

JOURNAL

OF

MORPHOLOGY.

THE DEVELOPMENT OF THE COXAL GLAND, BRANCHIAL CARTILAGES, AND GENITAL DUCTS OF LIMULUS POLYPHEMUS.

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I. INTRODUCTION.

THE work described in the following pages was mainly done, during the year 1895 and 1896, in the Biological Laboratory of Dartmouth College, and was continued through the summer season of 1896 in the Marine Biological Laboratory at Woods Holl.

The problem we had in mind at first was the development of the branchial cartilages, but it was finally deemed advisable to work out, in connection with this problem, the development of the genital ducts and the "coxal gland," or nephridia, as all three of these organs are closely associated with one another during development.

We were in a certain measure prepared for the independent origin of the nephric lobes and nephric duct by the discovery that in the adult kidney, as seen by the aid of injections, a distinct nephric duct is present, which could hardly be anything else than the tube seen by other investigators in the embryos.

This left the development of the nephric lobes to be accounted for in some other way than as a modification of the embryonic duct.

We are indebted to Professor Whitman for the privileges of the Marine Biological Laboratory at Woods Holl and to Dr. William A. Redenbaugh for assistance in working out the course and origin of the nerves found in the region of the nephridial lobes.

II. METHODS.

The *embryological material* was killed either in Perenyi's fluid, picro-nitric acid, picro-sulphuric acid, corrosive sublimate, or formalin. The most satisfactory results were obtained with Perenyi's fluid. The embryos remained in it overnight. In the morning the much distended membranes could be easily removed, and the eggs placed at once in large quantities of 95% alcohol, which was changed often to prevent the yolk from swelling and cracking. Low grades of alcohol must be avoided in the preliminary stages of hardening.

The embryos were stained *in toto* with Delafield's haematoxylin or with borax carmine, followed by Lyon's blue on the slide. The larvae and young Limuli were treated with Delafield's haematoxylin, and picro-acid fuchsin, or eosin.

The *nephric duct* may be injected by forcing a canula into its external opening at the base of the fifth leg, or it may be ligatured into the end of the duct, just below the shell.

Starch masses, or a thick celloiden mixed with lamp black, were used for injections. After celloiden injections are hardened in 80% alcohol for several days, the connective tissue may be dissected away from the duct, and its course followed without much difficulty. Complete casts of the duct may be obtained by maceration in strong hydrochloric acid; but in all cases the casts which were made showed a good deal of shrinkage. A good starch, asphalt, or celloiden mass will completely fill the duct and penetrate deeply into the nephridial lobes.

III. CRITICAL REVIEW.

The Nephridia of *Limulus* were first described by Packard in 1875, who concluded that they were renal organs, comparable with the green glands of Crustacea. In 1880, he compared them with the shell glands of the Entomostraca.

Lankester described them in 1880, and later ('82) discussed their histological structure in detail, comparing them with the coxal glands of scorpions and Mygale. Both Packard and Lankester saw only the lobes and the longitudinal stolon of the adult, and entirely overlooked the large thin-walled duct and its external opening. Packard tried to find a duct by injecting the gland, but failed. In 1895 Tower discovered the opening of the gland in the adult in the interarticular membrane on the posterior side of the base of the fifth leg. It is situated, as he correctly states, on a papilla, readily seen with the naked eye, and surrounded by a dark gray ring.

Gulland ('85) and Kingsley ('85 and '93) gave an incomplete description of the embryonic duct and its relation to the fifth coelomic cavity. They were deceived, however, in mistaking the developing duct and its end sac for the developing nephric

lobes, while the nephric lobes themselves were entirely overlooked. They were further misled by not knowing the existence of the adult duct with its permanent external opening, and other characters, which clearly distinguish it from the nephric lobes.

Kingsley ('93) supposed that the four nephridial lobes of one-half of the body arose from a portion of the coelom of the fifth somite, which had retained its position on the ventral side of the embryo after the other coelomic cavities had emigrated toward the dorsal surface. The fate of the dorsal portion of the fifth somite was not determined, but he states that it persists as a perfectly distinct cavity with epithelial walls on either side of the heart. From its position and from its posterior termination he is inclined to think that this portion of the coelomic epithelium is finally converted into the reproductive organs (p. 200). "For a considerable time," he says, the ventral portion of this coelomic cavity "shows no change worthy of remark." At length, however, it "begins to elongate and to become bent upon itself like the letter *U*, the rounded portion being directed anteriorly." An ingrowth of ectoderm unites with this tube to form its outer opening, situated on the posterior side of the coxa of the fifth leg. He also states that "in the neighborhood of the coxal gland at this stage may be seen numerous lacunae in the mesoderm, which is rapidly assuming the trabecular condition characteristic of the later stages" (p. 203). He was unable to trace any connection between these lacunae and the coelomic cavity, and is strongly of the opinion that none exists. Again he states (p. 204) that there is a "formation of trabeculae of mesodermal tissue which invade the cavity, and, passing from wall to wall of the proximal portion of the organ, tend to subdivide it and give it an anastomosing character." In the older stage he finds a fenestration in the region of the end sac, which is "the beginning of the anastomosing condition of the adult, while the proximal (internal) limb is thrown into a series of four outwardly directed diverticula, which are segmentally arranged and occupy somites 2-5" (p. 205). Kingsley suggested "that the whole organ of the adult is derived from the coelom of somite *V*, and that the apparently metameric lobes figured by

Packard . . . are not the remnants of the Nephridia of the corresponding somites, but are rather the derivatives of the diverticula of the duct" (p. 205). He maintains that, "besides an increase in the size of these lobes, all that is necessary to convert his reconstruction into the 'coxal gland' of the adult are closures of the external opening, more or less complete fusion of the two limbs of the duct, accompanied by an increase in the anastomoses, the result being to convert coelom and duct into the spongy tissue of the adult." Mr. Gulland's account does not differ materially from this, but is not as complete. He speaks of septa, which grow inside the tube and divide it. In one place he found "the tube continuous with connective-tissue spaces, which everywhere surround the gland."

Our own observations have shown that there is no division of the fifth somite into a dorsal and a ventral portion. It is very clear, also, that the nephric duct is not a transformation of the ventral portion of this somite, but a special epithelial outgrowth from it, and that the rest of the somite persists as the end sac only. There are no segmental diverticula of the duct, and no part of the duct is converted into the glandular tissue of the nephric lobes. It remains practically unchanged, except in length and in the number of its convolutions, as the permanent nephric duct of the adult. The nephridial lobes are derived from segmental clusters of cells which arise independently of the duct from the somatic layer of the second, third, fourth, and fifth thoracic somites.

It is obvious that the comparisons and conclusions of our predecessors in this line of work were based on incomplete anatomical and embryological data. We must still wait a more thorough study of the anatomy and development of the green glands, coxal glands, and shell glands of other arthropods before their relations to one another can be satisfactorily determined.

IV. FORMATION OF THE MESOBLASTIC SOMITES AND BLOOD SINUSES.

The Thoracic Somites.—Soon after the formation of the thoracic appendages, and before the abdominal ones have

appeared, a solid mass of mesoderm cells is seen at the base of each thoracic appendage. Each mass of cells gradually extends in a lateral direction to form a transverse band quite distinct from those on the opposite side of the body. Beneath the mesoderm, and enclosing the yolk, is a thin non-cellular membrane (Pl. XXIV, Figs. 35 and 36), which gradually separates from the central portion of each mesodermic band, but remains continuous with it around its margins. At the same time nuclei appear to migrate from these margins into the membrane. In this way an imperfectly closed sac, the *somite*, is formed with a very thick outer wall, the somatic, and a thin inner one, the splanchnic layer (Pl. XXIV, Figs. 37 and 38).

Each somite now grows rapidly in a lateral direction, making with its mate on the opposite side almost a half circle. The concave side of the posterior thoracic somites is directed backwards, that of the anterior somites forwards.

As the appendages grow in length, a space is formed between the ectoderm, forming the apex of the leg and the thick somatic mesoderm at its base. A few scattered mesoderm cells remain attached to the inner surface of the ectoderm, and they gradually form the walls of a spacious cavity, which later is converted into the blood channel that passes through the center of each leg. We could not certainly determine whether this space is to be regarded as a part of the coelom, as in the abdominal sinuses, or not.

The further history of the thoracic somites, except the fifth, has not been carefully studied.

The abdominal somites develop in a different way and are much more clearly defined. They separate from the paired bands of mesoderm formed by the primitive streak as hollow masses, with distinct and continuous walls of nearly uniform thickness. Each abdominal somite is at first quite separate from all the others, and its cavity remains closed for a long time. Figs. 2-8, Pl. XXII, are longitudinal sections showing the first three abdominal somites and a part of the primitive streak in an embryo in which the abdominal appendages are beginning to appear.

In this series the median ends of the somites are thin-walled

tubes; towards their lateral ends their somatic walls are much thickened to form the muscular tissues of the appendages.

The chelarial segment, it will be observed, contains a well-defined abdominal somite, although it is smaller than those in front or behind it. This fact shows conclusively that the chelaria are true appendages, having the same morphological value as the other appendages of the body.

The opercular somite is the largest, the following ones gradually diminish in size from before backwards.

Figs. 8 and 9, Pl. XXII, represent longitudinal sections through an older specimen. They show four fully formed abdominal somites, with a fifth one just separating from the anterior end of the primitive streak.

The somites grow over the surface of the yolk in a postero-lateral direction, till they unite in the median dorsal line. The circular bands of mesoderm thus formed become smaller and smaller in diameter from the opercular segment towards the posterior end of the body.

At an early period the distal ends of all the somites become continuous with a thickened band of proliferating cells that forms a well-defined margin extending round the whole embryonic area (see Patten, '90, p. 373).

The somites of the opercula and gill-bearing segments persist as the well-defined blood sinuses leading from the abdominal appendages to the pericardium.

V. THE DEVELOPMENT OF THE GENITAL DUCTS.

The genital ducts arise as diverticula from the median ventral side of the opercular somite, and extend towards the median line along the base of the opercular cartilages. They lose (?) their connection with the somites as soon as two or three gill leaves are formed on the first branchial appendage, and then remain in a very rudimentary condition until after the second larval stage. The distal ends of the diverticula finally unite with shallow invaginations of the ectoderm, while their proximal ends unite in a manner not yet determined with the genital organs.

The first traces of the genital ducts were found in embryos having three abdominal somites (Pl. XXII, Figs. 1-8). In this series the sections begin near the median line and extend towards the lateral ends of the somites. Near the median line are a few loose mesoderm cells. Those near the surface of the yolk are undergoing degeneration. Their nuclei contain large granules that stain deep red in borax carmine, and the nuclear membrane seems in some cases to have ruptured.

