A Laboratory Outline of Embryology

WITH SPECIAL REFERENCE TO THE

Chick AND THE *Pig*

New Printing with Corrections, 1956

By

FRANK R. LILLIE

and

CARL R. MOORE

THE UNIVERSITY OF CHICAGO PRESS

A Laboratory Outline of Embryology

WITH SPECIAL REFERENCE TO THE

Chick AND THE *Pig*

New Printing with Corrections, 1956

By

FRANK R. LILLIE

and

CARL R. MOORE

THE UNIVERSITY OF CHICAGO PRESS

THE UNIVERSITY OF CHICAGO PRESS, CHICAGO 37
Cambridge University Press, London, N.W. 1, England

 Published April 1919. Second Edition June 1923. Third Edition May 1934. Fourteenth Impression 1956. Composed and printed by THE UNIVERSITY OF CHICAGO PRESS, *Chicago, Illinois, U.S.A.*

A Laboratory Outline of Embryology

WITH SPECIAL REFERENCE TO THE

Chick AND THE *Pig*

PREFACE

THE original publication of the *Embryology of the Chick and the Pig*, with its revision (Lillie, 1904 and 1906), has served for the study of embryology for medical students in many schools since that time; practical experience in the meantime has proved to the writers that a course in embryology for medical students can be presented more effectively by studying the development of each organ system separately than by consideration of the entire embryo at time intervals.

These outlines, arranged by the junior writer, are the result of several revisions of the original draft, and have been employed in the medical course at the University of Chicago since 1915. It is hoped that they may prove useful in other institutions as an indication of a standard type of laboratory study for medical students. With proper additions, by an instructor, the outlines may be adapted to a more thorough laboratory course than is considered necessary for the medical student.

Specific directions have been included to enable the student to locate certain regions and structures; it is assumed that the sections are cut transversely to the longest axis of the embryo and that sections of approximately the following thickness are used: 33-hour chick, 10 μ–15 μ; 48-hour chick, 15 μ; 72-hour chick, 15 μ; 10-mm. pig, 15 μ.

References to figures and pages refer to the *Development of the Chick* (Lillie, 3d ed.) and to the *Developmental Anatomy* (Arey, 6th ed.), both of which the student is advised to have at hand in the laboratory.

HULL ZOÖLOGICAL LABORATORIES
UNIVERSITY OF CHICAGO
1952

PROVISIONAL TIME SCHEDULE

I.	*Morphology and gross anatomy*	
	Chick embryo, 33 hours, living and entire preparation	2½ hrs.
	Chick embryo, 33 hours, sections	3½ hrs.
	Chick embryo, 48 hours, living and entire preparation	3 hrs.
	Chick embryo, 72 hours, living and entire preparation	3 hrs.
	Chick embryo, five days	1 hr.
	Pig embryo, 10-mm. dissection	2 hrs.
	Pig embryos, 15-mm. and 25-mm. dissection	3 hrs.
II.	*Embryonic membranes, coelom, mesenteries, and placenta*	
	Chick embryos, 33 hours and 48 hours	3 hrs.
	Chick embryo, 72 hours	3 hrs.
	Pig embryo, 10 mm.	3 hrs.
	Placenta of pig	3 hrs.
III.	*Ectodermal derivatives*	
	Nervous system of chicks and pig	3 hrs.
	Organs of special sense, of chicks and pig	3 hrs.
IV.	*Entodermal derivatives*	
	Chick embryos, 33 hours, 48 hours, and 72 hours	3 hrs.
	Pig embryo, 10 mm.	3 hrs.
V.	*Mesodermal derivatives*	
	Urinogenital system of chicks and pig	3 hrs.
	Heart of chicks and pig	3 hrs.
	Arterial system of chicks and pig	5 hrs.
	Venous system of chicks and pig	6 hrs.
	Derivatives of a somite	1 hr.
		60 hrs.

TABLE OF CONTENTS

PROVISIONAL TIME SCHEDULE ix

METHODS 1

I. MORPHOLOGY AND GROSS ANATOMY 2

II. EMBRYONIC MEMBRANES, COELOM, MESENTERIES, AND PLACENTA 17

III. ECTODERMAL DERIVATIVES 25

IV. ENTODERMAL DERIVATIVES 32

V. MESODERMAL DERIVATIVES 37

METHODS

THE methods of study employed in the following outlines are: (1) Study of the living embryo. (2) Study of the entire embryo: (*a*) as an opaque object with the dissecting microscope; (*b*) with the compound microscope after killing, hardening, staining, clearing, and mounting. (3) Study of embryos by dissection, especially in later stages. (4) Study of organs and systems from the serial sections with the microscope.

