

GUIDES FOR VERTEBRATE DISSECTION

# NECTURUS

AN URODELE AMPHIBIAN

BY

J. S. KINGSLEY

*Professor of Biology in Tufts College*

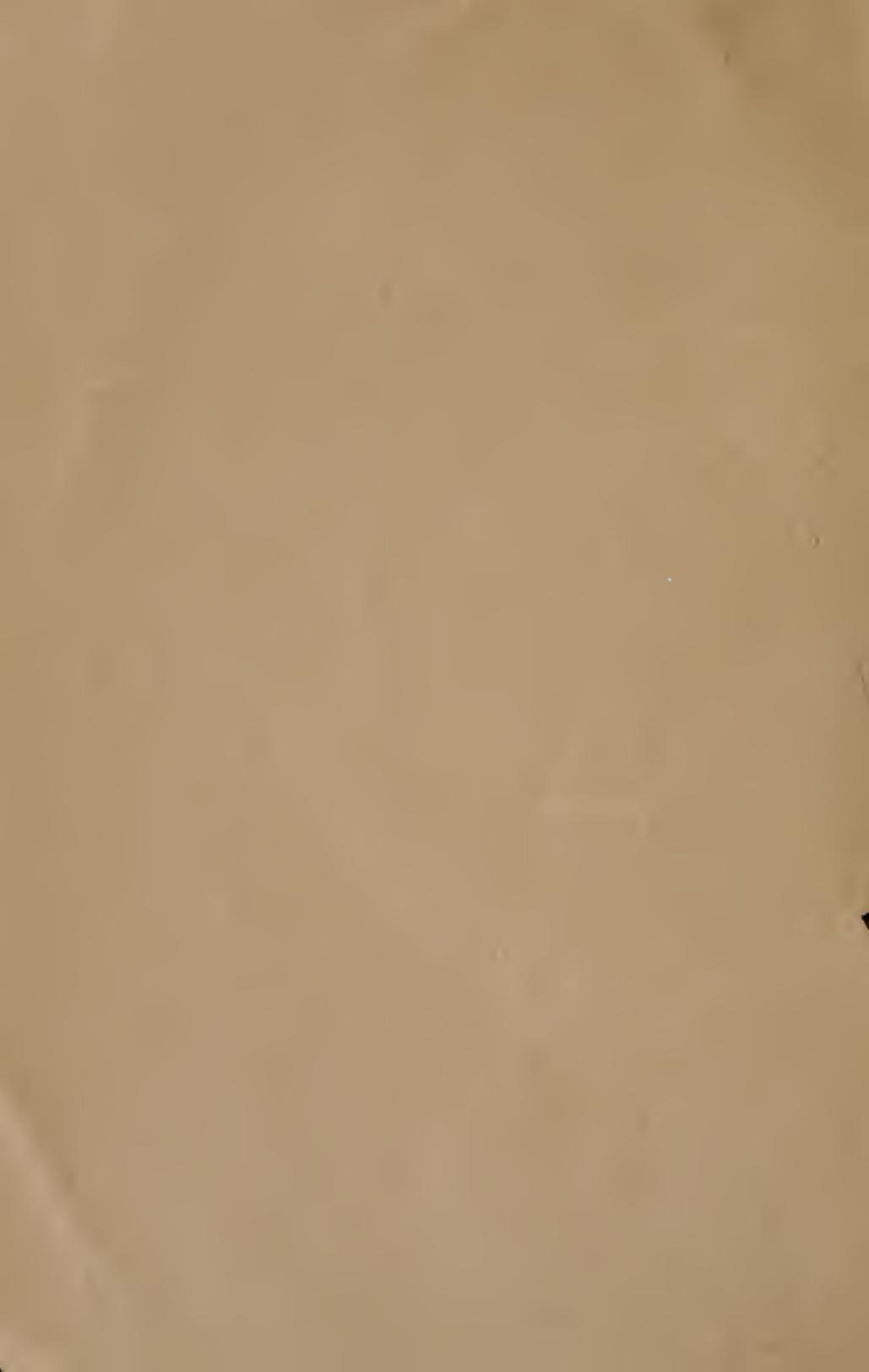


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## INTRODUCTION

THESE directions for dissection are intended eventually to include representatives of all the major groups of vertebrates. Each is complete in itself and is issued separately so that laboratories may select those forms best adapted for their courses. The directions have been tested by several years' use and are thought to have a distinct pedagogic value in that they do not so much tell the student what he will find, but instead ask him what he does find. He thus obtains his information from the specimen, not from the printed page. For similar reasons illustrations have been omitted; students sometimes find it easier to copy the published figure than to work out the points for themselves.

No attempt has been made to follow out every system of organs completely, but each has been traced far enough to give a good knowledge of the more important structures to use as a basis for comparisons. The student by following the directions may obtain a knowledge of the general anatomy of the animal studied, but this knowledge of itself has little value. More important is the benefit to be gained by comparing the different forms dissected, tracing as far as possible their resemblances and differences. Hence in his dissection the student should continually recall the conditions existing in all other animals as he is tracing out each part.

More than this: he should read the general statements given in manuals of vertebrate structure as he takes up each organ or system of organs, thus correlating his discoveries and making them a part of one general whole. It would be well to go farther and read the accounts of the development of the organs in question in some of the text-books of vertebrate embryology. It is only in this way that an explanation of many peculiarities of structure may be obtained.

Unless explicitly used otherwise the terms **right** and **left** in the following directions apply to the right and left of the animal being dissected, not of the student. **Anterior** and **posterior**

indicate relative position with regard to head and tail, while **dorsal** and **ventral** are used for the anterior and posterior of human anatomy. **Medial** is used to imply proximity to the middle line, **lateral** being the contrasting term. **Proximal** refers to that part of an organ or structure nearest to its centre or to its attachment to the body, **distal** being the opposite adjective. In speaking of muscles the fixed point of attachment is the **origin**, the attachment on the part to be moved is its **insertion**.

**INJECTING.**—In many cases it is almost impossible for the beginner to trace the blood-vessels unless they are filled with some colored substance which renders them more easily seen. This is especially true of the smaller vessels. Injection is also frequently convenient in tracing other vessels like those of the urogenital system.