The lumen of each somite increases in size as one moves away from the median line. At the same time the somatic layer increases rapidly in thickness, till just beneath the appendages it is many cells deep. The splanchnic layer contains only a few isolated nuclei. The lateral ends of the somites have no lumen and consist of mesoderm cells not arranged in distinct layers.

About midway between the median and lateral ends of the opercular somite the genital duct appears as a long diverticulum, extending from the coelom into the somatic layer (Pl. XXII, Fig. 6, *g.d.*). A similar diverticulum, although not as marked, is shown in the first gill in Pl. XXII, Fig. 5. It was not determined whether this was a rudimentary genital duct or the beginning of the branchial cartilage.

In Pl. XXII, Figs. 2 and 3, a body resembling an extra somite is present between the operculum and the first gill. Its median end was hollow, while its lateral extremity was united with the mesoderm of the first gill (Pl. XXII, Figs. 2-4, *SO*^o). Such a structure was not seen in any other series of sections of this age.

In the next stage five abdominal somites are present. They are shown in Pl. XXII, Figs. 9-20, which represent a series of longitudinal sections beginning near the median line and extending almost to the lateral ends of the somites.

In the opercular and first gill segment the somites have moved towards the yolk, leaving behind at the base of each appendage a *thick ring of mesoderm*, derived from the somatic mesoderm (Pl. XXII, Figs. 12-20, *SO*¹-*SO*⁵). As the appendage grows in length, a space is formed between its ectoderm and the ring of mesoderm at its base; and in these spaces and around the

somites are found numerous blood corpuscles, but free cells are never found at this stage inside the somites.

The chelarial somite is large and distinct near the median line (Pl. XXII, Fig. 9, SO^1), farther away from the median line it becomes constricted and nearly disappears (Pl. XXII, Fig. 11, SO^1), and still farther away it again becomes more distinct (Pl. XXII, Figs. 12 and 13, SO^1).

The genital duct at this stage is considerably enlarged. It forms a diverticulum on the ventral wall of the opercular

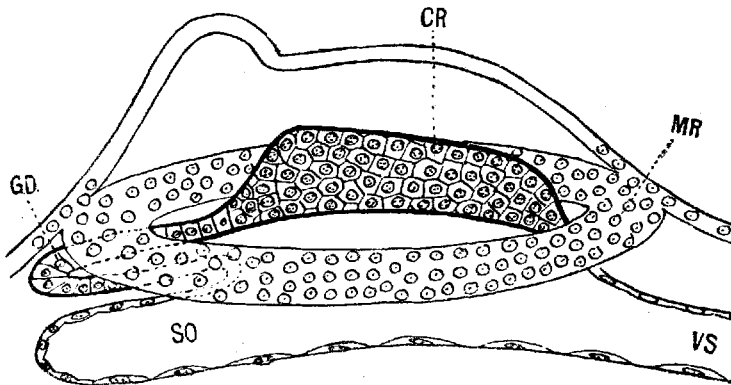


FIG. 1. — A diagram showing the relative positions and shapes of the cartilage, the genital duct, and the somite in the operculum. The somite is a large cavity at the base of the operculum, closed at the median side and extending on the surface of the yolk beyond the outer ectodermic margin of the appendage. On its ventral margin the somite pushes down into the space in the appendage and at the same time bends towards the median line, making a closed diverticulum. The diverticulum lengthens and becomes relatively smaller, and breaking free from the somite it finally becomes the genital duct (*g.d.*). On the ventral side of the diverticulum is a band of cells which form the cartilage rod (*c.r.*). Around the base of the operculum and on the ventral side of the somite is a ring of mesoderm cells. On its median and lateral sides it is continuous with the ectoderm.

somite, with its blind end directed towards the median line. By comparing the different sections of this series it will be seen that the opercular somite is swollen at its median end (Pl. XXII, Fig. 9, SO^2), and reduced to a narrow tube on the median side of the point where the genital duct arises from it (Pl. XXII, Figs. 11 and 12, SO^2). On the lateral side of this point the somite again enlarges to form a spacious chamber (Pl. XXII, Figs. 16–20, SO^2).

The relations of the genital duct, appendage, mesodermic ring, and cartilage to the opercular somite are shown in the accompanying diagrammatic cut (Fig. 1).

In the next series of sections (Pl. XXIII, Figs. 21-24) the genital duct is seen to open into the opercular somite, just median to a transverse plate of mesoderm cells that will ultimately develop into the opercular cartilage.

In a still later stage, where one gill leaf has formed on the posterior surface of the first branchial appendage (Pl. XXIII, Figs. 25-29), the ectodermic portion of the appendage extends a considerable distance beyond the ring of mesoderm around its base. Slender processes extend from the ectodermic cells of one wall to those on the opposite side. In the space beyond the mesoderm, blood corpuscles are frequently found. Pl. XXIII, Fig. 25, represents one of the sections from this series near the median line, showing the genital duct (*g.d.*) directly under the base of the cartilage. The cartilage has now grown out from the ventral wall of the somite in a median direction over the genital duct, and it also extends some distance ventrally into the appendage. Following this series of sections laterally, the somite is next seen at the posterior side of the genital duct (Pl. XXIII, Fig. 26) with a band of muscle cells (*m.*) upon its dorsal wall. The partition between the somite and the duct becomes thinner, until finally the duct and somite unite, as shown in Pl. XXIII, Fig. 27.

In the next stage to be described two or three gill leaves have formed on the first branchial appendage (Pl. XXIII, Figs. 30-32). The operculum is greatly elongated, and its opposite walls are united by numerous outgrowths of the ectoderm. The remnants of the mesodermic ring are still plainly visible at its base. The genital duct is relatively smaller than in the preceding stage and extends in a transverse direction along the dorsal edge of the opercular cartilage. Its proximal end now loses its connection with the opercular somite and remains for some time in a rudimentary condition. In the second larval stage it can be found at the base of the opercular cartilage as a short tube, still unconnected with either the ectoderm or the coelom.

In specimens half an inch long it was well developed and united at its distal end with an infolding of the ectoderm. The manner in which its inner end becomes united with the genital organs has not been determined.

VI. THE DEVELOPMENT OF THE BRANCHIAL CARTILAGES, MUSCLES, AND NERVES.

The cartilages in the abdominal appendages of *Limulus* first appear as solid outgrowths of the ventral wall of the somites. The following descriptions and drawings produced as proof of this statement are based in the main on what takes place in the operculum. The cartilages in the other abdominal appendages develop somewhat later, but in a very similar manner.

In an embryo of three abdominal segments, as shown in Pl. XXII, Figs. 1-8, where the genital duct is seen as a diverticulum on the median ventral wall of the somite, there is no trace of cartilage in the thick somatic layer of mesoderm. But when five abdominal somites are developed the cartilage can be easily distinguished (Pl. XXII, Figs. 9-20, *cr.*). At this time the somite has moved toward the yolk, leaving a large part of the somatic layer behind, as the ring of mesoderm around the base of the appendage. At the same time a transverse plate of cells is formed subtending the ring. This plate of cells is the "Anlage" of the opercular cartilage. Its ventral edge hangs freely into the cavity of the appendage; its median and lateral ends are continuous with the cells forming the mesodermic ring, while its dorsal edge is continuous with the ventral wall of the somite. The opercular cartilage is therefore derived from the ventral wall of the somite and lies just lateral to the point where the genital duct opens into it. At this time the cartilage cells do not differ in histological appearances from those in the surrounding mesoderm.

The cartilage of the first gill-bearing appendage develops in a similar manner, but more slowly than that in the operculum, as seen in Pl. XXII, Figs. 12-16, *SO*³. There are openings in the anterior wall of the first gill somite that appear to afford a normal communication between the coelom and the surrounding blood spaces. The posterior wall of the gill contains a mass of ectoderm cells in active division, evidently preparing for the formation of the gill leaves which appear there at a later period.

The relations of the mesodermic ring, cartilage, genital duct, and somite are shown in cut 1.

In the next stage, where one gill leaf is developed on the first branchial appendage (Pl. XXIII, Figs. 25-29), the opercular cartilage has grown rapidly; its ventral edge projects into the mesodermic ring, but the median and lateral sides are still continuous with it.

In the preceding stage (Pl. XXII, Figs. 15-20) the cartilage extended only from the point of union of the genital duct with the somite to the lateral margin of the mesodermic ring. In this stage (Pl. XXIII, Figs. 25-29) it has grown in a median direction along the ventral wall of the genital duct, so that the two structures are for a short distance completely fused with each other (Pl. XXIII, Figs. 25 and 26, *cr.*).

The cartilage cells now begin to show the features that characterize them so clearly in the later stages; *viz.*: (1) they are larger than the surrounding mesoderm cells and have distinct cell walls; (2) they are arranged in rather regular order; and (3) the protoplasm stains very lightly in borax carmine.

The first gill cartilage shows a very similar condition to that in the operculum.

In the next stage, with three gill leaves on the first branchial appendage (Pl. XXIII, Figs. 30-33), the cartilages form long flat plates that extend some distance beyond the ring of mesoderm into the appendage (Pl. XXIII, Fig. 33). The cartilage of the first gill is attached to the anterior wall of its appendage and extends from there to the somite at its base. A section nearer the lateral line would show a similar attachment of the opercular cartilage to the anterior wall of its appendage. The cartilage cells are of uniform size and have a characteristic appearance and arrangement in rows. The cell walls are sharply outlined against the clear homogeneous protoplasm. The nuclei are large and oval and deeply stained. The cartilage is surrounded by a thick hyaline membrane, which is continuous with the outer wall of the somite.

In the early Trilobite stage (Pl. XXIII, Fig. 34) the cartilages differ but little, except in size, from those in the adult. Each abdominal somite has now become a venous sinus bounded by a thin membrane. The dorsal wall of the sinus is composed of small oval cells, which take a very deep stain. On the ven-

tral side the cells are larger and gradually merge into the cells composing the branchial cartilages.

The ectodermic cells on the dorsal side of the embryo are long and slender and arranged in rows, with their nuclei near their outer ends. At the dorsal extremity of the cartilage there is a point where the boundaries between these ectoderm cells, the cartilage, and muscles merge together, and it is impossible to distinguish between them (Pl. XXIII, Fig. 34, *x*).

A perichondrium is now visible, composed of a layer of spindle-shaped cells, apparently derived from the mesodermic ring, and not from a transformation of the peripheral cartilage cells. The latter, as we have shown, are formed from the mesothelium of the somatic walls.