Lillie's *Development of the Chick* and Arey's *Developmental Anatomy* should be at hand continually. Notes consist of answers to questions.

STUDY OF THE LIVING EMBRYO OF THE CHICK

This is best performed under warm normal salt solution (0.75 per cent NaCl heated to about 38° C.). Open the egg by gradually picking away the shell at the broad end, making an aperture large enough to permit of the escape of the entire egg contents without breaking the yolk. Carefully tilt the egg to permit contents to flow out into the salt solution. The embryo may now be examined on the surface of the yolk, as the blastoderm almost invariably turns up, or may easily be made to do so. The blastoderm can now be separated by cutting through it *outside the area vasculosa* with fine scissors. It should be gently floated into a Syracuse watch crystal *submerged* in the salt solution. The crystal and its contents may then be lifted out. The vitelline membrane, a delicate *transparent membrane* covering the blastoderm, must next be removed.

I. MORPHOLOGY AND GROSS ANATOMY

A. CHICK EMBRYO WITH FROM TEN TO FOURTEEN SOMITES (TWENTY-NINE TO THIRTY-FOUR HOURS)

1. *The egg.*—While opening the egg, observe that the *shell membrane* is double. The *air chamber* at the broad end is between its two layers. After pouring the egg into the bowl, observe the twisted cords of albumen in the "white" (*chalazae*). They are attached to the denser layer of albumen (secreted by the glands of the oviduct) that closely surrounds the yolk. What function may they have? The *yolk* or *vitellus* is the true *ovum,* the other parts being merely *envelopes.* Observe the *vitelline membrane* surrounding the yolk. (Lillie, Fig. 31.)

2. *The living embryo.*—How much of the yolk is covered by the *blastoderm* (the living part of the egg)? In the center of the blastoderm is a transparent slipper-shaped area (*area pellucida*), in which the embryo appears as a narrow opaque streak. The part of the blastoderm external to the pellucid area is known as the *area opaca.* How much of the latter is occupied by the *area vasculosa?* The remainder of the opaque area is known as the *area vitellina.* Make a sketch of the egg and embryo to show these relations.

3. *Entire mount including the vascular area (stained embryo).*—

a) In the vascular area observe the irregular deeply stained masses (*blood islands*). At this stage it will be found that they are inclosed in wide anastomosing tubes, the *extra-embryonic blood vessels,* that later open peripherally into the bounding *sinus terminalis.* How are the extra-embryonic connected with the embryonic blood vessels?

b) The embryo. Make a careful drawing *to scale* (×*ca.* 25 or 30) of the embryo and *area vasculosa*. State whether your drawing is from the *upper* or from the *under* side. (Lillie, Figs. 69, 70, 71.)

The following structures can be readily identified (others will be seen, but are better understood after study of sections):

(1) The *neural tube* forms the axis of the embryo. In its anterior region identify *forebrain* (*prosencephalon*), *optic vesicles, midbrain* (*mesencephalon*), *hindbrain* (*rhombencephalon*). The secondary subdivisions of the handbrain are the *neuromeres;* how many? The portion of the neural tube (*cord, myelon*) back of the hindbrain is closed in front but open behind.

(2) The *auditory pit,* a slight depression of thickened ectoderm lateral to the fourth and fifth neuromeres, *may* be seen in the older embryos of this stage (13 to 14 somites). (Lillie, Fig. 77.)

(3) The *primitive streak* is inclosed by the diverging folds of the myelon.

(4) The *head fold*. The head of the embryo projects freely above the blastoderm. The fold uniting the ventral surface of the head with the blastoderm is known as the "head fold." (Lillie, Figs. 41, 57, 73.)

(5) The *mesoblastic somites,* appearing on each side of the neural tube. Number? The series is continued behind by the undivided *segmental plate*.

(6) The *heart* lies beneath the hindbrain in a special portion of the body cavity bounded in front by the head fold and behind by the converging limbs of the *splanchnopleure*. Its posterior (venous) end receives the *omphalomesenteric veins* from the vascular area. Its anterior

(arterial) end is prolonged into the *ventral aorta*. Its axis is somewhat bent at this stage. In what direction?

(7) The *proamnion*, a transparent two-layered portion of the blastoderm beneath and lateral and anterior to the head of the embryo.