Various substances ('injection masses') have been devised for filling the vessels. The essential features of a mass are that it have color, that it flow freely when injected, and that it soon harden so that it will not escape from a vessel accidentally cut. Within recent years a starch mass has been largely used, and as this answers all the purposes of these guides it is described here:

Corn-starch. . . . .	400 pts. by volume
2% chloral hydrate in water. . . . .	400 "
95% alcohol. . . . .	100 "
Color and glycerin (equal parts) . . . . .	100 "

The mixture should be thoroughly mixed by stirring and strained through cheese-cloth or paper cambric, stirring during the operation. The starch and color quickly settle, hence the mixture has to be stirred while using. It will keep indefinitely, but of course must be thoroughly mixed each time before using.

The colors commonly used are vermilion,\* insoluble Prussian blue, chrome green, and chrome yellow. The vermilion is usually used for the arterial, the blue for the venous system, but it is often advantageous to use chrome yellow instead of blue, as it contrasts better with dark organs like the liver and kidneys, while, when a blood-vessel occasionally bursts in injection, the viscera are not so badly stained.

\* Care should be taken to get true vermilion (mercuric sulphide), as much that masquerades under that name is red lead colored with eosine. This works disadvantageously as the eosine dissolves in the liquids in which the specimens are preserved and stains everything indiscriminately.

An extremely fine chrome yellow may be made by dissolving 200 parts of acetate of lead and 105 parts by weight of chromate of potash in separate dishes of water. After complete solution mix and allow the precipitate to settle. Pour off the supernatant fluid and wash the precipitate with several waters so as to remove the potassium acetate which would injure the specimen.

Many instruments—syringes, water-pressure apparatus, etc., have been proposed for injecting, but a considerable experience has led to the conclusion that for small animals there is nothing better than a large rubber bulb for the pressure. This is connected by rubber tubing with the canula which is inserted in the vessel to be filled. Use the largest canula possible and keep it free from precipitated mass.

**SKELETONS.**—The skeletons made by the average student are likely to be imperfect, but the knowledge which he obtains in preparing them is of value. The laboratory should have skeletons well prepared, but the student should clean those which he studies. In the case of fishes it is sufficient to remove the skin from the body, next to place the animal for a few minutes in water near the boiling-point, and then to remove the flesh by hand. With other animals the tissues are more resistant, and in these cases the animal, after removal of the skin, should be boiled in a soap solution made as follows:

Thoroughly mix with heat 75 grams of hard soap, 12 grams of potassic nitrate (saltpetre), 150 cc. of strong ammonia, and 2000 cc. of soft water. For use, one part of this 'stock' is diluted with three of water and the body is boiled in this, the length of time varying with the size and consistency of the animal, care being taken not to boil it long enough to soften the ligaments unless it be desired to separate the bones from each other.

For decalcification of skulls in order easily to get at the brains, nitric alcohol, made by mixing equal parts of ten per cent nitric acid and ninety-five per cent alcohol, is useful.

Material for dissection can be obtained from

Supply Department, Marine Biological Laboratory, Woods Hole, Mass.

Dr. F. D. Lambert, Tufts College, Mass.

H. H. and C. S. Brimley, Raleigh, N. C.

H. A. Ward, Rochester, N. Y.

Kny-Scheerer Co., 225 Fourth Ave., New York.



## NECTURUS

Necturus or Menobranchus, commonly called the mud-puppy, is distributed through the Mississippi and St. Lawrence basins, from whence it has found its way in recent years into other waters (the Hudson at Albany, etc.). Where it occurs it may be taken with hook and line, but it is better to capture it with nets or seines, on account of its habit of swallowing the hook.

Two specimens, a male and a female, besides a prepared skeleton, will be required by each student. One of the specimens, preferably the male, should be injected. The injection is best accomplished in the following order: First, with blue, inject forwards and backwards through the hepatic vein at the base of the liver, filling the heart, postcava, and the branches in the liver. Next, with red, into the dorsal aorta in both directions at about the middle of the cœlom. The postcava being already filled with blue, the aorta is more readily seen. Lastly insert the canula into the portal vein just before its entrance into the liver, and inject with blue forwards and backwards.

The preparation of the skeleton is a more difficult matter than with some animals, especially in the caudal region. Skeletons good enough for study purposes may be made by boiling with soap solution, following this by picking away the flesh with the forceps and finishing by careful brushing with a tooth-brush. The boiling should not be too prolonged because of the danger of separation of the parts. When not in use the skeletons should be kept in alcohol, but when studied they should be soaked in water, which swells the cartilages and restores them to their normal condition. The female specimen can be used for skeletal purposes after the urogenitals have been studied.

## EXTERNAL FEATURES

How many regions—head, neck, trunk, tail, etc.—can you distinguish? Do you find any indications of scales on or in the skin? Do you find any median or paired fins? Are any of them supported by fin-rays? Is the tail diphy-, homo-, or heterocercal? \* Where is the anus?

Examine the head for nostrils, eyes, ears, mouth, etc., noting the position of each. Is the mouth wide or narrow? Terminal or inferior in position? Are lips present? Are there any eyelids? Probe with a bristle through the nostril (**external naris**) and note the position of the opening (**choana** or **internal naris**) where it enters the mouth.

How many **external gills** do you find? Look between them for **gill-slits** (**visceral** or **branchial clefts**); how many on each side? How many pairs of legs occur? Can you find in each the same regions—thigh, upper arm, shank, forearm, ankle, wrist, etc.—as occur in your limbs? How many toes in each foot? Note the peculiar way in which the limbs stand out at nearly right angles to the body. Make out in each a dorsal and a ventral surface, an anterior, or **preaxial**, and a posterior, or **postaxial**, side.

In the trunk region notice the impression of the muscle-plates upon the skin. How many plates can be counted between fore and hind limbs?

Draw a side view of the animal, natural size, labelling the parts.

## INTERNAL STRUCTURE

## I. MUSCULAR SYSTEM

**REMOVE** the skin from the head and trunk by cutting down the middle line of the back and then skinning laterally until the whole is free. Take great care not to injure the underlying muscles. The external gills need not be removed with the skin.

\* **Diphycercal**, tail alike above and below, the parts symmetrically arranged around the vertebral column; **homocercal**, the upper and lower lobes of the tail alike, the vertebral column extending into the upper lobe; **heterocercal**, the lobes unequal, the vertebral column extending into the upper lobe.

**Muscles of the Head and Visceral Arches.**—Examine the lower side of the head, making out the following muscles and sketching each as you proceed.