The distal ends of the branchial cartilages finally fuse with the ectoderm that forms the anterior wall of the appendage. At these points the cartilage and ectoderm are so completely united that it is not possible to distinguish their original boundaries. Thus, an appearance is produced that might easily mislead one into believing that the cartilages were growing as inward proliferations from the ectoderm. But, aside from the fact of their union at these points, there is no evidence that such is the case.

The spaces in the distal ends of the appendages are crossed by fibrous columns arising from the ectodermic walls. At the base of each column are several nuclei, as though the columns were formed by the union of several cells. No mesoderm extends into the appendages beyond the distal ends of the cartilages.

The Abdominal Muscles. — Distinct muscles are first seen on the dorsal wall of the somite of the operculum and the first gill in specimens in which one gill leaf is developed (Pl. XXIII, Figs. 25-29, *m.*). They increase rapidly and grow into the yolk, forming the dorso-ventral muscles of the adult. There are also stout bands of longitudinal muscles which extend from the anterior end of one somite to the posterior end of the preceding somite. All the mesoderm cells of the mesodermic ring are apparently converted into muscles attached to the base of the abdominal appendages.

The Branchial Nerves.—In studying sections of young embryos the mesodermic ring is often found fused with the ectodermic wall of the appendage, so that it is impossible to distinguish between them. In some of these places were found what appeared to be large ganglionic or sensory cells, no doubt derived from the overlying thickenings of the ectoderm. They differed in general appearance and coloring from the surrounding cells, and were most numerous at the posterior margin of the appendages, between the mesodermic ring and the ectoderm. In older specimens nerve cells and fibers were found in corresponding positions (Pl. XXIII, Figs. 25 and 26, *n.*).

In a later stage (Pl. XXIII, Figs. 30 and 31) the nerves are clearly outlined in both the operculum and the first gill. In a still older embryo (Pl. XXIII, Fig. 33), on the posterior side of the first gill, is a proliferation of nerve cells and fibers resembling a sense organ. It represents the same proliferation seen in Pl. XXIII, Figs. 25–27. From it is formed the first gill leaf. The nerve fibers in question are the large ventral nerves that supply the abdominal appendages. They appear to develop, therefore, as fibrous outgrowths (of the central nervous system?) that receive at various points along their course fibers and cells from the overlying ectoderm, and especially from those points where the gill leaves are about to appear.

VII. THE NEPHRIC GLAND.

A. The Development of the Nephridial Lobes.—The nephridial lobes are formed from loose clusters of cells, situated near the base of each of the six thoracic appendages. The cells show a distinctly segmental arrangement, and are derived from what appears to be the median somatic wall of the mesoblastic somites. The nephridial cells of the first and sixth appendages degenerate and disappear after the second larval stage. Those in the second, third, fourth, and fifth appendages form the nephridial lobes of the adult.

The nephric duct arises in quite a different manner, as a tubular diverticulum of the somatic wall of the fifth thoracic somite; the nephridial cells of this segment do not appear till

long after the duct, and the lobes in the other thoracic segments are clearly differentiated.

Nephridial cells are first seen in the fourth segment at about the time the somite appears. They appear a little later in the third segment, and then in the second, first, sixth, and fifth in the order named.

The nephridial cells may be seen before the abdominal appendages are formed, or before the somites are clearly outlined, on the dorsal wall of the mass of mesoderm at the base of the appendages (Pl. XXIV, Fig. 35, *n.c.*). This mass of mesoderm corresponds to the mesodermic ring of the abdominal appendages, and later forms the somatic wall of the somite. The splanchnic layer is at this period represented by a thin non-nucleate membrane next the yolk.

The nephridial cells may be recognized by their larger size, their sharp nuclear stain, and by their lighter colored, transparent protoplasm. They are best developed near the center of the mesodermic mass at the base of the appendage, and from that point they gradually merge in all directions into the surrounding mesoderm.

In the next stage (Pl. XXIV, Fig. 36) the nephridial cells are larger and more numerous, and some of the oldest ones contain a few small granules.

In the next stage (Pl. XXIV, Fig. 37) they have increased greatly in size and numbers, and are very conspicuous, owing to the deep blue color they take when treated with Lyon's blue. The oldest cells have developed slender pseudopodia-like processes.

The membrane over the yolk contains distinct cells, forming the splanchnic layer of the somites, and the latter are as distinctly differentiated as at any time in their development. They do not at any time form closed sacs like the abdominal somites.

The section shown in Pl. XXIV, Fig. 38, passes through the posterior margin of the fourth leg of the same embryo, and shows the reduction in size and grade of development of the cells on the periphery of the future nephric lobes.

Pl. XXIV, Fig. 39, represents a section through the middle of the fourth nephric lobe in a still older stage. A few cells

appear to be leaving the central mass and moving along the walls of the somite towards the dorsal side of the egg. Some of them may possibly give rise to the nephric cells, which at a much later period are found around the pericardium.

The above description applies to the nephridial cells of the fourth leg; but a similar series of changes has also taken place in the chelicerae and the second and third legs. At a corresponding place in the fifth leg the duct of the nephridia has been developing. The true nephridial cells do not appear in that appendage until later. A longitudinal section (Pl. XXIV, Fig. 40), a little to one side of the median line, shows these bunches of developing nephridial cells. In the fifth leg the section passes through the end sac of the nephridial duct. In the sixth leg there is a cavity similar to the end sac, but it is not in the plane of the section.

The nephridial cells of the fourth and fifth leg are shown on a much larger scale ($\times 400$), in a longitudinal section, in Pl. XXIV, Fig. 41. Here the cells are crowded with large spherical granules that stain an intense blue in Lyon's blue. The cell walls are often very faint and easily overlooked. A few of these cells are seen in the fifth leg on the ventral wall of the end sac. The walls of all the thoracic somites have broken down and disappeared, except those of the fifth somite, which is now more clearly outlined as the end sac of the nephric duct (*e.s.*).

In a longitudinal section of an embryo, just before the Trilobite stage (Pl. XXV, Fig. 43), the segmental arrangement of the nephric cells is still clearly shown. The cells are relatively larger than before, and the granules are breaking up into smaller ones (Pl. XXIV, Fig. 41). During the Trilobite stage a marked change occurs in the character of the nephridial cells. The oldest ones have elongated and become irregularly cylindrical, the coarse granules have disappeared, and the finely granular protoplasm has collected around the periphery. The nucleus has also taken up its position just inside the cell wall (Pl. XXV, Fig. 45, *n.c.* and *g.n.c.*). These hollow elongated cells then unite end to end, forming a loose network of branching tubules (Pl. XXV, Fig. 47, *n.c.*).

These changes begin first in the center of each segmental mass of nephric cells, so that for a time each set of tubules is unconnected with the tubules in the adjacent segments.

Some nephric cells are still present like those described in the earlier stages (Pl. XXVIII, Fig. 80, *c* and *d*). On the periphery of the lobes are small granular cells that stain deeply in Lyon's blue, and which appear to be blood corpuscles (*s.r.c.*).

The formation of nephric tubules now begins to extend forward and backwards, forming at first a few slender chains of cells uniting the ventral ends of the nephric lobes with one another. As these connecting cells become canalated, the branching tubules in each lobe are united into one system. The various kinds of cells seen in the nephridial lobes are shown in Pl. XXVIII, Figs. 74, 75, 77, and 79.

In a crab about one inch long a cross-section in the region of the fifth leg (Pl. XXV, Fig. 48) shows that the nephridial lobes now consist of several distinct layers or strata, composed of cells in different stages of development. The end sac (*e.s.*) on the median side opens through numerous tubules into smaller ones, which again break up in the loose tissue of the gland. The tubules in the region of the end sac are large, and open with such wide mouths into the end sac that it is not possible to determine just where the sac ends and the tubular tissue begins. The layer of large tubules nearest the end sac is lined with pavement cells filled with coarse granules. These tubules are evidently formed from the hollow cell chains of the previous stage by the multiplication of the nuclei and the breaking up of the peripheral protoplasm into separate cells. Thus, the intracellular chain of vacuoles is changed into the intercellular lumen of a duct lined by many flattened cells.

The next layer is composed of chains of cells with finely granular protoplasm and conspicuous nuclei. Most of the cells are hollow, and united end to end to form a network of intracellular tubules like those seen in the second larval stage (Pl. XXV, Fig. 47).

The outer layer or cortex of the lobe consists of many small cells with very conspicuous nuclei. Among them are some

enormous cells filled with refractive spherules. One large spherule usually occupies the center, surrounded by many smaller ones, so numerous as to completely hide the nucleus (Pl. XXVIII, Fig. 77).

The division of the nephric lobes into concentric layers is not a sharp one, still it is clearly evident on careful examinations of the sections with moderately high powers. Pl. XXV, Fig. 48, was drawn on too small a scale to show these different layers well. There can be no doubt that the nephric lobes from now onward increase in size exogenously, and that the stratified appearance of the lobes in section is due to a succession of different stages of development that begins with indifferent mesoderm cells on the periphery and ends with the fully formed intracellular tubules in the center of the lobes.

The nephridial lobes of the second, third, fourth, and sixth leg resemble those in the fifth but the stratification of the layers in the latter is more clearly marked. Cross-sections of the stolons uniting these lobes also show very clearly the concentric strata.

The difficulty of following the history of the nephridial cells is much increased by the granules, which come and go, and which change the appearance of the nephridial cells so much that it is hard to recognize their various phases. They appear to accumulate in the cells until the canalization of the latter and their union to form a system of connecting tubules afford the necessary means for their discharge into the end sac, and from there to the exterior. It is certain that the granules begin to diminish in numbers about the time the nephric duct acquires an opening to the exterior.

B. *Structure of the Nephric Gland in the Adult.*—In young Limuli about two or three inches long the nephridial cells form compact masses of tissue easily distinguished from the surrounding organs. The cells at the base of the first and sixth appendages have disappeared; those at the base of the four remaining appendages form the four permanent lobes of the kidney.

In the adult the lateral surface of each lobe, except the first (Pl. XXVIII, Fig. 83), is flattened and lobulated, with a roughly

slipper-shaped outline. This is the growing surface of the lobe, and contains just beneath the outer layer the finest tubules and capillaries. The median side of the lobe is somewhat wedge-shaped, the coarsest ducts being nearest the apex of the wedge. At the ventral ends of the lobes the wedge-shaped surfaces gradually widen, as the coarser tubules diverge to meet those forming the stolon uniting the four lobes. The network of coarse longitudinal ducts of the stolon empty into the end sac situated in the middle of the fifth lobe (*e.s.*), and from there the secretions pass to the exterior through the long nephric duct.

