Please note the different location for this week's practical class: Wallace Wurth Teaching Lab 116.

**PRACTICAL CLASS PROGRAM:**

- Weekly Quiz + revision (15 minutes)
- Practical class activities (90 minutes)
- Practical Class Revision (15 minutes)

**PRACTICAL CLASS ACTIVITIES (90 minutes):**

1. Fertile egg dissections, stage definition, and annotations of structures (first 60 minutes)
2. Observation of skeletal preparations of chicken and mouse foetuses (first 60 minutes)
3. Group presentation of annotated embryo images (final 30 minutes)

**LEARNING OBJECTIVES:**

- Understanding early neurulation, mesoderm and heart development, and being able to identify the defining structures in the chicken embryo.
- Understanding craniofacial and limb development and being able to identify the defining structures in chicken embryos.
- Understanding the development of the musculoskeletal system and being able to identify the defining structures in chicken embryos.
- Be able to apply basic practical laboratory skills and work with embryo and regeneration models.
- Be able to work effectively within a small team to complete academic tasks.
- Be able to present embryonic observations effectively and appropriately to an audience
- Be able to self-manage and work independently with an ability to take responsibility for their own learning, and an appreciation of the value of learning.

**PRACTICAL CLASS ACTIVITIES**
In this practical class we will work in small groups of 3 students.

**Risks Associated with Practical**
Eggs have the potential to be contaminated with Salmonella. Wear gloves throughout and students should wash their hands before leaving the lab.

Dissection implements are sharp, so students should take care not to cut themselves or other students.

Students should wear a lab coat, gloves and enclosed shoes to protect themselves from egg splatter.

**Animal Ethics Compliance**
The procedures used in this practicum are in compliance with the UNSW Animal Care and Ethics Committee and the National Health and Medical Research Council "Australian code of practice" (8th edition, 2013).

**Activity 1: Fertile egg dissections**
The chicken (Gallus gallus) embryo is an excellent model for the study of early vertebrate embryogenesis and later organogenesis. The embryo is encased within a hardened eggshell which provides a natural incubator or culture dish. Through a hole in the eggshell, the embryo can be visualized and easily manipulated with microsurgical tools or gene constructs, then allowed to continue development in ovo to determine the consequence of the experimental manipulation.

Fertilized chicken eggs are readily available anywhere in the world and the equipment needed is minimal – a humidified incubator (39oC, no CO2 required), a dissecting microscope, microsurgical tools that can be prepared in the lab or purchased, and either a hand-held mouth pipette or a manufactured micromanipulator and picospritzer.

Fertilized eggs can be held at between 13-16oC for up to 1 week prior to incubation. They are incubated at 38ºC-39ºC to the desired stage in a humidified incubator with the eggs placed on their side (horizontal). For long-term post-operative survival, it is best that the eggs be left in the incubator until experimental manipulation. However, eggs can be removed from the incubator and held at room temperature to slow development.

For staging of chicken embryos:
https://embryology.med.unsw.edu.au/embryology/index.php/Hamburger_Hamilton_Stages