(8) The *head fold* of the *amnion* is beginning to fold over the anterior end of the head in the older embryos of this stage.

(9) The *fore-gut*, the lateral boundaries of which may be seen between the ectoderm of the head and the brain.

(10) The *notochord*, a dark streak apparently in the center of, but actually ventral to, the brain and neural tube.[1]

4. *Study of transverse sections.*—The embryo has been cut into a series of transverse sections of uniform thickness of 15 micromillimeters (0.015 mm.). The number of any section in the series, therefore, in comparison with the entire number, will enable the student to ascertain its position with relation to the entire embryo. *Each section drawn is to be located in its position by a line across the drawing of the entire embryo.*

Study each section drawn under the high power as well as under the low power. Some drawings of parts of sections should be made with the high power to show the character of the individual cells. For low-power drawings use colors to distinguish ectoderm (blue), mesoderm (red), and entoderm (yellow), and do not draw in cells.

After the segmentation of the ovum the multiplication of cells continues, and these gradually arrange themselves in such a manner as to form three distinct sheets or

1. The part of the embryo back to the last somite formed represents the head and cervical region of the bird. The remainder of the embryo is to be formed from the posterior unsegmented region.

laminae, which are named germ layers. These layers are designated: the outer as ectoderm, the inner as entoderm, and the middle as mesoderm. The student should familiarize himself with the appearance of these layers and the modification of each in the production of the various organs and systems. (For derivatives of germ layers see Arey, p. 83.)

Make *careful* drawings of transverse sections through the following regions, under the low power of the microscope:

(*a*) Primitive streak.
(*b*) Through region of the open neural tube.
(*c*) Through the sixth or seventh somite of the series.
(*d*) Posterior half of heart.
(*e*) Through midbrain.
(*f*) Through optic vesicles.

Identify, and *label fully,* all the parts to be observed.

a) The section illustrates the undifferentiated germ layers from which the embryo is formed, and the formation of mesoderm from the primitive streak. It represents, in a sense, the youngest part of the embryo; and, as development proceeds, the primitive streak as such disappears, becoming part of the embryo proper (see Lillie, chap. iv and Fig. 38). Note (1) that the entoderm (lower layer) is separate, while mesoderm and ectoderm are not separate layers in the median line but merge inseparably in the undifferentiated mass of cells; this is the *primitive streak.* The depression in the primitive streak is the *primitive groove.* (2) Mesoderm appears as wings from the thickened central portion and is split peripherally into two layers known as the somatic (upper) and splanchnic layers; the combination of the somatic layer with the ectoderm is known as the *somatopleure,* that of

the splanchnic layer with the entoderm as *splanchnopleure.* (3) The space between these two layers is the *body cavity* (coelom). What is the significance of the primitive streak? (Read Lillie, pp. 95–118.)

b) As one proceeds anteriorly from the primitive streak into an older, differentiated region of the embryo, the germ layers appear slightly modified. Note (1) that the *medullary plate* (thickened ectoderm) has folded upward, producing the *neural groove* (bounded by the *neural folds*) which is not yet closed above. (2) the rodlike *notochord* (reckoned as mesoderm) lies directly under the neural tube.[1] (3) The somatic and splanchnic layers of mesoderm are separated within the embryo producing the *coelom,* which is continuous with the extra-embryonic body cavity. Do blood vessels appear in this section, and if so where?

c) This section should be about halfway between the posterior end of the heart and the last somite and through the center of a mesoblastic somite (see Lillie, Fig. 74). The ectodermal folds (neural or medullary folds) have met dorsally in the median line, completing the closed *neural tube.* The *notochord* appears ventral to the neural tube. The mesoderm is much thickened and consists on each side of the median line of the following parts: (1) the *mesoblastic somite,* a block of cells that radiate from a central mass; (2) the *intermediate cell mass* (*nephrotome*) between (1) and (3); (3) the *lateral plate,* split into the somatic and splanchnic layers. Is there a *coelom* in this section? The two large blood vessels immediately beneath the somites are the *dorsal aortae.* Note the nu-

1. The notochord is the first indication of a skeletal axis and is the center about which mesenchyme (sclerotome) is converted, first into cartilage and finally into bone, to form the centra of vertebrae.

merous blood vessels in the splanchnopleure; do you find any in the somatopleure? The entodermal layer always appears as the ventral boundary of the embryo or blastoderm; in the region of the embryo it folds to produce the gut, and peripherally continues as the lining of the yolk sac. This relation should always be kept in mind.