(1) The **mylohyoid** muscle extends across the ventral surface of the lower jaw, its fibres running transversely. Can you distinguish any differences between its anterior and posterior portions, in their mode of attachment to the halves (rami) of the jaw? Cut the mylohyoid across in the median line, turn the halves back and see beneath

(2) The **geniohyoid** muscle, attached in front to the symphysis (tip) of the lower jaw and behind to the ventral fascia (see below) of the region of the neck, its fibres running at right angles to those of the mylohyoid.

Attached at about the middle of the geniohyoid on either side is (3) an **external ceratohyoid** muscle which runs obliquely outwards and backwards. Follow it towards the dorsal side: how far does it extend? and where is its insertion? To answer this last question it will be necessary to remove a fan-shaped muscle, (4) the **levator branchiarum**, from the side of the neck, which is attached at its smaller end to the region of the gills. The external ceratohyoid pulls the gills forward, the levator lifts their bases.

On the dorsal surface of the head are a pair of (5) **temporalis** muscles, the line separating them continuing back the length of the body. In what direction do the temporalis muscles run?

Lateral to each temporalis is (6) a large **masseter** muscle, which has its origin from the posterior and lateral regions of the skull. Its fibres run parallel to the external ceratohyoid, to be inserted on the lower jaw at the angle of the mouth.

Separate the masseter and the external ceratohyoid with the handle of the scalpel and cut across the levator branchiarum, exposing the small underlying muscle, (7) the **levator arcuum branchiarum**, which arises from the posterior part of the cranium and is inserted on the branchial cartilages at the base of the external gills.

Lateral and anterior to the levator arcuum is (8) the **depressor mandibulæ**, or **digastric** muscle, of about equal size. It is attached at one end to the cranium, and its fibres, running parallel to those of the external ceratohyoid, are inserted upon the angle of the lower jaw.

Turn to the lower surface, lift up the posterior margin of the external ceratohyoid and expose (9) the **internal ceratohyoid** muscle, extending outwards and backwards from the hyoid cartilage to its insertion on the cartilage of the first branchial arch.

Near the insertion of the internal ceratohyoid is (10) the **constrictor arcuum**, which extends across the branchial arches from the first to the third.

Farther out (i.e., more lateral) upon the branchial arches will be found fibres of (11) a **depressor arcuum branchialium**, the origin of which is best seen later, after the removal of the cucularis and omohyoid muscles.

**Muscles of the Shoulder-girdle.**—Just behind the mylohyoid muscle on the latero-ventral side of the throat will be found the anterior end of the procoracoid cartilage. From the anterior end of this the (1) **procoraco-humeralis** muscle extends backwards to the proximal end of the humerus. It is small and slip-like in character and serves to draw the limb forward.

The space between the two procoraco-humerales is occupied by (2) the broad **sternohyoid** muscle, its fibres in front being inserted upon the hyoid arch and forming the **fascia** to which the geniohyoid (*supra*) is attached. Posteriorly the sternohyoid is in part attached to the shoulder-girdle, while another portion continues backwards along the ventral surface of the body as the abdominal muscles (*vide infra*).

The **pectoralis** muscles (3) are fan-shaped. Each arises from the mid-ventral line, the posterior portion being continuous with the rectus abdominis muscles to be noticed later. The fibres converge upon the proximal ventral end of the humerus. The pectorales serve to draw the shoulders ventrally and backwards.

Cut across one of the pectorales in front, turn it back, and see (4) the **supracoracoid** muscle extending from the coracoid cartilage, between the two shoulders, to the humerus.

On the side of the body dorsal to the attachment of the fore limb is (5) the **latissimus dorsi** muscle, a broad sheet whose fibres converge ventrally upon the dorsal surface of the humerus. In front of this and between it and the procoracoid are several muscles as follows:

Just in front of the latissimus dorsi is (6) the **dorsalis scapulæ**, arising from the dorsal portion of the scapula and inserted, beside the others, upon the humerus. Next in order is (7) the

**cularis** muscle, arising by two heads from the sides of the trunk, behind the gills, and inserted, like the others, upon the humerus.

Between the **cularis** and the **procoraco-humeralis** is the (8) **omohyoid** muscle.

Separate these muscles at their origin and reflect them, exposing deeper muscles beneath as follows: Beneath the **latissimus dorsi** a rudimentary (9) **serratus magnus**, the fibres of which, running longitudinally, are attached to the posterior dorsal angle of the scapula. Beneath the **cularis** (10) the **levator scapulæ**.

**Muscles of the Fore Limb.**—In the fore limb, besides other muscles the following may be made out: (1) On the ventral surface a narrow **biceps** muscle, extending from the proximal end of the humerus to the anterior (radial) side of the forearm. Behind this, also on the ventral surface, is the larger (2) **coracobrachialis** muscle arising from the coracoid cartilage and inserted on the distal end of the humerus.

On the dorsal side is (3) the large **triceps** muscle which arises by three heads, attached respectively to the coracoid, scapula, and proximal end of the humerus, and is inserted on the ulna (posterior side of the forearm).

In the forearm the muscles may be grouped in two divisions: on the dorsal surface an **extensor communis**; on the ventral a **flexor communis**. Both flexors and extensors can be resolved into several components.

Draw dorsal, lateral, and ventral views of the head and body showing all the muscles. In the dorsal and ventral view put the superficial muscles on one side of the median line, the deeper muscles on the other.

**Muscles of the Trunk.**—In the trunk region the muscles of the two sides are distinct from each other, and on either side they are divided into dorsal and ventral portions by a lateral line. The ventral portions are the more modified. In each portion the muscles are subdivided by transverse non-muscular partitions—the **myosepta** or **myocommata**—into separate **myotomes** or **myomeres**. The dorsal myotomes constitute a **longissimus dorsi** muscle on either side, while the ventral region is composed of three layers of **oblique** muscles, external, middle, and internal, the latter best seen from the cœlom. Make out the direction of the muscle-fibres in each layer.

On either side of the mid-ventral line is a narrow **rectus abdominis** muscle, each being continuous in front with the pectoralis muscle, the two rectus muscles being separated by a non-muscular **linea alba**.

Draw a diagram of the trunk muscles showing all these points.

