

MOSTLY

VERTEBRATE EMBRYOLOGY

BIOLOGY 402

CONCORDIA COLLEGE

A LABORATORY MANUAL

Designed to be used with the following:

A Photographic Atlas of
Developmental Biology

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HISTOLOGY

Slide box h26 A (Histology cabinet)

Slide: Ileum, Monkey, x.s.
Turtox H5.53

Serosa (type of epithelium ?)

outer and inner muscle layers (type - smooth / direction - longitudinal or circular ?), submucosa (CT), muscularis mucosae (not always visible), lamina propria (CT), villi, epithelium (type - simple columnar), lumen, blood vessels (?)

Slide box h14 (Histology cabinet)

Slide: Esophagus and trachea

Ripon A110-9 Esophagus and trachea, c.s.

Wards' 93 W 4871 Trachea, c.s.

The Ripon slide shows all the structures listed below.

The Ward's slide has better detail for the lining of the trachea.

Esophagus stratified squamous epithelium (non keratinizing)

Trachea pseudostratified columnar ciliated epithelium with goblet cells
perichondrium, chondrocytes, cartilage matrix

Glands - simple cuboidal epithelium

Adipose cells (another connective tissue)

Skeletal muscle - peripheral nuclei, striations

(see web site for histology at <http://www.cord.edu/faculty/todt/336/lab/intro/slide.html>)

Slide Box #5

slide: Muscle types	skeletal, smooth, cardiac
Carolina H 1360	N=8

All three muscle types are on this slide. See if you can pick them out.

Skeletal tongue

- fibers are oriented in different directions
- look for a region of longitudinal section
 - multinucleated
 - peripheral nuclei
 - striations in longitudinal section
- look for region of myofibers in cross-section
 - peripheral nuclei

Cardiac muscle

- confusing section not uniform orientation like skeletal muscle
- look for a region of "longitudinal" fibers

- look for striations
- look for intercalated discs

- look for area of cross-section

- central nuclei
- cells about same diameter

Smooth muscle digestive system

- smooth muscle in the wall of the gut

- look for nuclei in longitudinal section

(see web site for histology at <http://www.cord.edu/faculty/todt/336/lab/muscle/3muscles.htm>)

MITOSIS

Preparation: Longitudinal sections of the uterus of *Ascaris* showing successive stages of cell division.

Instructions: Study the entire slide and become familiar with the different divisional stages before you start to draw. Draw each stage in the appropriate space. On each drawing label:

Shell, Extracellular Fluid, Plasma membrane, and Cytoplasm.

In addition, label the following features where appropriate:

Nuclear membrane, Nucleolus, Polar bodies, Pronuclei, Chromosome, Equatorial plate, Spindle fibers and Centriole, Asters.

RECENTLY FERTILIZED EGG (FIG. 5.15G)	PROPHASE
METAPHASE (LATERAL VIEW; FIG. 5.15H)	METAPHASE (POLAR VIEW)

MITOSIS (CONTINUED)

Preparation: Longitudinal sections of the uterus of *Ascaris* showing successive stages of cell division.

Instructions: Study the entire slide and become familiar with the different divisional stages before you start to draw. Draw each stage in the appropriate space. On each drawing label:

Shell, Extracellular Fluid, Plasma membrane, and Cytoplasm.

In addition, label the following features where appropriate:

Nuclear membrane, Nucleolus, Polar bodies, Pronuclei, Chromosome, Equatorial plate, Spindle fibers and Centriole, Asters.

ANAPHASE (EARLY) (FIG. 5.15I)	ANAPHASE (LATE)
TELOPHASE (CYTOKINESIS; FIG. 5.15J)	DAUGHTER CELLS

SPERMATOGENESIS

Slide box e1 Gametogenesis

Slide: spermatogenesis, rat testis Ward's 93 W 5441	n=12
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Find the following structures:

fixation artifact	
interstitial cells	(figs. 3.44, 3.45)
myoid cells (nuclei)	(figs. 3.44, 3.45)
blood vessels	(fig. 3.36)
seminiferous tubules with basement membrane and lumen	(figs. 3.34 to 3.61)
Sertoli cells (nucleus with nucleolus)	(fig 3.35)
Spermatogonia (ovoid nucleus)	(figs 3.42, 3.44, 3.46)
spermatocytes (primary and secondary)	(figs 3.42, 3.44, 3.46)
spermatids	(figs. 3.42, 3.46)
residual bodies	(fig. 3.45?)
sperm heads and tails	(figs. 3.42, 3.71)

Slide box e1 Gametogenesis

Slide: Bull sperm smear Turtox E17.16 or Ward's	n=30
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(see fig. 3.71; high dry magnification)

head
tail
general morphology

Slide box h40 (Histology cabinet)

