. 2), the tissue of the developing definitive opisthonephros began to increase in size (Fig. 8) and the dorsal mass of adipose tissue within the nephric fold was gradually reduced. During the early phase of the development of the definitive opisthonephros, the cross-sectional area of kidney tissue, expressed as a percentage of the whole cross-sectional area of the nephric fold, increased relatively slowly (Fig. 13, Table 1). The average percentages for the prometa-
morphic stage and stage I and II of metamorphosis were 4.2%, 12.1%, and 20.3% respectively. From the metamorphic stage II to the termination of metamorphosis, as determined by the onset of parasitic feeding, the cross-sectional area of the definitive opisthonephros increased rapidly and steadily. The average percentage areas for stage III, stage IV, and the macrophthalmia stage were 32.1%, 46.5%, and 74.6% respectively (Fig. 13, Table 1). The most extensive growth in kidney tissue occurred between stage IV and the macrophthalmia stage. At the same time the nephric fold did not increase in size (Table 1). The percentage of the cross-sectional area of the definitive opisthonephros in the lampreys after initial feeding on the host fish for a few days was 82.9%. There was a substantial increase in the areas of both the entire nephric fold and the kidney tissue (Table 1). Consequently, the cross-sectional area occupied by adipose tissue within the nephric fold became reduced as metamorphosis of the lamprey proceeded.

DISCUSSION

Schneider (1874) was the first to claim that the larval opisthonephros is completely replaced by an adult organ by the end of metamorphosis and this observation was subsequently supported by Vialleton (1890). Wheeler (1899) came to a similar conclusion by comparing the position of the larval opisthonephros before metamorphosis with the location of the definitive opisthonephros in mature adults. He further maintained that the atrophy of the opisthonephric kidney at the anterior end progresses simultaneously with the addition of new tubules to the posterior end throughout the entire larval life until the end of metamorphosis, and that the adult kidney forms progressively as a result of these two simultaneous processes. However, it is clear from the present study that the definitive opisthonephros of the lamprey, *Petromyzon marinus*, develops only during the period of metamorphosis and that there are two separate opisthonephroi during the life-cycle.

The developmental histories of the opisthonephroi in lamprey and amphibians are similar. Gray’s accounts (1930, 1932) of the development of the mesonephric (opisthonephric) kidneys in *Rana temporaria* and *Triton vulgaris* show that in these amphibians there are two distinct sets of excretory units, namely, the early units which develop in the larval stage, and the later units which develop into the definitive kidney of the adult. The early units are thought to play no role in the formation of the definitive units (Gray, 1932). The existence of two units of the opisthonephros in the course of amphibian development has been considered to be of some phylogenetic significance. It has been assumed that the early (larval) units represent an ancestral condition where a long larval life had no need for great speed in production of an adult kidney (Gray, 1930, 1932). The existence of two generations of opisthonephroi in lampreys is perhaps support for this interpretation in amphibians.
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The definitive opisthonephros of lampreys develops from a mass of mesenchyme, the nephrogenic cord, which has resulted from the dissolution of cells of the mesodermal nephrotomes (Goodrich, 1930). This has also been reported for the development of the ammocoete opisthonephros (Ooi & Youson, 1976) and for the kidneys of amphibians and higher vertebrates (Balinsky, 1975). However, there exist some basic differences in the mode of development of the larval opisthonephros (Ooi & Youson, 1976) and the definitive opisthonephros of lamprey. The nephrogenic cord of the prospective larval opisthonephros consists of serially arranged clusters of cells, the nephron rudiments. Beginning at the anterior end, new nephrons of the larval opisthonephros are added to the posterior tip through periodic differentiation of the nephron rudiments during the first three years of larval life. The larval opisthonephros continues to lengthen in an anterior to posterior direction only up to about the midpoint of the length of the body cavity (Ooi & Youson, 1976). Each definitive opisthonephros begins development at the very onset of metamorphosis by the simultaneous stimulation and differentiation of all the nephrogenic tissue which extends from near the posterior tip of the larval kidney to the cloaca. During metamorphosis further development of the definitive opisthonephros involves the elongation and branching of existing rudimentary nephrons, rather than the addition of new tubules at the posterior end, as in the larval opisthonephros (Ooi & Youson, 1976).

The simultaneous development of all nephron units throughout the whole nephrogenic tissue allows the adult kidney to be produced during the relatively short period of metamorphosis. This may reflect the urgent need for a functional organ of excretion and osmoregulation, owing to the massive degeneration of the larval opisthonephros (Youson, 1970). The morphological observations of the present study suggest that the definitive opisthonephros possesses the machinery to assume kidney function during late stage II of metamorphosis. This coincides with a significant increase in serum osmolality during early stages of metamorphosis in landlocked P. marinus (Mathers & Beamish, 1974). Although there are also significant changes at metamorphosis in the morphology of the other primary organs of osmoregulation, the alimentary canal and gills (Youson et al. 1977), the change in kidneys between ammocoetes and adults is one of the most dramatic morphological events of metamorphosis. The ammocoete opisthonephros is completely replaced by an opisthonephros with a different morphology (Youson & McMillan, 1970a, b). This suggests either that the kidney plays a significant role in osmoregulation during the parasitic phrase of the life-cycle, or that the changes occurring at metamorphosis reflect the phylogeny of the kidney in cyclostomes (Youson, Hansen & Campbell, 1974). The appearance of the parallel arrangement of tubules in the distal portion of the kidney in animals of stage III of metamorphosis suggests the early establishment of a functional unit of osmoregulatory importance to the lamprey (Youson & McMillan, 1971; Morris, 1972). However, landlocked
animals of this stage are unable to osmoregulate in even 10% artificial sea water (Mathers & Beamish, 1974).