The size and outline of the different lobes, especially the first one, vary a good deal in different individuals.

The nephric gland lies deeply imbedded in the muscles around the base of the legs, and can be readily recognized in the fresh condition by its brick-red color. In some specimens the surface is a pale yellow, or is mottled with red patches. The inside of the lobes, however, was always brick red. Each nephridial lobe had two ear-like lobules attached to its median ventral end near the stolon. On the first lobe they were large, massed one above the other, and entirely covering the collecting tubes. On the remaining lobes they were much smaller, and were connected with them by a slender stalk.

A short distance from where the pedal arteries leave the circum-oral ring a large blood vessel arises which extends laterally along the posterior ventral margin of each nephric lobe. It passes directly through the median portion of each lobe to supply the muscles and other tissue beyond. Before entering the nephric lobes many branches are given off, the anterior ones supplying the nephric lobes, the posterior ones the adjacent muscles. These blood vessels form a rich mass of capillaries round the nephric tubules.

Nerves. — Two sets of nerves pass close to or through the nephric glands. The haemal, or integumentary, nerves of the third, fourth, and fifth thoracic neuromeres pass through the stolon, and between the nephric lobes, to the sides of the carapace, without apparently giving off any branches to the gland (Pl. XXVIII, Fig. 83, *int.n.*, and Pl. XXVI, Fig. 49, *n.*).

Eight smaller nerves arise from the roots of the pedal nerves and supply the coxal muscles at the base of the coxite. There are two of these nerves to each lobe, one on either side (Pl. XXVIII, Fig. 83, *ex.n.*¹⁻⁸). The third, fourth, and seventh nerves pass directly through the stolon; the second, fifth, sixth, and eighth nerves pass over its dorsal, and the first over its ventral, side.

No branches could be found running from these nerves into the lobes, although sections show the presence of numerous fine nerve bundles ramifying through the lobes in all directions.

In sections of the adult gland one may distinguish five concentric layers, each layer containing nephridial tissue in different stages of development. Beginning at the center of the lobe, we have in order: (1) large collecting tubes (Pl. XXVI, Fig. 54); (2) small clear-walled tubules, Fig. 53; (3) tubes lined with granular cells, Fig. 52; (4) chains of vacuolated cells, Fig. 51; (5) large granular cells, Fig. 50.

The large granular cells are very numerous on the ventral and dorsal sides of the nephric lobes (Pl. XXVI, Fig. 49, *g.c.*). Under a higher power two or three nuclei are sometimes seen in a single cell. In borax carmine and Lyon's blue, or in Delafield's haematoxylin and eosin, the cell wall takes a dark stain and appears as a fine thread among the unstained granules. Wedged in between them were occasional bunches of from five to ten or more small dark-colored cells, probably blood corpuscles (Pl. XXVI, Fig. 50, *b.g.c.*).

Small bundles of nerve fibers are abundant in the granular tissue, especially on the median dorsal side of the nephridial lobe (Pl. XXVI, Fig. 50, *n.*). In fresh specimens this tissue has a dull orange color and resembles adipose tissue. A layer of loose connective tissue forms an indistinct boundary between the cells just described and the true nephridial cells.

The latter form the layer marked *h.c.* in Pl. XXVI, Fig. 49. It consists of small cells with large dark nuclei (Pl. XXVI, Fig. 51). The innermost cells are vacuolated, and have fine granular protoplasm on the periphery, and some have united end to end to form delicate intracellular tubules like those seen in

the early Trilobite and second larval stages. These cells and tubules represent the peripheral terminations of the system of tubules leading into the end sac. This layer nearly surrounds the lobe. It is thickest at its apex, becomes thinner on the median ventral side, and disappears entirely on the median dorsal side, where the longitudinal collecting tubes unite the lobe with one another. Within this layer is one formed of tubules, lined with large granular cells, as shown in Pl. XXVI, Fig. 49, *g.t.*, and Pl. XXVI, Fig. 52. They are surrounded by a loose connective tissue, containing nuclei larger than those in the granular cells. There are two kinds of nuclei in the walls of the tubules, one small, dark, and homogeneous, the others larger, and showing clearly the chromatin granules.

In the next layer (Pl. XXVI, Fig. 49, *t.p.*, and Pl. XXVI, Fig. 53) the cells have lost their granules and have flattened out to form a thin endothelial lining to the tubules. The tubules are large, and really form a meshwork of spaces separated by vacuolated connective tissue. Blood channels, containing large granular blood corpuscles, are abundant in the connective tissue surrounding the tubules. The large collecting tubules are best developed in the center of the lobe and on the dorsal surface at their median ends. The endothelium of these tubes (Pl. XXVI, Fig. 54) stains more deeply, and is vertically striated on its surface farthest from the lumen of the tubes, next the very distinct basement membrane. The tubes are widely separated by a spongy connective tissue, richly supplied with blood vessels.

VIII. THE NEPHRIC DUCT.

A. *The Development of the Nephric Duct.*—The nephric duct develops as an evagination of the somatic mesoderm of the fifth leg. The duct cells appear before the nephridial cells of that segment, and before the boundaries of the somite are clearly defined, as an oval plate of columnar cells, easily recognized by their large size and clear protoplasm (Pl. XXVI, Figs. 55–57). At the edges of the plate they pass gradually into the undifferentiated mesoderm that covers the yolk. Beneath the

plate is a noncellular membrane forming the boundary of the yolk. On the median side of the center of the plate is a shallow outfolding (Pl. XXVI, Fig. 56, *end.*) that marks the beginning of the tubular portion of the duct.

In the next stage this outgrowth (Pl. XXVII, Figs. 59-64) has formed a short tube, with its solid distal end growing towards the median line and meeting the ectoderm at the base of the fifth leg; the margins of the original plate now form the funnel-shaped opening (nephrostom ?) into the underlying space.

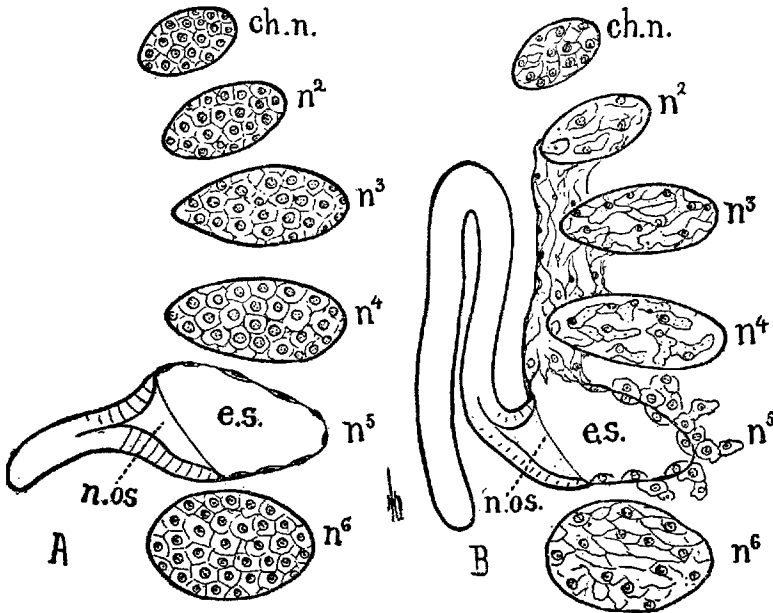


FIG. 2. — Diagrams representing two stages in the development of the nephridial lobes and the duct. They represent the left halves of the nephridia seen from the neural side. Clusters of the nephridial cells are seen in the chelicera, the 2d, 3d, and 4th legs. The end sac and nephridial duct are in the 5th leg. At this time the nephridial cells are large and granular. The end sac is a closed cavity with a few granular nephridial cells appearing on its ventral surface.

The lips of the funnel have gradually united with the membrane over the yolk, and at the same time nuclei migrate into the membrane. Thus, a closed sac is formed which we have called the *end sac*. The entire ventral wall is formed by the nephric plate, which represents the somatic layer of the fifth somite. The end sac apparently represents the coelomic cavity of that somite, and the dorsal wall is formed from the splanchnic layer of the somite.

A proliferation of the ectoderm on the posterior median side of the fifth leg is seen in Pl. XXVII, Fig. 63, *ect.p.*, marking the beginning of the ectodermic infolding, which in the following stage unites with the distal end of the nephric duct.

In the next stage (Pl. XXVII, Figs. 65-70) the distal end of the duct has united with the ectodermic infolding (Pl. XXVII, Fig. 71). The nephric plate is still visible as the flaring mouth of the duct (Pl. XXVII, Figs. 65 and 66), the lateral lip being much longer than the median one.

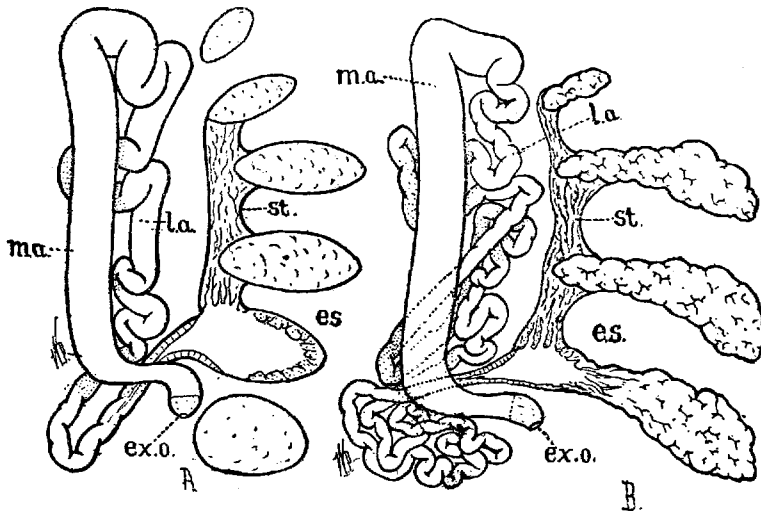


FIG. 3 A. — Diagram of the 2d larval stage, showing nephridial cells in the chelicera, the 2d, 3d, 4th, 5th, and 6th legs. The end sac is at the base of the 5th leg, surrounded by nephridial cells. The duct extends forward to the 2d leg, and backward to the 6th. The proximal arm is much coiled, the distal arm is nearly straight.

FIG. 3 B. — Diagram representing the adult condition. The median ends of the lobes have grown forward and backward and united to form the stolon. This portion of the nephridia is composed of large collecting tubules, which carry the excreta to the end sac in the 4th lobe; from there they pass into the nephridial duct.