**Muscles of the Pelvic Girdle and Hind Limb.**—Extending from the mid-ventral line to each hind limb are three muscles. In front, arising from the anterior end of the pubis is (1) the **pectineus** muscle, which is inserted on the proximal end of the femur. Behind this, but also arising from the pubis, is (2) the **pubo-femoralis brevis** muscle, inserted on the middle of the femur. The posterior, (3) the **gracilis** muscle, arises from both pubis and ischium and is inserted on the distal portion of the femur.

Lift the gracilis and find beneath it the (4) **adductor femoris** muscle with somewhat similar origin and insertion.

On the anterior side of the thigh is (5) the **rectus internus** muscle, arising from the anterior margin of the acetabulum and inserted on the proximal end of the tibia.

Behind this, on the ventral surface, is (6) the **vastus internus**, also extending from the acetabulum to the tibia. Behind the vastus internus is the distal end of the gracilis (3), already seen, while on the posterior margin of the limb is a part of (7) the **femoro-caudalis** muscle which arises from the pyriformis (see below) and is inserted on the fibula.

On the ventral surface of the shank are several muscles which may be grouped as (8) a **flexor communis**.

Dissect out the gelatinous tissue around and in front of the anus and then make out, on either side, two superficial muscles extending backwards to behind the vent. The more lateral is (9) the **pyriformis**, the more medial, (10) the **ileo-caudalis**. Trace each to its origin and insertion. Then lift the pyriformis and find beneath it (i.e., dorsal) the rest of the femoro-caudalis already seen.

Draw a ventral view of these parts with the muscles found.

In a dorsal view of the limb find the rectus internus, gracilis and femoro-caudalis seen on the dorsal side. Just behind the rectus internus is (11) the **rectus externus**, extending from the ilium and terminating by a tendon on the tibia. Next behind is (12) the **glutæus maximus**, arising from the ilium and inserted by tendon on the fibula. The **semimembranosus** muscle (13)

lies between the glutæus and the gracilis and extends from the ileo-ischial region to the fibula. The muscles of the dorsal surface of the shank act as an **extensor communis**.

Draw a dorsal view of the limb and the adjacent parts of the trunk, showing the muscles.

#### VISCERAL ANATOMY

Open the abdominal or peritoneal cavity of the injected specimen (**cœlom**) by a longitudinal incision a little to the right of the mid-ventral line, taking care not to injure the underlying organs or to cut the pectoral or pelvic arches. Make lateral cuts on the right side so that the wall may be folded outwards. See the thin membrane (**mesohepar**) connecting the liver with the ventral body wall. How far does it extend in either direction? Do you find any blood-vessels in it?

Now make similar cuts in the other side, cut the mesohepar, and pin out the body walls. Tip the liver to the right, slightly separate the viscera, without cutting or tearing any of the membranes, and draw the parts exposed, as follows:

The **liver**, dark-colored and nearly median in position. How many lobes to it? Lift the lobes and find a small vesicle, the **gall-bladder**, on their dorsal surface. Trace the **bile-duct** from the gall-bladder to the liver and to the intestine. To the left of the liver is the **stomach**. Can you distinguish where stomach ends and intestine begins? Cut into the intestine behind the entrance of the bile-duct and carry the incision forward. The abrupt change noticeable in the character of the lining marks the boundary (**pylorus**).

Follow the intestine back to the pelvic region. See the lobulated **pancreas** near the entrance of the bile-duct. Examine the inner surface of the intestine for the opening of its duct. To the left of the stomach is the elongate, dark-colored **spleen**. Look on either side of the liver in the dorsal part of the cœlom for the elongate **lungs**. Which lung, right or left, is the larger?

Lift the intestine and see that it is bound to the dorsal body wall by a thin membrane, the **mesentery**, and that the stomach is supported in a similar way by a **mesogaster**. A membrane, the **gastro-hepatic omentum**, extends from stomach to liver, while liver and intestine are connected by an **hepato-duodenal omen-**

**tum.** That part of the mesentery which supports the hinder end of the intestine is the **mesorectum**.

Now examine the extent of the body cavity. It is lined everywhere by a smooth membrane, the **peritoneum**, which also covers mesenteries and viscera so that all organs really lie outside the peritoneum. In front note that the body cavity is bounded by a thin membrane, the **false diaphragm** or **septum transversum**.

Various blood-vessels course in the mesenteries and omenta. Make out the following, drawing them in a separate sketch as you proceed, the arteries in red, the veins in blue.

In the middle line of the dorsal wall of the cœlom is the large artery, the **dorsal aorta**. From this arise several arteries. In the mesogaster is the **gastric artery**, which soon branches and supplies both sides of the stomach. Which of the branches, right or left, gives off twigs (**splenic artery**) to the spleen?

Posterior to the gastric artery are segmental **intercostal arteries** leading into the muscles of the back, and, in the female, more numerous and smaller arteries supplying the oviducts.

At about the middle of the cœlom the dorsal aorta gives off, into the mesentery, a **cœliac axis** which soon divides into a **gastro-splenic artery**, going to the stomach and spleen, and a **hepato-pancreatic artery**, which supplies not only the pancreas and liver but sends branches to the stomach as well.

The intestine is supplied by several (how many?) **mesenteric arteries** each of which branches in the mesentery into fine twigs (**intestinal arteries**) which enter the intestinal walls.

The **mesenteric vein** runs in the mesentery parallel to the intestine. It is formed by the union of numerous **intestinal veins** from the intestinal wall, and is to be traced forward, through the pancreas, receiving a **pancreatic vein** in its course, to its union with the gastro-splenic vein to form the **hepatic portal vein** which enters the liver near the bile-duct. The **gastro-splenic vein** is formed by the union of **gastric** and **splenic veins** coming from stomach and spleen respectively. The hepatic portal vein runs for some distance along the dorsal surface of the liver and is reinforced in its course by a **ventral abdominal vein** which passes dorsally from the ventral body wall. Trace the branches of the portal vein on and in the liver.

A second vein, the **postcava (vena cava inferior)** extends from about the middle of the dorsal surface of the liver dorsally

and posteriorly to about the middle of the body cavity. Cut into it near the liver, and with probe and scalpel trace the postcava through the liver to the false diaphragm, noting the entrance of small vessels, the **hepatic veins**, during its course. At about the point where the postcava leaves its dorsal position to enter the liver it connects with the hinder ends of a pair of **postcardinal** ('azygos') **veins** into which pass small veins from the anterior genital organs and the spinal region.

Add these vessels to your sketch.