d) Trace the parts shown in (*c*) forward to this section. The splanchnopleure folds ventrally and has given rise to two new structures in this region: the *pharynx* or anterior division of the *fore-gut*, produced by the growing-together ventrally of the splanchnopleure from the two sides, and the heart, produced in the same manner from splanchnic mesoderm. The *fore-gut* appears as a crescentic cavity with lateral projections. What do these signify? Notice again at the open end of the fore-gut (a few sections posterior to *d*) that the gut is formed by the gradual folding and growing-together of the splanchnopleure. The *heart,* formed from the splanchnic layer of mesoderm, is attached by a dorsal mesentery (mesocardium) to the under wall of the pharynx. Note its two layers; *muscular* (*myocardium*) and *endothelial* (*endocardium*), both of which are mesoderm. The cavity in which it is situated (*pericardial*) is an enlarged part of the general body cavity or coelom. Do the dorsal aortae appear in this section? To what level of the embryo do they extend anteriorly?

e) Choose a section through the oral plate, the point of fusion between ventral ectoderm of the head and entoderm of the ventral side of the pharynx. This is the site of the future mouth (see Lillie, Figs. 61, 73). The fore-gut in the region of mid- and hindbrain is known as the pharynx. Identify *dorsal* and *ventral aortae, proamnion, parietal cavity* (extra-embryonic), *somatopleure, splanch-*

nopleure. Find out, by tracing both forward and backward, if the ventral aortae have any connection with heart or dorsal aortae. Compare the mesoderm of this section with that of section *c;* the loose unorganized mesoderm is known as mesenchyme. Is the embryo connected with the blastoderm in this section?

f) Do you find entodermal structures in this section? Where did the notochord disappear? Draw in the accompanying section of the blastoderm. What is the proamnion? Identify and label parts.

B. CHICK EMBRYO WITH FROM TWENTY-FOUR TO TWENTY-NINE SOMITES (FORTY-FOUR TO FORTY-EIGHT HOURS)

1. *The living embryo*.—Describe carefully the changes visible to the naked eye since the thirty-fourth hour. Observe the beating of the heart and the circulation of the blood. Can the heartbeat after stopping be renewed by slight heating? Make a sketch of the embryo on the egg showing the relation of the various areas of the blastoderm. Remove the embryo with the entire vascular area and preserve it.

2. *Entire mounts*.—The most striking changes concern the region of the head. By more rapid growth of the dorsal surface the head has become bent (*cephalic flexure*) in the region of the midbrain, so that the forebrain and part of the midbrain form almost a right angle with the rest of the head. Moreover, the head has become so far free from the blastoderm, and so compressed laterally, that it now lies on its side (which side?). The dorsal side of the trunk, on the other hand, is still turned up, so that there is a twisting of the embryo just back of the heart. About the head three layers may be seen: *brain wall, ecto-*

derm of head, and *amnion.* The *tail fold* is not yet formed, or has just begun. (Lillie, Fig. 98.)

a) Brain: The prosencephalon now exhibits two divisions (*telencephalon* and *diencephalon*) and the rhombencephalon likewise two (*metencephalon* and *myelencephalon*); the mesencephalon is never divided.

b) The *optic vesicles* are relatively smaller in relation to the brain than in the 33-hour chick (not actually smaller, of course). To which division of the forebrain are they attached? Distinguish *inner* and *outer layers* of the *retina,* the *lens,* and the *choroid fissure.*

c) The *auditory vesicles* (*otocyst*) are now closed (?) sacs. Above which visceral arch do they lie?

d) The heart has grown greatly in length, and, its two ends being fixed, it has become doubled on itself. Identify *atrium, ventricle,* and *bulbus arteriosus.* What is the relation of the heart to the main afferent and efferent blood vessels?

e) Two or three *visceral* (*pharyngeal*) *grooves* are now visible. They may be found ventral to the region of the myelencephalon. The first or *hyomandibular* groove is bounded in front by the *first visceral* or *mandibular arch,* and behind by the *second visceral* or *hyoid arch;* the second groove is bounded in front by the *hyoid,* and behind by the *third visceral arch;* the third groove is bounded in front by the *third visceral arch* and behind by the *fourth* (see Lillie, pp. 218–24). Running through each visceral arch note the smaller clear blood vessel, the *aortic arches.* Why called arches?

f) How many mesoblastic somites are there? What is the condition of the mesoblastic segmental plate?

g) How far back is the fore-gut closed?