The relative size and positions of these parts are shown in a little earlier stage in Pl. XXVI, Fig. 58.

In the next stage the duct elongates very rapidly, and as each end is fixed a Ω -shaped tube is formed with the loop reaching forward to the middle of the fourth leg.

The ectodermic portion of the duct is very short (Pl. XXVII, Fig. 73), and may be readily distinguished by its small deeply stained nuclei and by its delicate internal lining of chitin.

During and after the Trilobite stage the lateral arm of the duct becomes convoluted and a second loop is formed near its proximal end, directed backwards and medianly, and lying dorsal to the median arm of the first loop (cuts 3 A. and 3 B.). The median arm of the first loop becomes considerably dilated, and apparently acts as a reservoir for the secretions of the gland. It remains a straight tube throughout life. The anterior end of the lateral arm (*l.a.*) is smaller and somewhat convoluted, the foldings increasing in number and extent towards the posterior loop.

The structure of the nephric duct during the Trilobite stage is shown in Pl. XXV, Fig. 45. The cells lining the duct now have no distinct cell walls, although the walls are easily seen in the preceding and in the following stages.

The anterior loop of the duct now extends as far forward as the second leg. The following parts may be distinguished (cut 3 A.); *viz.*: (*a*) the short ectodermic portion; (*b*) the dilated median arm of the anterior loop; (*c*) the slightly coiled lateral arm of the anterior loop; (*d*) the much coiled posterior loop; (*e*) the end funnel; (*f*) the end sac.

B. *The End Sac.*—The early stages in the formation of the end sac out of the fifth thoracic somite have already been described.

Before the Trilobite stage a longitudinal section shows the presence of a few enlarged finely granular cells in the walls of the end sac. These granules increase in size and numbers till the cells present the appearance shown in Pl. XXIV, Fig. 42, *e.s.* They now resemble those cells from which the nephridial tubules develop.

In the Trilobite stage (Pl. XXVIII, Fig. 80) the coarse granular protoplasm has nearly disappeared, and the sac is lined with a delicate layer of protoplasm, with here and there a nucleus. Numerous finger-like evaginations of the wall of the sac have developed, the walls of which have the same structure as those of the sac itself.

It was not possible to determine the exact manner in which these evaginations were formed. A careful study indicates that the large tubes opening directly into the sac were formed as

evaginations of the walls of the sac, and that the outgrowths were subsequently increased in length by the addition of nephridial cells to their distal ends. These cells in turn become hollowed out and united with the cells forming the nephric lobes. In this way the system of tubules in the nephric lobes becomes continuous with those leading into the end sac.

There seem to be two sets of tubules opening into the sac; one set arises from its anterior wall and leads into the longitudinal tubules of the stolon, and hence to the three anterior nephric lobes; the others lead into the tubules of the fourth lobe. On its median side the sac opens through a small neck into the nephric duct.

Finally, in the adult, the end sac becomes so irregular through the formation of the numerous large tubes opening into it that its original boundaries cannot be distinguished.

C. *The nephric duct of the adult* lies along the edge of the plastron dorsal to the nephric lobes, and extends backwards from the base of the second leg to the anterior side of the sixth. The thin-walled transparent tube is easily torn, and, unless injected, it is very difficult to trace out its various convolutions. On careful dissection the course of the duct is seen to be as follows: At the distal end of the duct, just before it opens to the exterior, is the ectodermic portion (Pl. XXVIII, Fig. 83, *ect.* and cut 3 B.). It is sharply marked off from the rest of the duct by its thick walls lined with chitin. From this point the duct turns at right angles and extends in a dorsal direction, till it reaches the plastron, along the lateral edge of which it extends as far as the first nephric lobe (Pl. XXVIII, Fig. 83). It then bends directly backwards, diminishing rapidly in size up to the angle of the second loop, which in Pl. XXVIII, Fig. 83, is seen on the median side of the fourth nephric lobe. From here on the calibre of the tube remains about the same. It now turns forwards, parallel to the dorsal limb of the loop, as far as the posterior margin of the third lobe, and then backwards to form a large mass of coils, lying a little behind and dorsal to the fourth lobe. From this coil the proximal end of the duct issues and passes forwards and ventrally to the end sac, buried in the interior of

the fourth lobe. From the end sac many tubes lead forward into the stolon. The latter consists of a coarse network of anastomosing tubes, from which branches are given off that extend along the median dorsal face of each lobe, diminishing in size as they go. The entire substance of the lobes may be colored a deep red by injecting red gelatine into the main duct.

Along the walls of the duct are here and there short, blunt evaginations or pockets ending blindly. In some cases the pockets of one tube may unite with those of another, thus forming communications between the separate coils (Pl. XXVII, Figs. 81 and 82, *po.* and *c.n.t.*). They are most numerous in the extensive coil lateral to the fourth nephric lobe.

IX. CELLS OF DOUBTFUL SIGNIFICANCE.

During the Trilobite stage certain cells appear on the nephridial lobes, which may be readily recognized by peculiarities of shape and coloring (Pl. XXV, Fig. 45; Pl. XXVIII, Figs. 74 and 80, *s.r.c.*). They are dark purple when stained in Lyon's blue and borax carmine. These cells were found only in the vicinity of the hollow nephridial tubules, with which they were often so closely connected that it was impossible to ascertain with certainty whether they were inside or outside the tubules. Occasionally they were on the outer margin of the tubules, and it would then appear as if they were about to separate from them (Pl. XXVIII, Fig. 74, *c.*). In Pl. XXVIII, Fig. 80, at the dorsal side of the end sac, about a dozen were collected, which suggested a point of proliferation either by cell division among themselves or from the nephridial tubules or sac. At the left (*v.c.*) one of the cells is much larger than the others and shows vacuolations in the protoplasm. A few of these cells were found in the second larval stage, as shown in Pl. XXV, Fig. 46, *r.c.*, and also in the region of the heart (Pl. XXVIII, Fig. 76, *r.c.*), after which they entirely disappeared. A large number of granular cells appear at this time, and it seems probable that they are different conditions of the same cells, although no convincing proof of it could be found.

The last-named cells may be found in the second larval stage distributed throughout the nephridial lobes (Pl. XXV, Fig. 47, *g.c.*), and they occur in large masses along their lateral margins. These cells are round or oval, and are filled with great numbers of coarse granules, which usually completely conceal the nucleus. They are well shown in Pl. XXV, Fig. 46, *g.c.*, and Pl. XXVIII, Fig. 75, *g.c.*

In larvae three-quarters of an inch long the cells on the lateral margins of the lobes are enormous (Pl. XXV, Fig. 48, and Pl. XXVIII, Fig. 77). Similar cells were found throughout the body, from the proventriculus to the first gill. In the anterior sections they are most numerous on the dorsal and lateral sides of the proventriculus. They also extend laterally on the ventral side of the body close to the ectoderm. Posterior to this they are less abundant around the alimentary canal, but are thickly massed around the base of the legs. In the sixth leg and in the operculum they are more numerous than in any other place. Similar cells are found in the region of the heart. The origin and fate of these cells were not determined with certainty. They agree in some respects with the granular cells seen in the early stages of the nephric lobes, and which, as we have seen, subsequently cleared up and formed the nephric tubules.

During the Trilobite and second larval stage, cells are found in the pericardial region that closely resemble nephridial cells. They are most abundant on the dorsal side of the pericardium in the sixth thoracic segment and over the proventriculus. Many cells are hollow and united end to end, forming loose-branching tubules like those in the nephridial lobes of the Trilobite stage (Pl. XXVIII, Fig. 76, *h.c.t.*). Among these cells are a few of the large granular ones (*g.c.*), and some of the small dark red cells (*r.c.*) like those seen in the nephridial lobes. All these cells probably arose from the nephridial "Anlagen" at the base of the legs, and were carried to their present position by the growth of the somites over the dorsal surface of the egg.

The same kind of cells are also found in the chelicerae and in the sixth leg. Those in the cheliceral segment (Pl. XXIV, Fig. 40) disappear early. Those in the sixth leg appear before

the Trilobite stage (Pl. XXV, Fig. 43, *n.c.*⁶). These cells first become granular, then vacuolated, and then united end to end (Pl. XXVIII, Fig. 79). In Limuli about three-quarters of an inch long the cells were still present, but they were not united with the permanent nephric lobes, and appeared to be degenerating.

X. SUMMARY.

1. *Branchial Cartilages*.—A thick ring of somatic mesoderm forms at the base of each abdominal appendage. The gill cartilage arises as a plate of somatic mesoderm attached by its dorsal end to the ventral wall of the somite, and continuous on either side with the ring of mesoderm. The ventral end of the cartilage finally extends through and beyond the mesodermic ring and becomes attached to the anterior wall of the corresponding appendage.

2. The ventral ends of the abdominal somites persist as venus sinuses.

3. The *genital ducts* arise as diverticula of the median ventral side of the opercular somite. They remain in a rudimentary condition until after the second larval stage.

4. *Nephric Duct*.—A nephric plate is formed from a single layer of columnar cells of the somatic mesoderm on the median side of the fifth somite. The plate is gradually evaginated to form a funnel, opening by a wide mouth into a thin-walled end sac that represents the fifth somite; the opposite end unites with a shallow ectodermic invagination at the base of the fifth leg. The tube becomes much convoluted, and is converted directly into the adult nephric duct. Finger-like outgrowths of the end sac finally unite with the hollow cell chains of the adjacent nephric lobes.

5. *The Nephric Lobes*.—A mass of nephric cells arises independently of the duct from the median dorsal portion of the somatic layer of each of the six thoracic somites. The cells become enlarged and filled with coarse granules. The granules become smaller or disappear, and a vacuole appears in each cell. The latter elongate and unite end to end to form irregular masses of branching intracellular tubules. The cell masses

in the second, third, fourth, and fifth legs form the four lobes of the adult organ. Offshoots extend forward and backward from the median end of each lobe, that unite with each other and with the end sac to form the longitudinal ducts of the stolon. The nephric cells of the first and sixth somite disappear. In the stolon, and in the larger tubules on the dorsal side of each lobe, the nephric cells become flattened, and by repeated divisions are finally converted into a pavement epithelium, thus changing the intracellular lumina into intercellular ones. New tubules are formed throughout life by the transformation of indifferent cells on the ventral surface of each lobe.

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INDEX LETTERS TO PLATE XXII.