## EXPERIMENTAL PROCEDURES

### Opening of the egg (11:15 am - 12:15 pm)

1. Eggs were incubated for several days at 38.5°C, and
2. Each student group will open 2-3 eggs.
3. Each student group should have an egg holder, a pair of blunt forceps and a pair of scissors, 3 petri dishes, PBS, disposable pipet, syringe and 23G needle, 10 ml tube with fixative, dissection microscope.
4. Put the egg into the holder with the blunt end up (pointed end down).
5. Use the pointy end of the scissors to tap a hole in the top of the egg into the air chamber, be very careful not to push the scissors too far into the egg.
6. Use the forceps to pick bits of shell out. Do not remove eggshell beyond the air chamber. You will see the air chamber and the vitelline membrane.
7. Carefully remove the vitelline membrane at the top.
8. If you do not see an embryo (a ring of blood vessels should be visible) then gently swirl the egg so that it floats up to the top of the yolk. If this does not work, you will need to take another egg if available.
9. Use the forceps to break off pieces of shell down to the yolk so that the embryo (visible as a ring of blood vessels) is exposed and the rim of the shell is just above the surface of the egg white or albumen.
10. You may be able to see the heart beating without magnification. If not, put the egg under the dissection microscope.
11. Cut off part of the blunt end of a transfer pipet to make a spoon, and prepare a petridish with PBS.
12. Very carefully lift the embryos out of the egg into the Petri dish containing PBS.
13. Study embryos under a dissection microscope, determine Hamburger & Hamilton (HH) developmental stages, and identify all the embryonic structures.
14. Make a photo of your embryos, annotate the embryonic structures that you have identified, and clearly label it with the developmental stage (HH).
15. Post your annotated embryo images on the Padlet Application (Groups will present their annotated embryo images from the Padlet App at the end of the practical class)
16. Transfer each of your embryos to a separate 10 ml tube containing fixative.
17. Annemiek will process the embryos for alcian blue/alizarin red skeletal staining (protocol in final page of the practical class notes) and bring the skeletal preparations to one of the next practical classes.

**Observation of other developmental stages (11:15 am - 12:15 pm)**
Move around the class to study the embryonic chicken stages of your colleagues. Identify the following structures:

- Neural groove and folds, neuropores
- Somites (how many can you count?)
- Brain vesicles (identify each of them)
- Developing heart and vasculature
- Optic vesicles
- Otic vesicles
- Branchial arches (how many can you count?)
- Nasal placodes
- Nasal pits and nasal processes (watch how these fuse over time)
- Beak
- Limb buds (note how the limb bud is ahead in development compared to the hind limb bud)
- Autopod, stylopod and zeugopod (note how the limb bud is segmented in these three regions over time)
- Hand plate and digits (note how the hand plate is shaped over time)
- Liver
- Genital tubercle

**Observation of skeletal preparations of mice and chicken (11:15 am - 12:15 pm)**
These are skeletal preparations of chicken embryos (9-10 dpf) and mouse foetuses, which have been cleared, and cartilage is stained blue and bone is stained red.

- Note that the embryonic skeletons consist mostly of cartilage, whereas the fetal skeletons have a lot more bone.
- Observe the bones of the mandible, the calvaria and the clavicles. Through what type of ossification are these bones formed?
- Observe the embryonic chicken skeleton and note the ossification centres in the developing long bones of the limbs and the axial skeleton. Through what type of ossification are these bones formed?
- Identify the cartilages and bones of the craniofacial region including the larynx. From which branchial arches are these cartilages and bones derived?
- Identify the bones in the developing zeugopod, stylopod and autopod. Through what type of ossification are these bones formed?
- Identify the phalanges. Through which process does the limb bud develop the separate digits?

**Group presentations (12:15 – 12:50 pm)**
Student groups will present their annotated embryo images that they uploaded on the Padlet Application to the class in 3-5 minute presentations discussing developmental stage and annotated embryo structures. We will follow the time line of development.