During the early phase of development in the definitive opisthonephros in animals of the prometamorphic stage and stage I of metamorphosis, the presence of numerous mitotic figures and cells labelled with $[^3]$H thymidine indicates intense proliferative activity. There is a marked decrease in the number of cells synthesizing DNA during the later stages of metamorphosis. A similar decrease in DNA synthesis is observed in the mesonephros during larval development of *Xenopus laevis* (Goldin & Fabian, 1975) and in proliferative activity during the postnatal development of the metanephric kidney in the mouse (Litvak & Baserga, 1964). A marked reduction in the number of cells synthesizing DNA has also been shown during morphogenesis of many organs, such as the postnatal liver (LeBouton, 1976) and the heart (Jeter & Cameron, 1971; Chacko, 1973). Following the onset of differentiation of the nephrons from the nephrogenic tissue, the definitive opisthonephros increases in size through proliferation of cells in the nephrons, for no new rudimentary nephron units are formed. The kidney is thus an example of an expanding cell population (Messier & Leblond, 1960; Leblond, 1972). However, the expansion of the cell population is not uniform. The differential growth of certain segments of the tubular nephrons during various stages of metamorphosis is reflected in changes in the distribution of cells that synthesize DNA and divide in the definitive opisthonephros. In lampreys of the prometamorphic stage and stage I of metamorphosis, the distribution of labelled cells appears mainly in the proximal portions of the developing tubules. In animals of late stage II and the later stages of metamorphosis, cells synthesizing DNA become progressively localized more distally in the position of the presumptive distal and collecting segments. A similar pattern of differential distribution of labelled cells after the injection of $[^3]$H thymidine has been reported for the postnatal growth of the adrenal cortex (Ford & Young, 1963), the submandibular gland (Chang, 1974), and the simple liver acinus in neonatal rats (LeBouton & Marchand, 1970; LeBouton, 1974).

The relative amount of stored lipid increases in ammocoetes as they approach metamorphosing size (Lowe, Beamish & Potter, 1973) and enter an 'arrested growth phase' (Hardisty & Potter, 1971), in preparation for their non-trophic period of 5–6 months (Beamish & Potter, 1972). The progressive decrease in lipid content of animals as they proceed through metamorphosis suggests the utilization of lipid as a source of energy (Lowe *et al.* 1973). Two of the sites for the deposition of lipid in ammocoetes are believed to be the dorsal part of the nephric fold in the ammocoete opisthonephros and the entire fold extending from the posterior tip of the ammocoete opisthonephros to the cloaca, destined to become the definitive opisthonephros (Percy & Potter, 1977). During metamorphosis the larval opisthonephros and the nephric fold associated with it degenerates (Ooi, 1977), while the remaining nephric fold houses the developing
definitive opisthonephros. The progressive increase in cross-sectional areas of the definitive opisthonephroi as metamorphosis proceeds therefore occurs at the expense of adipose tissue, which is in line with the observation that the lipid content of landlocked *P. marinus* decreases curvilinearly during metamorphosis (Lowe *et al.* 1973).

The archinephric duct provides an inductive stimulus for the differentiation of nephrons in both the opisthonephroi of amphibians and in the mesonephroi of chick embryos (for reviews see Fraser, 1950; Gruenwald, 1952; Burns, 1955; Fox, 1963; Torrey, 1965). However, there are no experimental studies of induction of nephrons in the opisthonephroi of fishes. In the present investigation it was observed that the epithelium and related tissue of the archinephric ducts of lampreys were stimulated into DNA synthesis as a result of metamorphosis. Although the present observations do not show whether any induction was involved, evidence from other investigations suggests a two-step process (Gossens & Unsworth, 1972) involving an inductor, nephrogenic mesenchyme, and the surrounding non-induced mesenchymal cells. In most anamniotes studied, the archinephric duct provides the inductive stimulus (Torrey, 1965). It may therefore be significant that the archinephric duct was stimulated into DNA synthesis throughout its entire length, including the portion within the larval opisthonephros which contained no associated nephrogenic tissue and which was destined to degenerate. As DNA synthesis was absent in other parts of the larval opisthonephros, with the exception of the haemopoietic tissue, this indicates that the archinephric duct may have specific properties which are different from all elements of the fully differentiated nephrons and also from the cells of the nephrogenic cord. The relationship of the archinephric duct to kidney morphogenesis in fishes requires further investigation; the transformation of the nephric system during metamorphosis of lamprey may prove to be a model system for this study. The development of the definitive opisthonephros during metamorphosis also provides an ideal opportunity to examine the various steps in the differentiation of the segments of the tubular nephron and renal corpuscle in a complex kidney of a lower vertebrate.

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**REFERENCES**


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