<i>a.p.</i> = anal plate.	<i>p.f.</i> = primitive furrow.
<i>B.c.</i> = blood corpuscles.	<i>p.s.</i> = primitive streak.
<i>ch.</i> = chelaria.	<i>SO</i> ⁰ = unexplained cavity appearing only in this series.
<i>c.r.</i> = opercular cartilage.	<i>SO</i> ¹⁻⁵ = somite of the chelaria, operculum, and first to third gills, respectively.
<i>ect.</i> = ectoderm.	
<i>g.</i> ' = first gill.	
<i>g.d.</i> = genital duct.	
<i>op.</i> = operculum.	

EXPLANATION OF PLATE XXII.

All the sections were outlined with a camera and drawn to the same scale.

FIGS. 1-8 were drawn from a series of longitudinal sections through the region of the chelaria (*ch.*), operculum (*op.*), and the first gill (*g.*') of an embryo in which the abdominal appendages were beginning to show in surface views. Borax carmine, 15 μ .

FIG. 1. Section No. 1, through the median line of the embryo. A few scattered mesoderm cells lie between the ectodermic layer and the yolk. There were also a very few large nuclei in the yolk, and others from which chromatin granules were escaping, as if they were beginning to degenerate. $\times 200$.

FIG. 2. Section No. 2. The median ends of the somites are shown by four bunches of cells. *SO*¹ is the somite of the chelaria; *SO*², that of the operculum; *SO*⁰, one which disappeared shortly after this stage; *SO*³, somite of the first gill about to separate from the primitive streak (*p.s.*). $\times 200$.

FIG. 3. Section No. 3. The somatic cavities are distinct; *SO*⁰ merges with the ectoderm on the ventral side of the somite. $\times 200$.

FIG. 4. Section No. 6. *SO*¹, *SO*², and *SO*³ are larger than in the previous sections. The cavity of *SO*⁰ has disappeared, and in its place are a few mesoderm cells which disappear in the next section. $\times 200$.

FIG. 5. Section No. 11. The operculum and the first gill are much larger than formerly, and are now filled with mesoderm. Their somatic cavities remain distinct. $\times 200$.

FIG. 6. Section No. 13. *SO*² shows a diverticulum, the beginning of the genital duct (*g.d.*). $\times 200$.

FIG. 7. Section No. 18. *SO*² remains large. *SO*³ has almost closed. $\times 200$.

FIG. 8. Section No. 23. *SO*² is the only abdominal somite remaining; its lumen disappears a few sections farther toward the lateral side. $\times 200$.

FIGS. 9-20 were drawn from a series of longitudinal sections through the region of the chelaria, operculum, and the first gill of an embryo somewhat older than the one in the preceding series. In this embryo there were four perfect abdominal somites; the fifth was just breaking free from the primitive streak. The series

begins with Fig. 9 near the median line and extends laterally almost to the outer edge of the appendages. Borax carmine and Lyon's blue, 10 μ .

FIG. 9. Section No. 1, near the median line, showing five abdominal somites, SO^{1-5} . SO^5 is attached to the primitive streak (*p.s.*); the point of separation is indicated by a slight furrow on the dorsal side. The enlargement at the anterior end of the primitive streak will form the sixth somite. $\times 200$.

FIG. 10. Section No. 3. SO^5 extends in a lateral direction beyond the point of attachment to the primitive streak. SO^{1-4} are distinct and free from the mesoderm at the base of the appendages. $\times 200$.

FIG. 11. Section No. 6. The median end of the genital duct (*g.d.*) may be seen in the operculum. SO^2 is small and lies close to the surface of the yolk. $\times 200$.

FIG. 12. Section No. 7. The genital duct is larger than in the previous section, and is nearer SO^2 . $\times 200$.

FIG. 13. Section No. 12. The genital duct and SO^2 are in contact. In the first gill the mesoderm cells are arranged in a row, extending down into the mass of mesoderm from the ventral side of the somite to form the cartilage (*c.r.*) of the first gill. $\times 200$.

FIG. 14. Section No. 13. The cavity of SO^2 and the genital duct are separated by a thin membrane only. $\times 200$.

FIG. 15. Section No. 14. SO^2 and *g.d.* have united. A deep furrow on the anterior side indicates the point of union. $\times 200$.

FIG. 16. Section No. 17. A line of cells on the ventral margin of the opercular somite shows the median limit of the opercular cartilage. $\times 200$.

FIG. 17. Section No. 20. The opercular cartilage is free from the mesoderm, and consists of a single row of cells on the ventral side of SO^2 . $\times 200$.

FIG. 18. Section No. 22. The end of the cartilage rod is broader than before, and a thin membrane connects it with the adjacent mesoderm. $\times 200$.

FIG. 19. Section No. 26. The opercular cartilage has disappeared. The ventral margin of the somite connects with the posterior bunch of mesoderm cells. The somites show a tendency to bend in a posterior direction. $\times 200$.

FIG. 20. Section No. 32. $\times 200$.

INDEX LETTERS TO PLATE XXIII.

<i>B.c.</i> = blood corpuscles.	<i>n.</i> = nerve.
<i>Ch.</i> = chelaria.	<i>n.p.</i> = proliferation of nerve cells and fibers.
<i>c.r.</i> = cartilage rod.	<i>o.p.</i> = operculum.
<i>cu.</i> = cuticular membrane.	<i>SO</i> ¹ = somite of the chelaria.
<i>ec.p.</i> = ectodermic proliferation.	<i>SO</i> ² = somite of the operculum.
<i>ect.</i> = ectoderm.	<i>SO</i> ³ = somite of the first gill.
<i>end.</i> = endothelium.	<i>v.s.</i> = venous sinus.
<i>g.</i> = first gill.	<i>x.</i> = fusion of cartilage, ectoderm, and mesoderm.
<i>g.d.</i> = genital duct.	<i>y.k.</i> = yolk.
<i>m.</i> = muscle cells.	
<i>ms.</i> = mesoderm.	

EXPLANATION OF PLATE XXIII.

FIGS. 21-24 represent longitudinal sections through the region of the chelaria, operculum, and first gill of an embryo a little more advanced than the one from which Figs. 9-20 in Plate XXII were drawn. The sections were 15 μ thick and stained in borax carmine and Lyon's blue.

FIG. 21. Section No. 1, near the median line. The somites of the operculum and the first gill have extended so far in a lateral direction that the somite of the chelaria could not come in the same longitudinal sections with them, but would be found in the sections nearer the median line. The somites of the operculum (*o.p.*) and the first gill (*g.*) are large and distinct. The genital duct (*g.d.*) shows at the base of the operculum. The lumen seen here, ends in a solid mass of mesoderm in the preceding section. $\times 200$.

FIG. 22. Section No. 5. The genital duct is larger than in the preceding section, and has approached *SO*². $\times 200$.

FIG. 23. Section No. 10. The genital duct and *SO*² have united. A few cartilage cells (*c.r.*) are seen on the dorsal wall of the somite. $\times 200$.

FIG. 24. Section No. 10. Both *SO*² and *SO*³ show a tendency to bend in a posterior direction. The opercular cartilage (*c.r.*) is still present. $\times 200$.

FIGS. 25-29. Longitudinal sections through the region of the operculum and the first gill from an embryo with two gill leaves on the first gill. The sections were 15 μ thick and stained with borax carmine and Lyon's blue.

FIG. 25. Section No. 1, near the median line. The opercular cartilage and genital duct extend toward the median line some distance beyond the somite. The opercular cartilage is attached to the genital duct between bunches of mesoderm at the base of the operculum. The first gill cartilage is now visible. In the spaces between the ectoderm and mesoderm of the appendages are a few blood corpuscles (*B.c.*). Some of the mesoderm cells on the yolk show muscular

striations (*m.*). At the posterior side of the first gill are nerve fibers beneath the place where the first gill leaf is forming (*n.*). $\times 200$.

FIG. 26. Section No. 5. The genital duct and opercular cartilage are larger than in the preceding drawing. SO^2 shows at the posterior side of the operculum. SO^3 is greatly enlarged. $\times 200$.

FIG. 27. Section No. 8. The genital duct (*g.d.*) and SO^2 are separated by a thin membrane. $\times 200$.

FIG. 28. Section No. 10. The genital duct and SO^2 have united. The opercular cartilage remains on the dorsal side of the somite. SO^2 and SO^3 show a tendency to extend in a posterior direction. $\times 200$.

FIG. 29. Section No. 19. The somites extend a long distance laterally and posteriorly as closed cavities. $\times 200$.

FIGS. 30-33. Longitudinal sections through the operculum and first branchial appendage of an embryo in which the third gill leaf had commenced to form. The embryo appears older than that of the preceding series as the appendages have lengthened considerably.

FIG. 30. Section No. 1, near the median line. Shows the median end of the genital duct at the base of the operculum. As the somite has grown laterally and the genital duct toward the median line, they no longer appear in the same longitudinal sections. Nerve fibers (*n.*) are found at the posterior side of the base of the operculum and the first gill. $\times 200$.

FIG. 31. Section No. 3. Shows the genital duct with a small lumen and the median edge of the opercular cartilage. $\times 200$.

FIG. 32. Section No. 10. Shows the lateral end of the genital duct connected with the base of the opercular cartilage. The genital duct is relatively much smaller than in the preceding series and separate from the somite (SO^2). The first gill has a well-formed cartilage. $\times 200$.

FIG. 33. Section much farther from the median line. It shows the long, slender cartilage plates; that of the first gill is attached to the ectoderm on the anterior side of the appendage. A similar condition would be found in other sections in the operculum. The cartilage cells are placed in rows and show a characteristic appearance, and take a lighter stain than the mesoderm. The cartilages are surrounded by a thick membrane. Each somite has been transformed into a large venous sinus, and extends from the base of the cartilage through the yolk to the dorsal side of the embryo. A number of nerve fibers and cells (*n.*) are shown in the gill leaf in *g.* $\times 200$.

FIG. 34. Longitudinal section through the operculum and the first gill of a specimen with five gill leaves on the first gill, showing the opercular cartilage attached to the anterior wall of the operculum and to the venous sinus at the base of the appendage. The cartilage is surrounded by a membrane, the perichondrium (*pc.*). At the apex of the leg slender processes reach from one ectodermic wall to the other. The outer wall is covered by a thin cuticular membrane. $\times 200$.

INDEX LETTERS TO PLATE XXIV.