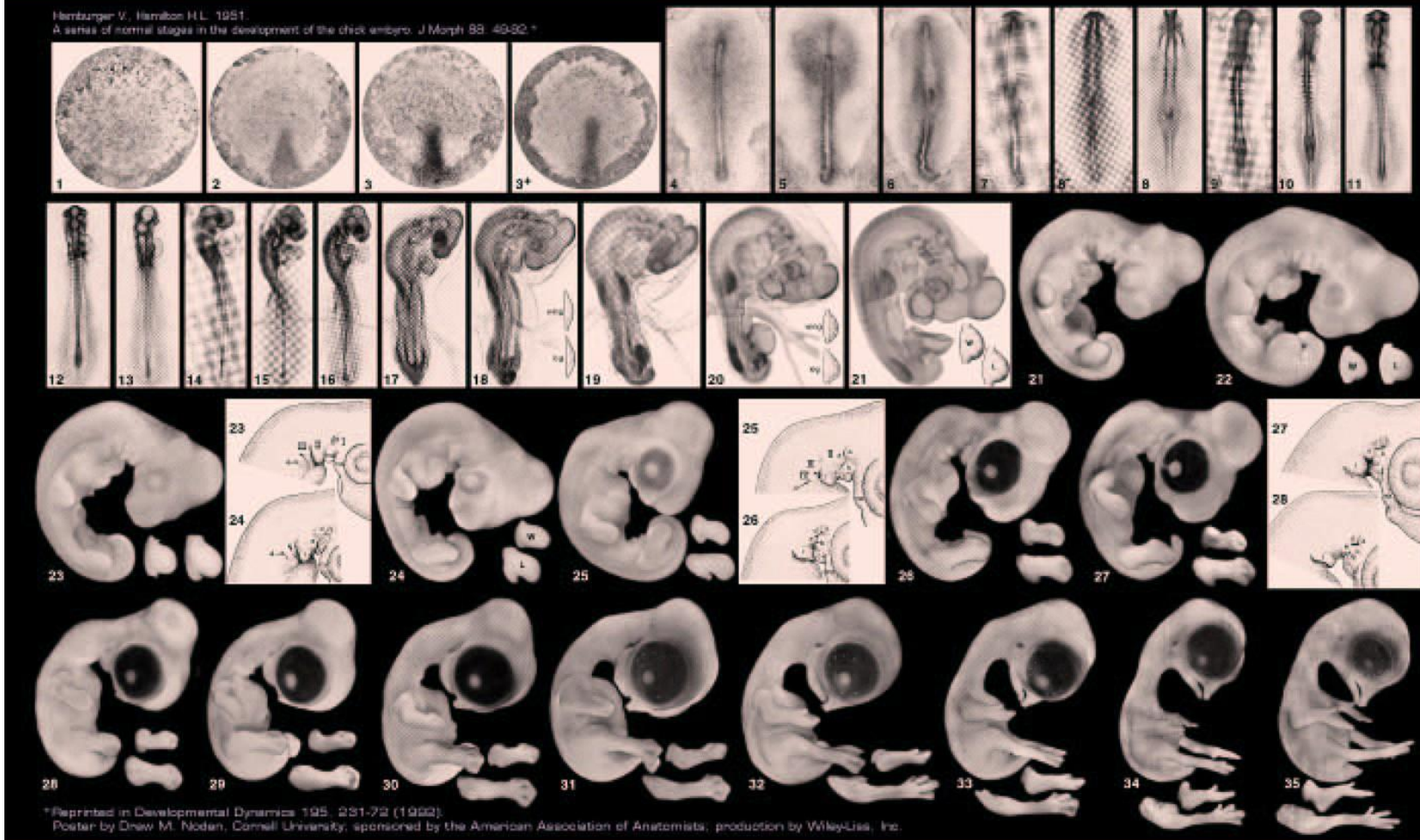
**Tidying up after the prac (from 12.50 pm)**
- Please discard egg waste and disposable consumables into the yellow bins (not the paper bins).
- Dispose of Syringes and needles in sharps bins
- Wash forceps and scissors with water, and wipe with ethanol, place in provided containers on the sink.
- Clean surfaces (microscope stages, benches) with water and ethanol, and wipe dry.