Slide: testis, human interstitial tissue Turtox H9.415	n=7
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similar to figs. 3.43 to 3.46

interstitial tissue
myoid cells (nuclei)
basement membrane (basil lamina)

OOGENESIS

Slide box h39 Ovary

Slide: Ovary, mature follicle, cat. Ward's 93 W 5532 n=14

Find the following structures:

General (figs. 4.36 to 4.39):

- germinal epithelium (simple squamous)
- tunica albuginea (dense connective tissue)
- cortex / medulla (hard to define boundary between them)

Primordial follicle (fig 4.25):

- small primary oocyte (lots of them located just under the tunica albuginea)
- thin follicular cells surrounding oocyte

Primary (growing) follicle (Figs 4.26 to 4.28):

- larger primary oocyte
- "thicker" follicular cells (they look like a simple cuboidal epithelium)
- younger follicles will have a simple cuboidal covering of follicular cells
- older follicles will have a stratified cuboidal covering of follicular cells

Secondary (antral) follicle (figs 4.29 to 4.31):

- larger primary oocyte
- developed follicular cells with a visible antrum or cavity

Mature (Graafian) follicle (figs 4.32 to 4.34):

- theca externa (fibrous)
- theca interna (cellular)
- stratum granulosum
- cumulus oophorus
- oocyte
- zona pellucida (blue in these slides)
- antrum with liquor folliculi (often appears granular)

Corpus luteum (figs. 4.42 and 4.43):

- hypertrophied granulosa cells (pinkish color)
- what do these cells secrete ?

ECHINODERM CLEAVAGE

Slide box e3 Whole-mounts of stained starfish embryos-various stages. (see fig. 6.12 to 6.15)

Slide: starfish composite
other labels too

Instructions: Draw and label the following structures (where applicable) of the stages below:

fertilization membrane, plasma membrane, nucleus, nuclear membrane, nucleolus, cytoplasm, blastocoel, blastoderm, animal pole and vegetal pole.

UNFERTILIZED OVUM (Primary Oocyte; FIG. 5.24 B)	FERTILIZED EGG (FIGS. 5.24D, 6.15 B)
TWO-CELL STAGE (FIG. 6.15 D,E,F)	EIGHT-CELL STAGE (FIG. 6.15 I, J)
EARLY BLASTULA (FIGS. 6.15 M, N)	LATE BLASTULA (FIG. 6.15 P)

ECHINODERM GASTRULATION

Slide box e3 Whole-mounts of stained starfish embryos - various stages.

Slides: starfish composite, starfish all stages young larvae (late gastrula) (# 4917) Bipinnarian Larvae (# 5250)

Instructions: Draw and label the following structures (where applicable) of the stages below:

plasma membrane, cytoplasm, fertilization membrane, blastocoel, blastoderm, blastopore, gastrocoel (archenteron), animal and vegetal poles, endoderm, ectoderm, mouth, esophagus, stomach, intestine, anus.

LATE BLASTULA (AGAIN FIG. 7.8 A)	EARLY GASTRULA (FIG. 7.8 C)
GASTRULA (LATERAL VIEW; FIG. 7.8)	GASTRULA (POLAR VIEW) (FIG. 7.8 B)
LATE GASTRULA (FIG. 7.8 G)	BIPINNARIAN LARVA (FIGS. 7.9, TO 7.12)

EARLY AMPHIBIAN DEVELOPMENT

Slide boxes e3, e4, and e5 (see figs 6.26, 6.27, 7.19 and 7.20)

Instructions: Using slides from the indicated boxes draw and label the following stages & structures.
 Cleavage planes (when identifiable), animal and vegetal poles, blastocoel, blastopore
 dorsal and ventral lips of the blastopore, archenteron, yolk plug.

box e3	slide 5100	box e4	
EARLY CLEAVAGE (8-CELL STAGE; FIG. 6.27 A)		EARLY BLASTULA (FIG. 6.27 C)	
box e4	slide 5120	box e4	slide 5134
LATE BLASTULA (FIG. 6.27 D, 7.20 A)		EARLY GASTRULA (FIG. 7.20 B)	
box e5	slide 5140		
LATE GASTRULA (YOLK PLUG; FIG. 7.20 F)			

CHICK PRIMITIVE STREAK

Slide box e8

Slide: 18 hr chick, wholemount

figs. 7.27 to 7.30 (see also 7.22 to 7.25)

Identify the following:

primitive streak (folds and groove), primitive knot, notochord if visible
margin of mesoderm (cranial border of mesoderm) and proamnion
the head fold may not be visible
area pellucida and area opaca
(we will subdivide into vitellina and vasculosa and blood islands later)

Slide box e8

Slide: 18 hr chick, serial sections

figs. 7.33 and 7.34 (see also 7.26)

Identify the following:

neural plate (see also fig 7.30)
epiblast, mesoderm, hypoblast, subgerminal cavity
primitive knot, primitive groove, primitive folds
know which way is anterior and posterior
you may not be able to distinguish the area opaca (if no yolk is visible)

FROG NEURULATION

Look to find the successive stages of neurulation using the following slides and figures as a guide. Keep in mind that because we don't have the entire embryo in serial section, it may be hard to be absolutely sure where any particular section is taken from along the embryonic axis. So what you see on the slide may not correspond to exactly what you see in the lab manual.