Cut through the stomach at about the middle and through the intestine in the pelvic region. Cut the alimentary canal loose by trimming the mesogaster, mesentery, etc., close to the intestine. Also remove most of the liver, leaving the anterior portion, together with part of the postcava, attached to the septum transversum. You can now study

**The Urogenital Organs.**—In the male these consist of the testes and their efferent ducts together with the mesonephroi (the so-called kidneys); in the females, besides the mesonephroi, of the ovary and its ducts. Two specimens will be needed to make out all the features.

In the male the **testes** are a pair of solid oval bodies lying in the posterior part of the roof of the body cavity. Each is supported by a fold of the peritoneum, the **mesorchium**, in which blood-vessels, **spermatic arteries** and **spermatic veins**, may be traced, the former coming from the dorsal aorta to the testes, the latter passing from the testes to enter the postcava. Add these vessels to the sketch of the circulation already made.

Next find the ducts leading from each testis, the **vasa efferentia**, these uniting to form a common convoluted tube, the **vas deferens** or **Leydig's duct**. Trace this latter in both directions. How far forward does it extend? This portion in front of the testis is functionless. Sketch the reproductive organs on one side of the body as far as made out. Then cut away the testis of the opposite side. This will expose the **mesonephros** (**Wolffian body**) lying between the vas deferens and the median line. Sketch this and the related structures on the other side of the drawing. Note that the mesonephros is narrower in front than behind. See that the vasa efferentia pass through this narrower portion in order to reach Leydig's duct. Farther back see the urinary tubules leading from the mesonephros to Leydig's duct, which

therefore must serve for **ureter** as well as for vas deferens; in other words it is urogenital in function.

Cut through the pelvis and trace the posterior end of the alimentary canal and urogenital ducts. That part into which the ducts empty is the **cloaca**. On its ventral side is a thin-walled **urinary bladder**. Inflate through the vent and notice its shape. Beginning at the vent split the cloaca a little to one side of the mid-ventral line; lay open and find the opening of the urinary bladder, and in the sides the openings of Leydig's ducts.

In the female the much lobulated **ovary** occupies a position comparable to that of the testis in the male. It is attached to the peritoneal wall by a membrane, the **mesovarium**. Notice through the ovarian walls the eggs of various sizes. Make a slight incision in the wall of the ovary and inflate, noting that the organ is saccular. What relations do the ova bear to its wall? Also note the **ovarian arteries** going from the dorsal aorta to the ovary.

Dorsal to the ovary is a long convoluted tube, the **oviduct (Müllerian duct)**; trace it forward and back. Cut into it near its anterior end. Note that it opens directly into the body cavity in front (**ostium tubæ**). Trace it also into the cloaca, inflating and cutting if necessary. In the posterior part of the cœlom between the oviduct and the middle line is the mesonephros. Do you find vasa efferentia similar to those in the male? Notice on the lateral margin of the mesonephros a delicate tube, the **ureter (Wolffian duct)**. Trace it backwards: does it unite with the oviduct or does it open separately into the cloaca?

What is the most marked difference between the urogenital ducts in the two sexes?

Draw the urogenital system of both sexes, bringing out the points discovered.

**Posterior Circulation.**—Follow the postcava backward from the point of its connection with the postcardinals, sketching this part as well as the **renal** and **genital veins** which it receives.

Cut through the pelvis and see, on either side of the hinder part of the cœlom, an **iliac artery** which soon divides, one branch (**femoral artery**) passing into the hind limb, the other passing to the ventral side of the body, where it forms a **hypogastric artery**,

giving off a twig to the urinary bladder in its course. Trace the femoral artery along the posterior side of the thigh to its division at the knee and trace both branches into the foot.

The **femoral vein** also occurs on the posterior side of the leg. Trace it proximally to its junction with a **renal portal vein**, which extends along the outer side of the mesonephros, reaching forward to the genital region. Behind, the renal portal veins of the two sides unite and receive a **caudal vein** from the tail as well as smaller pelvic veins. From each femoral vein there also arises a **pelvic vein**, the two uniting to form a **ventral abdominal vein**, which runs forward in the ventral body wall and eventually passes into the liver through the mesohepar, to unite with the hepatic vein. Trace the renal portal vein of either side forward to the anterior limits of the coelom. The portion of the vessel in front of the mesonephros is called **posterior cardinal vein**.

Add these parts to your sketch.

Now trace the connection of the femoral artery with the aorta and trace the aorta, now called the **caudal artery**, into the tail, noting the cloacal branches. Do you find spinal or intercostal arteries (p. 8) in this region?

Add these to the sketch together with the **pulmonary artery and vein**, the latter on the ventral, the former on the dorsal, side of the lung. Trace both as far forward as the septum.

**The Heart.**—Cut through the ventral wall of the body just in front of the fore legs, laying open the **pericardial cavity** in which the heart lies. Note that the cavity is lined with a smooth membrane, the **pericardium**, comparable to the peritoneum. In the heart make out the following parts: Behind and attached to the septum transversum, the **sinus venosus**. Anterior to this on the ventral side, the thick, muscular **ventricle**, from which a tube, the **truncus arteriosus**, leads to the anterior wall of the pericardial cavity. Dorsal to the ventricle are a pair of **auricles**.

Inflate by blowing into the part of the postcava left attached to the liver. Does this demonstrate a connection of postcava and sinus venosus?

Lay open the ventricle from the ventral surface and, if necessary, remove the blood or injecting mass from the interior. Follow forward with the incision, splitting the ventral wall of the truncus arteriosus. Do you find any valves between ventricle

and truncus? Farther forward the walls of the truncus are thickened and muscular, forming a **bulbus arteriosus**. How many openings do you find from the auricles into the ventricle? Cut into the auricles: are the chambers entirely distinct from each other? Do both connect with the sinus venosus? If not, which one, right or left, has such a connection?

Make a diagrammatic sketch of the heart, showing the relations of the chambers.

**The Afferent Branchials.**—Follow the truncus arteriosus forward in front of the pericardium by removing the covering muscles and notice that it branches almost immediately into right and left trunks, the portion before the branching being all that remains of the **ventral aorta**. Trace out one of the branches and see how it divides into **afferent branchial arteries**. How many are there of these? and what relation does each bear to branchial arches, gill-slits, and external gills? Cut off one of the gills and see the vessels in it. How would you describe their position?

Add the heart and the afferent branchial arteries to the sketch.