| HH Stage | Time and Features |
|---|---|
| 2 | 6-7 hr: Initial primitive streak, 0.3-0.5 mm long |
| 3 | 12-13 hr: Intermediate primitive streak |
| 4 | 18-19 hr: Definitive primitive streak, ±1.88 mm long |
| 5 | 19-22 hr: Head process (notochord) |
| 6 | 23-25 hr: Head fold |
| 7 | 23-26 hr: 1 somite; neural folds |
| 7 to 8- | ca. 23-26 hr: 1-3 somites; coelom |
| 8 | 26-29 hr: 4 somites; blood islands |
| 9 | 29-33 hr: 7 somites; primary optic vesicles |
| 9+ to 10- | ca. 33 hr: 8-9 somites; anterior amniotic fold |
| 10 | 33-38 hr: 10 somites; 3 primary brain vesicles |
| 11 | 40-45 hr: 13 somites; 5 neuromeres of hindbrain |
| 12 | 45-49 hr: 16 somites; telencephalon |
| 13 | 48-52 hr: 19 somites; atrioventricular canal |
| 13+ to 14- | ca. 50-52 hr: 20-21 somites; tail bud |
| 14 | 50-53 hr: 22 somites; trunk flexure; visceral arches I and II, clefts 1 and 2 |
| 14+ to 15- | ca. 50-54 hr: 23 somites; premandibular head cavities |
| 15 | 50-55 hr: 24-27 somites; visceral arch III, cleft 3 |
| 16 | 51-56 hr: 26-28 somites; wing bud; posterior amniotic fold |
| 17 | 52-64 hr: 29-32 somites; leg bud; epiphysis |
| 18 | 65-69 hr: 30-36 somites extending beyond level of leg bud; allantois |
| 19 | 68-72 hr: 37-40 somites extending into tail; maxillary process |
| 20 | 70-72 hr: 40-43 somites; rotation completed; eye pigment faint grey |
| 21 | 3.5 da: 43-44 somites; pharyngeal arch IV, pharyngeal cleft 4; eye pigment faint |
| 22 | 3.5 da: Somites extend to tip of tail; eye pigmentation distinct |
| 23 | 3.5-4 da: Dorsal contour from hindbrain to tail is a curved line |
| 24 | 4 da: Toe plate in leg-bud distinct; 4th pharyngeal cleft reduced to small pit |
| 25 | 4.5 da: Elbow and knee joints; 3rd & 4th pharyngeal clefts reduced to small pits |
| 26 | 4.5-5 da: 1st 3 toes; 3rd and 4th pharyngeal clefts no longer visible |
| 27 | 5 da: Beak just barely recognisable |
| 28 | 5.5 da: 3 digits, 4 toes; beak outgrowth distinct in profile |
| 29 | 6 da: Rudiment of 5th toe; beak more prominent; no egg tooth |
| 30 | 6.5 da: Feather germs; scleral papillae; egg tooth |
| 31 | 7 da: Web between 1st and 2nd digits; feather germs dorsally continuous from brachial to lumbosacral level |
| 32 | 7.5 da: Anterior tip of mandible has reached beak |
| 33 | 7.5-8.0 da: Web on radial margin of wing and 1st digit |
| 34 | 8 da: Nictitating membrane |
| 35 | 8 - 9 da: Phalanges in toes |

| | |
|---|---|
| 36 | 10 da: Length of 3rd toe from tip to middle of metatarsal joint = 5.4±0.3mm; length of beak from anterior angle of nostril to tip of bill = 2.5mm; primordium of comb; labial groove; uropygial gland |
| 37 | 11 da: Length of 3rd toe = 7.4±0.3mm; length of beak = 3.0 mm |
| 38 | 12 da: Length of 3rd toe = 8.4±0.3 mm; length of beak = 3.1 mm |
| 39 | 13 da: Length of 3rd toe = 9.8±0.3 mm; length of beak = 3.5 mm |
| 40 | 14 da: Length of beak = 4.0 mm; length of 3rd toe = 12.7±0.5 mm |
| 41 | 15 da: Length of beak from anterior angle of nostril to tip of upper bill = 4.5 mm; length of 3rd toe = 14.9±0.8 mm |
| 42 | 16 da: Length of beak = 4.8 mm; length of 3rd toe = 16.7±0.8 mm |
| 43 | 17 da: Length of beak = 5.0 mm; length of 3rd toe = 18.6±0.8 mm |
| 44 | 18 da: Length of beak = 5.7 mm; length of 3rd toe = 20.4±0.8 mm |
| 45 | 19-20 da: Yolk sac half enclosed in body cavity; chorio-allantoic membrane contains less blood and is "sticky" in living embryo |
| 46 | 20-21 da: Newly-hatched chick |