Slide box e5

Slide: frog, neural plate
e.g. E 1680

see page 111 (figs. 8.14 to 8.16)

Learn and be able to identify #1 to 12 in fig, 8.16

Slide box e6

Slide: frog, neural fold / groove
e.g., E 14.35

see page 112 (figs. 8.17 to 8.19)

Learn and be able to identify #1 to 15 in fig, 8.19

liver diverticulum = outpocketing of foregut (see fig. 8.21)

Slide box e7

Slide: frog neural tube
e.g., E 14.41 cross section
E 14.42 sagittal section

see pages 113 to 115 (figs. 8.20 to 8.23)

Learn and be able to identify #1 to 24 in fig, 8.21 (with exceptions below)

prosencephalon, mesencephalon and rhombencephalon= parts of brain (later)

don't worry about terms 7, 8, and 11 of fig. 8.20

don't worry about terms 8, 9, 10, 14, 22, and 24 of fig. 8.21

CHICK NEURULATION

24 HR OF INCUBATION

Slide box e9

Slide: 24 hr chick, wholemount

figs 8.33

Identify all of the structures listed in fig. 8.33 (also in 8.29 to 8.32)

Slide: 24 hr chick, serial sections

Identify all of the structures in figs. 8.37

Fig 8.36 gives reference locations for each figure.

Figs 8.34 and 8.35 may also help to put things in perspective.

CHICK DEVELOPMENT

33, 48 AND 72 HRS

18 different slide boxes contain sets representing each stage.
Sets may contain several slides. Please keep the sets intact.
Please be careful that slides do not get **broken**.
(a set with a slide missing is not worth much)

For all the figures, learn the labeled structures and be able to follow them in space as you move forward and backward in the embryo. Refer to the next three pages for structures to know.

(the glossary in back of the lab manual is helpful for terms we have not yet covered in class)

Slide: 33 hour chick, serial sections

Figs. 11.5 to 11.10 show wholemounts

Fig 11.15 shows representative cross-sections

(Figs. 11.12 and 11.13 have longitudinal sections.)
(We don't have corresponding slides).

Slide: 48 hour chick, serial sections

Fig. 11.24 (wholemount) (and other figures for perspective)

Fig. 11.25 (cross-sections)

Slide: 72 hour chick, serial sections

Fig. 11.37 (wholemount) (Figs. 11.28 to 11.36 show more views, etc.)

Fig. 11.38 (cross-sections)

Lab Intro and Lab Exam

At the beginning of each lab, an introduction will be done to some part of the chick that we have covered in class. The introductions will be done using the projecting microscope or DVD. This format will be used for the lab exam on the chick material. **All questions** will come from structures seen in the serial, cross-sections of the embryos at 33, 48 or 72 hours.

Chick 33 Hr
Figure

(Wright)
Fig. 11.7
wholemout

Fig. 11.15
cross sections

Fig. 11.15 (cont.)
cross sections

area opaca
area pellucida
area vasculosa
area vitellina
blood islands
conotruncus
cranial intestinal portal
epidermis
foregut
head fold
heart
Hensen's node
infundibulum
lateral plate mesoderm
mesencephalon
neuromeres
optic vesicle
primitive streak
proamnion
prosencephalon
rhombencephalon
segmental plate mesoderm
somite
spinal cord
ventricle
vitelline vein

aortic arch 1
conotruncus
cranial cardinal vein
cranial intestinal portal
dorsal aorta
dorsal mesocardium
ectoderm
embryonic coelom (intra-)
endocardium
endoderm
epidermis
epimyocardium
extraembryonic coelom
foregut
head mesenchyme
infundibulum
intermediate mesoderm
lateral plate mesoderm
mesencephalon
midgut
neural crest
neural fold
neural groove
notochord
optic vesicle
oral membrane

pericardial cavity
primitive fold
primitive groove
primitive streak
proamnion
prosencephalon
rhombencephalon
segmental plate mesoderm
sinoatrium
somatic mesoderm
somatopleure
somite
spinal cord
splanchnic mesoderm
splanchnopleure
stomodeum
subcephalic pocket
ventral aorta
ventricle
vitelline plexus
vitelline vein
yolk