**The Mouth.**—Insert strong scissors at the angle of the mouth, the points of the blades extending just dorsal to the external gills; cut, and lay back the floor. Make a sketch of the roof of the mouth showing the distribution of teeth in three pairs of patches upon the premaxillaries, vomers, and the anterior end of the palatopterygoids. Find the **internal nares (choanæ)** by probing from the external nostrils.

In the floor of the mouth see the short **tongue**. Recognize in it, by feeling, the hyoid cartilage and extending outwards and backwards from this the branchial cartilages. What relation do these bear to the gill-slits? Insert gill-clefts and teeth in a sketch of the floor of the mouth, and, by dotted lines, the positions of the cartilages. Note the projections on the margins of the gill-clefts; to what in fishes are they to be compared? Do you find internal gills? In the median line behind is a narrow slit, the **glottis**. Insert a blowpipe in it and inflate. What happens to the lungs? Notice the change in the lining of the throat just behind the glottis, indicating the line between **pharynx** and **œsophagus**.

Indicate these features in the sketch of the floor of the mouth.

Slit up the œsophagus, lay open the roof of the mouth cavity

and trace the aorta and postcardinal veins forward, stripping off the skin to see the parts more clearly. At the level of the shoulder-girdle the aorta gives off a **subclavian artery** on either side, in front of which it divides into two vessels, the **radices aortæ**. Lateral to the radices are the anterior ends of the post-cardinal veins. Trace them as they bend to enter the sinus venosus. This portion is called the **ductus Cuvierii**.

Follow one **radix aortæ** forward into the gill region, noting the **vertebral artery** which it gives off on its medial side. In front of the origin of the vertebral the radix bends laterally to pass, as the second **efferent branchial artery**, along the dorsal side of the middle gill, receiving in its course a third efferent artery from the last gill. At the point of bending the radix is connected by a smaller vessel with a similar first efferent branchial artery which comes from the first gill. From this first efferent artery an **external carotid artery** is given off which passes downward and forward, while the common trunk of radix and first efferent continues toward the middle line as the **internal carotid artery**.

Also note, proceeding backwards from the third efferent branchial, the **pulmonary artery**, which should be traced to the lung of the same side.

Add these vessels to your drawing.

The venous trunks of the head are more difficult to follow. Follow the **anterior cardinal** or **jugular vein** forward from the ductus Cuvierii, noting its branches: first a **subclavian vein**, next a division into **internal jugular**, coming from the brain, and **external jugular vein**, from the face and lower jaw. See also a **lateral vein** entering the Cuvierian duct of either side and trace it back into the side walls of the body.

Add these vessels to the sketch and then follow out and add the remainder of the pulmonary artery and the subclavian artery and vein. Note, arising from the subclavian artery and passing back near the mid-ventral line of the coelom, a **cutaneous artery**.

(The head may now be cut from the trunk and placed for a day or two in nitric alcohol to decalcify it for the study of the brain. While this is being done the skeleton (p. 15) may be studied.)

**The Brain.**—Take the head from the nitric alcohol, rinse for 15 minutes in running water, and then very carefully remove muscles and decalcified bone from the roof until the whole top

of the brain is exposed. In this make out the following parts:

In front a pair of elongate **cerebral hemispheres**, **telencephalon** or **prosencephalon**, each prolonged in front as a narrower olfactory nerve.\* Between and behind the hemispheres is the **choroid plexus**, which is followed by the **'twixt-brain** (**di-** or **thalamencephalon**) and **midbrain** (**mesencephalon**), which are separated externally by a slight furrow. On the roof of the diencephalon is a flattened vesicle, the **pinealis**. The **cerebellum** (**metencephalon**), a slight transverse fold, lies behind the mesencephalon, and bounds anteriorly the **fossa rhomboidalis**, a triangular opening into the large posterior region of the brain, the **medulla oblongata** (**myelencephalon**). (If very carefully opened it is possible to preserve the roof of the third ventricle in the diencephalon and the large choroid plexus covering the fossa rhomboidalis.)

Draw the brain from above  $\times 6$ , and then insert the nerves arising from it, as follows:

I. The **olfactory nerve**, arising from the anterior end (olfactory lobe) of the telencephalon. II. The **optic nerve**, passing out from the floor of the diencephalon and appearing at the sides, beneath the hinder angles of the cerebrum. III. The **oculomotor nerve**, appearing in the angle between mes- and myelencephalon. IV. The **trochlearis nerve**, not readily found except by use of the microscope. It arises from the roof between mes- and metencephalon. V. The **trigeminal nerve**, arising as a strong trunk from the anterolateral portion of the myelencephalon and passing obliquely outwards and forwards. Follow it through the cranial wall to its enlargement, the **Gasserian ganglion**. From this ganglion three branches are easily followed: (*a*) an **ophthalmicus profundus**, passing straight forward; (*b*) a **maxillaris superior**, following more closely the margin of the upper jaw; and (*c*) the **mandibularis**, going to the lower jaw. VII-VIII. The **facialis** and **auditory nerves** arise just behind the trigeminal by several superimposed roots. The dorsal root goes straight to meet the Gasserian ganglion, while the other roots fuse and send laterally a strong **facialis proper** and, behind, branches to the inner ear. (The sixth nerve is closely associated with the facial so that it is

\*The division of what are apparently cerebral hemispheres into hemispheres proper and olfactory lobes is not very evident, being marked by a very slight constriction.

not readily distinguished from above.) IX-X. Four roots one after the other form the next nerve. The most anterior of these is the **glossopharyngeal nerve**, the rest compose the **vagus nerve**. All unite to form the **vagus ganglion**, from which several nerves can be traced.

Remove the brain from the skull and draw its ventral surface. Note the origin of the optic nerves (they really cross) and the large **hypophysis**, a structure in the median line between the floor of the 'twixt-brain (**infundibulum**) and the medulla oblongata. The roots of the sixth nerve (**abducens**) may be found near the middle line of the lower surface of the medulla nearly between the roots of the glossopharyngeal.

### SKELETON

The skeleton consists of **axial** and **appendicular** portions. The axial skeleton includes the vertebral column, ribs, and skull; the appendicular consists of the framework of the limbs and the arches or girdles by which they are connected to the trunk. The vertebral column may be divided into four regions: **cervical** (neck), **dorsal** (trunk), **sacral** (articulation of girdle of hind limbs), and **caudal** (tail).