<i>ap.</i> ²⁻⁶ = the second to the sixth thoracic appendages.	<i>m.r.</i> = marginal ring.
<i>b.c.</i> = blood corpuscle.	<i>n.c.</i> = nephridial cells.
<i>br.</i> = brain.	<i>n.c.</i> ¹⁻⁵ = nephridial cells of the first to the fifth appendages, respectively.
<i>b.s.</i> = blood space.	<i>ped.n.</i> = pedal nerve.
<i>b.v.</i> = blood vessel.	<i>s.c.</i> = sensory cells.
<i>che.</i> = chelicera.	<i>SO</i> = somite.
<i>ect.</i> = ectoderm.	<i>sop.</i> = somatopleure.
<i>e.s.</i> = end sac.	<i>sp.c.</i> = spinal cord.
<i>g.n.c.</i> = granular nephridial cells.	<i>spl.</i> = splanchnopleure.
<i>mes.</i> = mesoderm.	<i>y.k.</i> = yolk.

EXPLANATION OF PLATE XXIV.

FIGS. 35-39 were drawn from cross-sections through corresponding regions of the third and fourth thoracic appendages of embryos of varying ages. The sections are all arranged so that the median line is at the upper margin of the plate. The sections of the younger embryos are 3μ to 5μ thick, the older ones 10μ .

FIG. 35. Cross-section through the middle of the fourth appendage of an embryo in which none of the abdominal appendages had been formed. On the dorsal margin of the mass of mesoderm lying near the median side of the base of the appendage may be seen four or five larger cells, the "Anlage" of the nephridial lobe (*n.c.*). $\times 200$.

FIG. 36. Cross-section through the middle of the third appendage of an embryo slightly older than that in Fig. 35. The nephridial cells are more numerous, larger, and have faint granulations. A thin non-cellular membrane covers the yolk at the base of the appendage. A space is formed between the mesoderm and the apex of the appendage, which later develops into the blood space of the legs. $\times 200$.

FIG. 37. Cross-section through the middle of the fourth appendage of an embryo older than that in Fig. 36. The somites are imperfectly formed in the thoracic appendages. The somatic layer is several cells thick; the splanchnic layer is represented by a thin membrane with a few nuclei. The nephridial cells are larger than in the preceding figures. They possess slender pseudopodia, have become finely granular, and take a deep stain in Lyon's blue. $\times 200$.

FIG. 38. Drawn from the same series and the fourth section back of that in Fig. 37. It shows the nephridial cells smaller than at the middle of the base of the appendage and without pseudopodia. The somite extends out laterally as a closed cavity. $\times 200$.

FIG. 39. Cross-section through the middle of the fourth appendage. Large granular cells with long processes are shown on the dorsal margin of the meso-

derm. The largest of these cells lie beyond the lateral base of the appendage. $\times 200$.

FIG. 40. A longitudinal section from an embryo of about the same age as that in Pl. XXIII, Figs. 30-32. The somites of the thorax have disappeared, except the one in the fifth appendage, which remains as the end sac to the nephridial duct. Bunches of nephridial cells are found in the chelicerae and in the second, third, and fourth appendages. Nephridial cells appear later in the sixth appendage. $\times 100$.

FIG. 41. Longitudinal section through the fourth and fifth appendages. The nephridial cells are filled with large granules, among which the larger nuclei are visible. Often the cell boundaries were very indistinct or else entirely invisible, giving the appearance of several nuclei in the same cell. In the fifth appendage the end sac shows as a closed cavity, with a few of the larger nephridial cells on its ventral wall. $\times 400$.

FIG. 42. Longitudinal section through the fourth and fifth legs. It shows a bunch of large, granular, nephridial cells at the base of the fourth leg. In the fifth leg the end sac is lined with granular cells which are similar to those in the fourth leg, except that they are smaller. There is a blood space between the appendages and at the apex of the appendages. $\times 300$.

INDEX LETTERS TO PLATE XXV.

<i>ap.²⁻⁶</i> = the second to the sixth appendages, respectively.	<i>l.n.c.</i> = longitudinal section through nephridial cells.
<i>ant.n.d.</i> = anterior arm of the nephric duct.	<i>n.c.¹⁻⁶</i> = nephridial cells in the first to the sixth appendages, respectively.
<i>b.r.</i> = brain.	<i>n.c.</i> = nephridial cell.
<i>b.s.</i> = blood space.	<i>n.d.</i> = nephric duct.
<i>b.v.</i> = blood vessel.	<i>n.l.³, n.l.⁴</i> = third and fourth nephridial lobes.
<i>c.n.c.</i> = cross-section through nephridial cell.	<i>n.t.</i> = nephric tubules.
<i>e.s.</i> = end sac.	<i>oe.</i> = oesophagus.
<i>ex.n.d.</i> = nephric duct near the external opening.	<i>pl.</i> = plastron.
<i>g.c.</i> = granular cells.	<i>post.n.d.</i> = posterior arm of the nephric duct.
<i>g.n.c.</i> = granular nephridial cells.	<i>r.c.</i> = red cells.
<i>g.t.</i> = tubules lined with granule cells.	<i>a.t.</i> = areolar tissue.

EXPLANATION OF PLATE XXV.

FIG. 43. Longitudinal sections through the second to the sixth legs of an embryo a little younger than the Trilobite stage. The chelicera have remained near the median line, so that they are not included. Bunches of nephridial cells are seen at the base of the second, third, fourth, and sixth legs, and the nephric duct in the fifth leg. $\times 100$.

FIG. 44. A transverse section through the region of the second, third, and fourth legs of the Trilobite stage. Nephridial cells are seen at the base of the third and fourth legs. The duct has grown anteriorly nearly as far as the second leg, and both the distal and proximal limbs are shown in the figure. $\times 100$.

FIG. 45. Enlarged drawing through the dorsal region of the fourth and fifth legs from the same series as Fig. 44, showing the nephric duct and the nephridial cells in various stages of development. $\times 400$.

FIG. 46. Horizontal section through the fourth and fifth legs of a specimen in the second larval stage. As the section was cut near the external opening of the duct, only one portion of it is seen. The nephridial lobes form a definitely marked area, and the portions in the second, third, fourth, and fifth legs have united with one another. The nephridial lobes are composed of a lacuna tissue, in which are large granular cells of varying sizes. $\times 200$.

FIG. 47. Cross-section through the fourth leg of a younger specimen of the second larval stage than the preceding one. The dorsal side of the section is on the right. The nephric duct is much coiled, and several sections through it are shown. Cylindrical cells with fine granules around the periphery are uniting end

to end, forming long, narrow tubules. These tubules are found on the median and dorsal side of the nephridial lobes. A number of small cells with large granules (*g.c.*) show on the lateral side of the lobes. $\times 300$.

FIG. 48. Cross-section through the fifth leg of a crab about one inch long. The dorsal side of the drawing is on the right and the median at the lower margin of the page. It shows sections through the nephridial duct and the end sac with its long, branching diverticula. The structure of the lobe differs in different places. Near the end sac it is composed of long, branching tubules, lined with cells containing coarse granules. Outside this layer the tubule cells are smaller; on the lateral side of the lobe the tissue is aerolated. On the lateral margin are large cells filled with very coarse granules. $\times 100$.

INDEX LETTERS TO PLATE XXVI.

<i>a.</i> = anterior.	<i>m.</i> = median.
<i>art.</i> = artery.	<i>m.art.</i> = main artery.
<i>c.</i> = cells filled with fine granules.	<i>mes.</i> = mesoderm.
<i>cap.</i> = capillaries.	<i>m.r.</i> = marginal ring.
<i>c.is.</i> = connective tissue.	<i>m.y.</i> = membrane on the yolk.
<i>c.t.</i> = collecting tubules.	<i>n.</i> = nerve.
<i>e.n.d.</i> = evagination of nephric duct.	<i>n.c.</i> = nerve cord.
<i>e.s.</i> = end sac.	<i>n.d.</i> = nephridial duct.
<i>ex.op.</i> = external opening of the nephric duct.	<i>n.f.</i> = nerve fibers.
<i>f.g.c.</i> = fine granular cells.	<i>p.</i> = posterior.
<i>g.c.</i> = granular cells.	<i>p.n.d.</i> = nephric plate.
<i>g.t.</i> = granular tubules.	<i>s.l.</i> = striated layer.
<i>h.c.</i> = hollow cells.	<i>so.</i> = somite.
<i>int.n.</i> = integumentary nerve.	<i>t.</i> = tubules.
<i>l.</i> = lateral.	<i>t.p.</i> = tubular portion.
	<i>y.k.</i> = yolk.

EXPLANATION OF PLATE XXVI.

FIG. 49. Longitudinal section through the middle of the second lobe of an adult nephridium. The section was $5\ \mu$ thick and was stained in borax carmine and Lyon's blue. The median side of the lobe is at the right. The lobe is composed of four distinct regions or layers, each of which has a characteristic structure. $\times 16$. (1) The outer portion is formed of very large granular cells (*g.c.*) (see Fig. 50) most abundant at the lateral end of the lobe on both dorsal and ventral sides. Two smaller groups are shown on the median side. Small nerve fibers penetrate this tissue. (2) *h.c.* A dark layer which surrounds the lobe, except for a short distance on its median ventral side. It is composed of small cells with fine granules around the periphery. They are probably hollow cells (*h.c.*) which are uniting end to end (see Fig. 51). (3) *g.t.* A faintly stained layer inside of *h.c.* and surrounding the entire lobe. It is composed of small tubules which are lined with large granular cells (see Fig. 52). (4) The larger part of the lobe is contained in this tubular portion (*t.p.*) (see Fig. 53). (5) *c.t.* The collecting tubular portion is similar to (4), except that the tubules are larger. They connect the small tubules of each lobe with the collecting tubes of the stolon. It is shown on the dorsal side of the figure and near the median end of the lobe (see Fig. 54).

FIG. 50. An enlarged drawing through the large cells which surround the lateral end of Fig. 49 (*g.c.*). They are filled with small granules, which did not stain in either borax carmine and Lyon's blue, or Delafield's haematoxylin and eosin. Several nuclei are often found in the same cell. Nerve fibers penetrate

throughout the tissue. A bunch of small, round cells, with fine granules around their periphery (*fg.c.*), is seen, apparently within the large cell on the left. $\times 270$.

FIG. 51. Some of the hollow cells which nearly surround the nephridial lobe (*h.c.*, Fig. 49). It shows cells with a finely granular periphery, apparently uniting end to end. $\times 270$.

FIG. 52. Enlarged section through the tubules lined with large granular cells. $\times 270$.

FIG. 53. Enlarged section of the tubular part (*t.p.*) of the lobe. The tubules are surrounded by a loose connective tissue, in which not infrequently were large, round cells with granular protoplasm (*c.*). The tubules form an anastomosing network throughout the central portion of the lobe. They have a cellular lining which is separated by a dark membrane from the connective tissue (*ctis.*). $\times 270$.