Chick 48 Hr Wright
Figs. 11.24 and 11.25
wholemound and sections

amnion	ecotderm	otic vesicle
amniotic cavity	embryonic coelom (intra-)	pericardial cavity
aortic arch 1	endocardium	peritoneal cavity
aortic arch 2	endoderm	pharygneal pouch 1
aortic arch 3	epidermis	pharygneal pouch 2
aortic sac	epimyocardium	pharygneal pouch 3
atrium	extraembryonic coelom	pharynx
branchial groove I	foregut	pigmented retina
C.N. V Trigeminal	head mesenchyme	pleural cavity
C.N. VII Facial	infundibulum	Rathke's pouch
C.N. VIII Acoustic	laryngeotracheal groove	roof of myelencephalon
caudal cardinal vein	lateral amniotic fold	segmental plate mesoderm
caudal intestinal portal	lateral body fold	sinus venosus
caudal liver diverticulum	lateral plate mesoderm	somite
chorion	lens vesicle	spinal cord
chorionic cavity	mandibular process	stomodeum
closing plate I	maxillary process	tail bud
common cardinal vein	mesencephalon	telencephalon
conotruncus	metencephalon	thyroid rudiment
cranial cardinal vein	midgut	transvers septum
cranial intestinal portal	myelencephalon	ventricle
cranial liver diverticulum	neural retina (sensory)	vitelline artery
descending aorta	notochord	vitelline blood vessel
diencephalon	olfactory placode	vitelline vein
dorsal aorta	optic cup	vitelline vessel
dorsal mesocardium	optic stalk	yolk sac
duodenum	oral membrane	

Chick 72 Hr Wright
Figs. 11.37 and 11.38

allantoic vein	diencephalon	optic cup
allantois	dorsal aorta	optic stalk
amnion	dorsal mesentery	otic vesicle
amniotic cavity	dorsal pancreatic rudiment	pericardial cavity
aortic arch 1	ductus venosus	peritoneal cavity
aortic arch 2	duodenum	pharyngeal pouch 1
aortic arch 3	embryonic coelom	pharyngeal pouch 2
aortic arch 4	epidermis	pharyngeal pouch 3
aortic arch 6	epiphysis	pharyngeal pouch 4
aortic sac	esophagus	pharynx
apical ectodermal ridge	extraembryonic coelom	pigmented retina
atrium	head mesenchyme	pleural cavity
branchial arch 1	hindgut	plfactory placode
branchial arch 2	infundibulum	postanal gut
branchial arch 3	internal carotid artery	Rathke's pouch
branchial groove 1	lamina terminalis	roof for myelencephalon
C.N. III Occulomotor	laryngotracheal groove	second closing plate
C.N. IX Glossopharyngeal	lateral body fold	sinus venosus
C.N. V Trigeminal	lateral ventricle	somite
C.N. VII Facial	leg bud	spinal cord
C.N. VIII Acoustic	lens vesicle	spinal ganglion
C.N. X Vagus	liver	stomach
caudal cardinal vein	lung bud	stomodeum
caudal intestinal portal	mandibular process	tail bud
caudal liver diverticulum	maxillary process	telencephalon
cerebral hemisphere	mesencephalon	third ventricle
chorion	mesonephric duct	thyroid rudiment
cloaca	mesonephros	ventral mesentery
cloacal membrane	metencephalon	ventricle
common cardinal vein	midgut	vitelline artery
conotruncus	myelencephalon	vitelline vein
cranial liver diverticulum	neural retina	vitelline vessels
cranial cardinal vein	neuromere	wing bud
cranial intestinal portal	notochord	yolk sac
descending aorta	olfactory pit	

PIG DEVELOPMENT

10 mm

Small slide boxes each contain a pig embryo serially sectioned. There may be up to or more than 20 slides in a set. Please be careful not to break slides or to get the sets messed up with each other.

We have serial sections of the 10 mm pig embryo. The corresponding figures in the lab manual are figures 8.4 to 8.40.

For all the figures, learn the labeled structures and be able to follow them in space as you move forward and backward in the embryo.

(the glossary in back of the lab manual is helpful for terms we have not yet covered in class)

Using your lecture notes as a guide will give you some focus on your observations. Most (all) of the test questions will be material that we have talked about in lecture and are labeled or clearly seen in the lab manual.

As with the Chick, at the beginning of each lab, an introduction will be done to some part of the pig. The introductions will be done using the projecting microscope and video. **All questions** on the final lab test will come from structures seen in the serial, cross-sections of the 10 mm pig embryo.