Study a vertebra from about the middle of the dorsal region, making out the following features. On the ventral side in the middle line a **body** or **centrum** shaped like an hour-glass. Is it concave in front (**proccœlous**), behind (**opisthocœlous**), or at both ends (**amphicœlous**)? Dorsal to the centrum is the **neural arch** enclosing a **spinal canal** for the spinal cord. This arch consists of two plates (**neurapophyses**) which meet above and bears on its dorsal surface a projection, the **neural spine**.

In front each neurapophysis bears a projection, the **prezygapophysis**, which articulates with a similar **postzygapophysis** developed from the vertebra in front. How do these **zygapophyses** overlap?

Extending laterally from each vertebra is a **transverse process**, directed outwards and backwards and composed of two parts, a **diapophysis**, arising from the neural arch, and a **parapophysis**, from the centrum. Dia- and parapophyses are connected by a thin plate, the **vertical lamina**, and the parapophysis is expanded at its base by a similar **horizontal lamina** which unites with the

centrum for nearly its whole length. (In carefully prepared specimens dia- and pleurapophyses contain cartilages with which the ribs articulate, but usually these are lost.) The horizontal lamina is perforated by the **foramen ventrale** (for the collateral vertebral vessels), which passes, posterior to the pleurapophysis, to a deep pit, the **posterior vertebral fossa**, bounded by centrum, transverse process, and postzygapophysis.

• The ribs are short, tapering somewhat distally, while proximally each is forked, presenting two surfaces, a dorsal **tubercular** and a ventral **capitular** head, for articulation with dia- and parapophyses.

Draw a vertebra and ribs from different sides showing these points.

Compare other vertebræ with this one. Where is the neural spine largest? Do all bear transverse processes? Is the ventral foramen present in all?

The first vertebra is the **atlas**. What is the relative length of its centrum? Is it amphicœlous? On its anterior surface are a pair of slightly concave surfaces (**condylar facets**) for articulation with the base of the skull. Does the atlas bear ribs?

The vertebra with which the hind legs are connected (by means of the pelvic girdle) is the **sacrum**. Could the element intervening between this vertebra and the pelvic arch be considered as a rib? Has it two heads? Counting the atlas as 1, what is the number of the sacrum in your specimen? Compare with other skeletons in the laboratory; is the numerical position of the sacrum constant?

Study the caudal vertebræ. On what one does an arch (**hæmal arch**) on the ventral side, comparable to the neural arch, appear? Is there a disappearance of parts towards the tip of the tail? Are transverse processes constant throughout the series? Are ribs present on any of the caudal vertebræ? How many caudal vertebræ are present?

(**Sternum**.—Wilder has described cartilages in the ventral body wall, unconnected with other parts, which are regarded as **sternebræ**, or sternal elements. They are lost in skeletons prepared in the ordinary way.)

The **Skull** is composed of two parts, the **cranium**, containing the brain and special sense-organs, and the **visceral skeleton**, connected with the alimentary tract. In the cranium, in turn,

distinct parts, cartilaginous and osseous, are to be recognized. In the dry skull the cartilaginous parts are not readily made out, but by soaking in water they usually become visible.\*

The cranium is at first developed in cartilage forming a **chondrocranium**, which, while incomplete in parts, can be compared to that of the elasmobranch. As development proceeds this cartilage is in part converted into bone (cartilage bone) and in part covered by other bones (**membrane bones**) which never pass through a cartilage stage but are developed from the deeper layer of the skin. The distinctions between these two kinds of bone cannot be recognized in the adult, but only by tracing the history. A list of each group occurring in the cranium of Necturus is given here.

Cartilage Bones: 2 exoccipitals, 2 epiotics, 2 prootics, 2 pterygoids, 2 stapes, and 2 columellas.

Membrane Bones: 2 parietals, 2 frontals, 2 premaxillaries, 2 vomers, 2 squamosals, and 1 parasphenoid.

Quadrates, pterygoids, and premaxillaries rightly belong to the visceral skeleton, but they are so intimately united to the cranium that they may be considered with it.

In studying the skull, after recognizing the position of any element, carefully trace its boundaries with the hand-lens, noting the exact position of the joints (**sutures**) between it and the adjacent bones.

The cranium presents a base, where it connects with the trunk, and dorsal and ventral surfaces. In the base is a large opening, the **foramen magnum**, through which the brain connects with the spinal cord. On either side of this foramen is an **exoccipital** bone, which bears on its posterior surface an **occipital condyle** for articulation with the atlas.

On the side the exoccipital articulates with an **opisthotic** bone which extends to both dorsal and ventral surfaces, while laterally it forms the postero-lateral angle of the skull.

Draw the cranium from behind,  $\times 4$ .

The dorsal surface of the skull is covered behind by a pair of large **parietal** bones which meet in the middle line, while in front they articulate with a pair of **frontal** bones which extend forward nearly to the tip of the snout. The tip of the cranium

\* It is well to keep the skulls in 95% alcohol in which a little Lyons blue is dissolved. When required for study they may be placed in water which swells the cartilages while the blue has stained these parts so that they are distinctly seen. At the close of the exercise return them to the alcohol.

is formed by a pair of **premaxillary** bones which meet at an angle, each sending backwards an **ascending process** which partly covers the anterior end of the frontal of the same side. The rest of the premaxillary is formed by a tooth-bearing **alveolar process**.

Wedged into the angle between the antero-lateral part of the frontal and the alveolar process of the premaxillary is the upper surface of the **vomer**, the posterior portion of which is covered by the cartilaginous antorbital process of the chondrocranium to be studied later.

Extending obliquely outwards and forwards from the external surface of the opisthotic is a slender splint-like **squamosal** bone which articulates in front with the quadrate, while at about the middle of its lower margin it gives off a short **opercular process** which articulates with the stapes (*infra*). The **quadrate**, just referred to, is a splint-like bone just inside the squamosal and expanded at its anterior end where it bears a shallow fossa with which the lower jaw is articulated. The posterior portion lies close to the **quadrate cartilage** from which it has arisen by ossification.

At its posterior end the quadrate meets the lateral surface of the **prootic** bone, parts of which are visible in both dorsal and ventral views of the skull, the more medial portions being covered by the parietals above and the parasphenoid below.

Extending inwards and forwards from the quadrate to the vomer is the **palatopterygoid** bone, the limits of which are best seen in the ventral view of the cranium.

Draw a dorsal view of the skull  $\times 4$ .