FIG. 54. Enlarged portion of the longitudinal collecting tubules (*c.t.*). The tubules are large and branching. They have a heavily striated lining, which is separated by a dark membrane from the connective tissue. Nerves and capillaries are shown in the connective tissue, and also a few large granular cells, similar to those in Fig. 53. $\times 270$.

FIG. 55. Cross-section through the anterior part of the fifth appendage of a specimen the same age as that in Pl. XXIV, Fig. 36 (5 μ , haematoxylin). The section shows the nephric plate of mesoderm cells at the base of the appendage. It is continuous on both the lateral and median sides with a cellular membrane which lies upon the yolk. $\times 200$.

FIG. 56. The fourth section posterior to Fig. 55, showing an evagination of the nephric plate to form the nephric duct. $\times 200$.

FIG. 57. The third section posterior to Fig. 56, showing the posterior margin of the nephric plate. $\times 200$.

FIG. 58. Reconstructed outline of the nephridial duct and end sac, made from a specimen of the same age as Pl. III, Fig. 40. $\times 400$.

INDEX LETTERS TO PLATE XXVII.

<i>ap.⁵</i> = fifth appendage.	<i>mes.</i> = mesoderm.
<i>ect.p.</i> = proliferation of ectoderm.	<i>m.r.</i> = marginal ring.
<i>e.n.d.</i> = ectodermic portion of nephridial duct.	<i>n.c.</i> = nerve cord.
<i>e.s.</i> = end sac.	<i>n.d.</i> = nephric duct.
<i>g.n.c.</i> = granular nephridial cells.	<i>p.n.d.</i> = nephric plate.
<i>l.n.d.</i> = lip of the nephric duct.	<i>s.m.</i> = sphincter muscle.
	<i>so.</i> = somite.

EXPLANATION OF PLATE XXVII.

FIGS. 59-64 were drawn from a series of cross-sections through the fifth appendage of an embryo of the same age as that in Pl. XXIV, Figs. 37 and 38. The sections were cut 8μ thick and stained with Delafield's haematoxylin.

FIG. 59. Section No. 1, showing a mass of mesoderm cells at the base of the appendage, in the middle of which are a few larger and lighter-colored cells which mark the anterior margin of the nephric duct. $\times 200$.

FIG. 60. Section No. 2, showing the nephric plate folded on itself, making a double layer of large, clear cells, which extend toward the ectoderm. The somite is a closed cavity dorsal to the nephridial duct. $\times 200$.

FIG. 61. Section No. 4, showing the nephric duct extending out to the ectoderm on the median margin of the appendage. $\times 200$.

FIG. 62. Section No. 6. The nephric duct and somite are much reduced in size, but retain the same relative position as before. $\times 200$.

FIG. 63. Section No. 7, showing the somite reduced to a long, narrow space on the surface of the yolk. In place of the nephric duct is a row of large mesoderm cells with a slight outward projection. $\times 200$.

FIG. 64. Section No. 10. The nephric duct is represented by a row of large cells, continuous on the median and lateral sides with the yolk membrane. The mesoderm at the base of the appendage and the somite have entirely disappeared. $\times 200$.

FIGS. 65-70 are drawn from a series of cross-sections through the fifth appendage of an embryo somewhat older than the one in the preceding series. The proximal end of the duct has grown away from the median line, changing its general direction somewhat.

FIG. 65. Section No. 1 shows the anterior margin of the nephric plate. $\times 200$.

FIG. 66. Section No. 3 shows the mouth of the duct opening with a broad lateral lip on the ventral side of the somite. $\times 200$.

FIG. 67. Section No. 5 shows cross-section through the middle of the duct. $\times 200$.

FIG. 68. In Section No. 9 the lumen of the duct has disappeared. $\times 200$.

FIG. 69. Section No. 10, showing the distal end of the duct. $\times 200$.

FIG. 70. Section No. 11, showing the union of the ectodermic invagination with the mesodermic portion of the duct. $\times 200$.

FIG. 71 is a longitudinal section through the middle of the fifth appendage in the early Trilobite stage, showing the opening of the nephric duct to the exterior. $\times 200$.

FIG. 72. Section on the lateral side of the base of the fifth appendage in a late Trilobite stage. It shows the end sac, which extends beyond the base of the appendage on the surface of the yolk. Granular cells (*g.c.*) are developing on its ventral margin. $\times 200$.

FIG. 73. Longitudinal section through the fifth appendage of a specimen somewhat older than that of the preceding figures. It shows the distal arm of the nephridial duct and its opening at the posterior side of the appendage. The ectodermic portion is characterized by numerous small cells, which at the very end seem to be forming a sphincter muscle around the opening. The mesoderm cells are large, with a faintly colored protoplasm. In many specimens a lumen was continuous throughout the mesodermic and ectodermic portions. $\times 300$.

INDEX LETTERS TO PLATE XXVIII.

<i>c.n.t.</i> = cross-section through a nephridial tubule.	<i>l.r.c.</i> = large red cells.
<i>c.t.</i> = connecting tubes.	<i>mus.</i> = muscle.
<i>ec.p.</i> = ectodermic part of nephric duct.	<i>n.l.¹⁻⁴</i> = nephridial lobes.
<i>e.s.</i> = end sac.	<i>p.a.</i> = pedal artery.
<i>e.s.t.</i> = tubule of the end sac.	<i>pl.</i> = plastron.
<i>ex.o.</i> = external opening of the nephridial duct.	<i>p.n.</i> = pedal nerves.
<i>g.c.</i> = granular cells.	<i>po.</i> = pockets in the walls of the duct.
<i>h.c.t.</i> = hollow cells forming tubules.	<i>r.c.</i> = red cells.
	<i>v.c.</i> = vacuolated cells.
	<i>s.r.c.</i> = small red cells.

EXPLANATION OF PLATE XXVIII.

FIG. 74. A few characteristic nephridial cells of the Trilobite stage. *a.* Longitudinal section through a cell with finely granular protoplasm around the periphery. As the section passes near the surface of one end of the cell the granules show a reticulated arrangement. *b.* A cross-section through two cells similar to *a.* The nucleus adheres to the side of the cell. *c.* Shows a small red cell on the surface of *b.* *d.* Red cells, with a clear protoplasm which took a deep stain in Lyon's blue. $\times 475$.

FIG. 75. Sections through the nephridial cells from one of the older specimens of the second larval stage. *a.* Longitudinal section through a tubule. *b.* Cross-section through a tubule. *c* and *d.* Cells in which large, dense-looking granules cover the nucleus. $\times 475$.

FIG. 76. Cross-section through the region on the dorsal side of the heart of *Limulus* in the second larval stage, showing hollow cells similar to those in the nephridial lobes. Several cells have united end to end to form branching tubules. Other cells are present filled with large granules (*g.c.*), while others of the same size have only a few granules in them. $\times 300$.

FIG. 77. Section through a granular cell of a young *Limulus* three-fourths of an inch long. (See Pl. XXIII, Fig. 48.) It shows one enormous granule in the center with smaller ones around it. $\times 475$.

FIG. 78. Section through nephridial cells, from the younger specimens of the second larval stage. *a.* Longitudinal section through tubules which show a granular periphery. *b.* Section through two cells which have united. *c.* Cross-section through a tubule. *d.* A small cell with the nucleus surrounded by granules. $\times 475$.

FIG. 79. Section of cells in the sixth leg of a specimen in the second larval stage. $\times 475$. *a.* Long hollow cells uniting, similar to those in the nephridial and pericardial regions of the same age. *b.* Large triangular cells uniting.

- c.* Cross-section through a hollow cell. *d.* Section through a granular cell.
e. Section through a triangular cell filled with granules.

FIG. 80. Cross-section through the end sac of a specimen in the Trilobite stage. The section is posterior to the point where the nephridial duct opens into the end sac. The end sac is lined with small and finely granular cells. One large cell is shown on the dorsal side of the sac, filled with small granules, and with pseudopodia extending out from its free margins. At the lateral side the end sac shows a projection similar to both the wall of the end sac, and to the long hollow cells which are characteristic of this age. Ventral to this sac is another of similar structure, which unites with the end sac in the second section posterior to this. These projections are either outgrowths from the wall of the end sac, or nephridial tubules united with it. Scattered among the nephridial cells were a number of small red cells. Some of them had a faintly granular protoplasm, others were vacuolated, and still others in which nothing but the cell walls could be distinguished outside the nucleus. $\times 400$.

FIG. 81. Drawing of the injected nephric duct of an adult *Limulus* from the dorsal side. The main part of the duct is coiled and folded upon itself many times, the distal arm alone remaining straight, running from a point in front of the anterior transverse process of the plastron along the edge of the plastron as far as the fourth nephric lobe. It then passes between the muscles through the median end of the last nephridial lobe to the exterior. Along the free margin of the duct, slight projections, or pockets (*po.*), are found. In other places small connecting tubes (*c.t.*) unite different portions of the duct.

FIG. 82. In this case the duct has been dissected apart along its entire length. In many places small tubes were found, connecting one fold of the duct with another which lay either beneath or beside it. In most cases these connecting tubes had to be cut in order to free and unfold the duct. A few of them are left untouched (*c.n.t.*). In other places small evaginations or pockets in the wall of the duct were found along its free margin (*po.*). The nephric duct lies below the outer edge of the plastron, deeply imbedded in muscle, the genital organs, and in the hepatic caeca. The fourth lobe of the Nephridia shows from below.

FIG. 83. Drawing of the nephric region of an adult *Limulus*, showing the nephric duct, nephridial lobes, and the blood vessels and nerves.

The four nephridial lobes lie at the base of the second, third, fourth, and fifth legs. They are connected along their median dorsal ends by a band of collecting tubes—the stolon. A short distance from the oral ring small branches arise from the pedal arteries of the second, third, fourth, and fifth legs, and pass along the ventral surface of each of the nephridial lobes, supplying the muscles beyond. During its course, each artery sends off alternate branches to the muscles and to the lobe. The arteries which pass to the lobes break up into small branches, which fill the nephridia with a network of vessels.

Large integumentary nerves (*int.n.*), arising from the haemal side of the brain, pass out between each nephridial lobe to the sides of the carapace. On either side of each lobe a smaller nerve arises from the haemal side of the pedal nerve which supplies the tergo-coxal and the plastro-coxal muscles on the posterior and anterior sides of the base of the coxite. The nephric duct lies near the lateral dorsal side of the lobes. The distal arm passes through the posterior median end of the fourth lobe to the external opening at the base of the fifth leg. The ectodermic portion of the duct extends from the external opening to the fourth lobe.