# In Memory of Viktor Hamburger: 1900-2001

## NORMAL STAGES OF CHICK EMBRYONIC DEVELOPMENT




The stage series of normal chick embryonic development is one of the most frequently cited and enduring biomedical articles ever published, and set the standards for amniote species-specific stage series that have followed. The coauthor of this paper, Viktor Hamburger, died in June, 2001, just a few weeks prior to his 101st birthday. In recognition of the tremendous importance of this stage series to countless research and teaching laboratories around the world, and in tribute to Prof. Hamburger's many contributions to developmental biology spanning seven decades, the editors and publisher of *Developmental Dynamics* commissioned the poster that accompanies this issue. Stages 1-35 (incubation days 0 through 9) were scanned from the original 1951 publication. Embryos have been resized and cropped for this poster format, and digitally enhanced to better highlight key features of each stage. Sketches of early limb bud and branchial (pharyngeal) arch development are also included; keys for these are in the original publication.

*Reprinted in Developmental Dynamics 195: 231-72 (1992).
Poster by Drew M. Noden, Cornell University; sponsored by the American Association of Anatomists; production by Wiley-Liss, Inc.

DEVELOPMENTAL DYNAMICS
WWW.INTERSCIENCE.WILEY.COM/DEVELOPMENTALDYNAMICS
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**Skeletal staining of Chicken Embryos**
(Modified from Kelly and Bryden, 1983)

Reagents:

| | | |
|---|---|---|
| - Glacial acetic acid: | 2.5L | @18.30 (upper campus Store) |
| - $H_2O$ | | |
| - Alcian Blue: | 10G | @ $252 (Merck) |
| - Alizarin Red: | 25G | @ $127 (Merck) |
| - Ethanol: | 2.5L | @ $16.48 (upper campus store) |
| - KOH: | 250G | @ $37.50 (Sigma) |
| - Glycerin/Glycerol: | 1L | @ $150 (Sigma) |

Solutions:

- Ethanol + 1% Glacial acetic acid
- Alcian Blue Solution: 20 mg alcian blue + 80 ml 95% ethanol + 20 ml acetic acid (make fresh, stir for 1+ hours)
- Alizarin Red Solution: 0.002% (w/v) in 0.5% KOH (make fresh)
- 95% ethanol
- 75% ethanol/$H_2O$
- 40% ethanol/$H_2O$
- 15% ethanol/$H_2O$
- 2.0% KOH
- 0.5% KOH

Protocol:

- Fix embryos in Ethanol + 1% Glacial acetic acid for 24 hours at room temperature
- Rinse fixed embryos in 95% ethanol at room temperature
- Stain embryos overnight in freshly prepared Alcian Blue Solution at room temperature (16 hours)
- Rinse fixed embryos in 95% ethanol at room temperature for 6 hours or overnight
- Rehydrate embryos at room temperature >=2 hours per step (95, 75, 40, 15)
- Stain in Alizarin Red Solution for 24 hours at room temperature
- Rinse embryos in 0.5% KOH at room temperature
- Incubate embryos in 2.0% KOH for 4 hours at room temperature
- Transfer to 3:1 0.5% KOH/glycerine solution for 8-24 hours at room temperature
- Transfer to 1:1 0.5% KOH/glycerine solution for 8-24 hours at room temperature
- Transfer to 1:3 0.5% KOH/glycerine solution for 8-24 hours at room temperature
- Store in 100% glycerine solution