On the ventral surface of the cranium in the middle line is a large **parasphenoid** bone, the anterior end of which lies between the palatine processes of the two vomers. The **vomers** (already seen from above) present, besides this palatine process, a tooth-bearing **alveolar process** which articulates behind with the anterior end of the palatopterygoid, this latter having teeth in front continuous with those of the vomer. The palatopterygoid articulates behind with the quadrate bone and the quadrate cartilage.

In drawing the ventral view of the cranium, insert these parts as well as the quadrate, prootic, opisthotic, and exoccipital bones recognized in the dorsal view.

The chondrocranium (cartilaginous skull) can be studied by taking the head of a fresh or alcoholic (not formalin) specimen, removing the skin and dissolving the flesh, etc., by warm soap solution, taking care that it does not get hot enough to injure the cartilage. Then, with the scalpel the membrane bones, enumerated above, may be readily removed, leaving but the chondrocranium and the bones ossified in it. The nasal capsules are usually so injured that they are not mentioned here.

The chondrocranium consists behind of a pair of irregularly oval hollow bodies, the **otic capsules** connected above with each other by a narrow cartilage band, the **synotic tectum**, below by a broader cartilage, the remains of a primitively much larger **parachordal plate**. On the medial side each otic capsule has a large foramen for the passage of nerves and blood-vessels, while on the inferolateral surface an opening, the **fenestra ovalis**, is occupied by the stapes. The walls of the capsule are made up largely of pro- and opisthotic bones, the extent of which can now be clearly seen.

From the anterior end of each otic capsule a slender cartilage bar, the **trabecula**, extends to near the snout, where the trabeculæ of the two sides are connected by a median **ethmoid plate**, beyond which the trabeculæ continue as the **cornua trabecularium**. From each trabecula, near its anterior end, an **antorbital process** \* is directed outwards and slightly forwards, in front of the eye.

The quadrate bone and cartilage are best considered together. They extend outwards and forwards from the otic capsule to the articular surface for the attachment of the jaw and to the hinder end of the pterygoquadrate. From this as a centre they give off two diverging processes, an **otic**, extending to the prootic bone, and an **epipterygoid process**, reaching to the base of the trabecula.

This cartilaginous skull will also show well the **stapes**, a rounded plate of bone, with a prominent **columellar process**, fitting in the fenestra ovalis.

The lower jaw consists of two halves loosely united in front, and each half consisting of three bones arranged around a cartilage bar. These bones are a tooth-bearing **dentary** in front and on the outer side; a plate-like, tooth-bearing **splénial** at about

\* Sometimes regarded as the anterior part of the pterygoquadrate arch, but not proved to be such.

the middle of the inner surface and a large **angular bone** making up most of the inner surface. Between these bones runs **Meckel's cartilage**, its posterior end enlarged for articulation with the quadrate. (In many vertebrates this end ossifies as an **articular bone**.)

Draw the lower jaw from the inner and outer surfaces.

The rest of the visceral skeleton is composed of a series of cartilage arches in the floor of the mouth and throat. These arches, of which there are four, are connected together by a longitudinal median portion, the **copula**. The most anterior arch is the **hyoid**, each half of which is composed of a small medial **hypohyal** and a larger and more lateral **ceratohyal**. The copula between the hyoid and the next (the **first branchial**) arch is the first **basibranchial**. Each half of the first branchial arch is composed of a **ceratobranchial** next the middle line, and a larger distal **epibranchial**. Branchial arches 2 and 3 are more incomplete, the ceratobranchial elements being small or absent. Behind the first ceratobranchials the copula is continued by a slender bone, the **second basibranchial**.

Draw this hyoid-branchial apparatus.

The **pectoral**, or **shoulder girdle**, is that arch of cartilage and bone which supports the fore limbs. Its two halves are not connected, but overlap ventrally. Each half consists of dorsal and ventral portions. In the ventral portion, which is entirely cartilaginous, distinct elements cannot be recognized but merely regions, a **procoracoid** in front, a **coracoid** behind, the limits between the two being slightly indicated by a small opening, the **coracoid foramen**, passing through the cartilage and by the pit, the **glenoid fossa**, by which the arm is articulated to the girdle. The dorsal half of the girdle consists of a bony element, the **scapula**, continued dorsally by a larger cartilaginous plate, the **suprascapula**.

The bone of the upper arm (**humerus**) is cartilaginous at either end, but has a bony shaft. On the lower surface it presents a strong **crista deltoidea** for the attachment of the thoracic muscles. In the forearm are two bones, the **radius** on the thumb or anterior side, the **ulna** on the other; both have cartilaginous ends, the proximal of the ulna extending beyond the elbow-joint as a strong **olecranon process**.

In the wrist (**carpus**) there are six or seven cartilages arranged

as follows: a proximal row of two, a **radiale** on the radial side, and an **ulnare** on the other, to which is usually fused a middle element, the **intermedium**. The distal row consists of three **carpales**, while a **centrale** lies between the two rows.

The carpus is followed by the palm, **metacarpus**, and this in turn by the **digits**. How many metacarpal bones, and how many bones (**phalanges**) in each finger? (The thumb—**pollex**—or first digit is absent.)

The pelvic girdle is largely cartilaginous, its two halves being fused in the mid-ventral line. On either side the pelvis bears a bony element, the **ilium**, which articulates by the intervention of a cartilage with the sacrum. At the base of the ilium is a pit, the **acetabulum**, for the articulation of the hind limb. The ventral half of the girdle forms a flat plate, the anterior part of which is the **pubis**, the posterior the **ischium**. In the ischial region is an ossification, and the limits between ischium and pubis are indicated by a small **obturator foramen**.

The bones of the hind limb correspond very closely with those of the arm—**femur** to humerus, **tibia** and **fibula** to ulna and radius, **tarsus** to carpus (**tibiale**, **fibulare**, **centrale**, **tarsales**), **metatarsals** to metacarpals—except that there are two ridges (**cresta ventralis**, **c. lateralis**) on the femur. As in the hand the first digit (**hallux**) is lacking.

Draw both girdles and the skeleton of each limb, indicating in your drawing bone and cartilage.

## LITERATURE

THE following list of papers upon the morphology of the Urodeles contains the more important works relating to the group, as well as some of the lesser notes relating to Necturus. It includes, besides articles on the anatomy, others upon the organogeny which are necessary to the understanding of the structures dissected.

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