## *Appendix II*

# Normal Table of Hamburger and Hamilton (1951; 1992)

(Magnifications are those supplied by Hamburger and Hamilton)

A fuller description of each stage is given in the original publications (1951, reprinted 1992), the following being abbreviated and with minor modifications. Reproduced by permission of John Wiley and Sons, Inc.

Stages 1 and 2 probably correspond to stages XII–XIV of Eyal-Giladi and Kochav (Appendix I). Approximate incubation times are indicated for each stage in parentheses. Notes in italics have been added by the present authors; those in square brackets, derived from the table of Freeman and Vince (1974), describe internal features and their approximate times of appearance.

References to 'HH Plates' refer to plates in this normal table.

**Stage 1.** ×20 Pre-streak.
An 'embryonic shield' may be visible towards the posterior half of the blastoderm.

**Stage 2.** ×20 Initial streak (about 6–7 h).
The primitive streak is short and conical (0.3–0.5 mm in length).

**Stage 3.** ×20 Intermediate streak (12–13 h).
The primitive streak extends to about the centre of the area pellucida.

**Stage 3+.** This stage was inserted by Hamburger and Hamilton but no description was given.

**Stage 4–.** This critical stage (illustrated in *Plate 9* of this book) was not included in the Hamburger and Hamilton table. The primitive streak has reached its maximum length and Hensen's node is visible. The distinction between stage 4– and 4 is essential because of changes in cell migration, cell adhesion and gene expression.

**Stage 4.** ×20 Definitive streak (18–19 h).
The primitive streak has reached its maximum length (about 1.88 mm). The primitive groove, primitive pit and Hensen's node are present. The area pellucida has become pear-shaped and the primitive streak extends over about two-thirds to third-quarters of its length. *(NB: The beginning of the head process is already visible in the photograph as a dark region at the anterior tip of the primitive streak.)*

**Stage 5.** ×20 Head process (19–22 h).
The head process extends anteriorly from Hensen's node.

**Stage 6.** ×20 Head fold (23–25 h).
No somites yet visible.

Stages 7–14 are based primarily on the numbers of pairs of somites visible.

**Stage 7.** ×20 One pair of somites (23–26 h).
This is actually the second somite pair, the first not yet clearly defined. Neural folds are visible in the head region.

**Stage 8.** ×20 Four pairs of somites (26–29 h).

The neural folds meet at the level of the midbrain. Blood islands are present in the posterior half of the blastoderm.
*[Nephric ducts are starting to develop.]*

**Stage 9.** ×20 Seven pairs of somites (29–33 h).
Primary optic vesicles are present. Paired primordia of the heart begin to fuse.
*[Inner ear is beginning to form.]*

**Stage 10.** ×12 Ten pairs of somites (33–38 h).
First indication of cranial flexure. Three primary brain vesicles are clearly visible. Heart primordia are fused, bent slightly to right.
*[Haemoglobin synthesis begins; thyroid anlage differentiates; pronephros forming.]*

**Stage 11.** ×12 Thirteen pairs of somites (40–45 h).
Slight cranial flexure. Five neuromeres of the hindbrain are distinct. Anterior neuropore is closing. Optic vesicles are constricted at the bases. Heart is bent to the right.
*[Rathke's pouch is forming; pronephros degenerates; mesonephros differentiates; amniotic head fold appears; neurohypophysis begins to form.]*

**Stage 12.** ×12 Sixteen pairs of somites (45–49 h).
Head is turning onto the left side. Anterior neuropore is closed. Telencephalon is indicated. Primary optic vesicles and optic stalk are well established. Otic pit is deep but wide open. Heart is slightly S-shaped. Head fold of amnion covers entire region of the forebrain.
*[Circulatory system is developing; ductus venosus and liver primordia appear; telencephalon is differentiating; amniotic tail fold.]*

**Stage 13.** ×12 Nineteen pairs of somites (48–52 h).
Head is partly to fully turned onto the left side. Cranial and cervical flexures make broad curves. Enlargement of telencephalon. Atrio-ventricular canal is indicated by constriction. Head fold of the amnion covers forebrain, midbrain and the anterior part of hindbrain.
*[Proventriculus and gizzard differentiate; interatrial septum forms; allantoic bud and hindgut develop.]*

**Stage 14.** ×12 Twenty-two pairs of somites (50–53 h).
Cranial flexure: axes of forebrain and hindbrain form almost a right angle. Cervical flexure is a broad curve.
Rotation of body as far back as somites 7–9.
Visceral *(pharyngeal)* arches 1 and 2, and clefts *(grooves)* 1 and 2 are distinct. Optic vesicle begins to invaginate and lens placode is formed. Opening of otic pit is constricted. Rathke's pouch is recognizable. Ventricular loop of the heart is now ventral to the atrio-ventricular canal. Amnion extends to somites 7–10.
*[Pancreatic anlage differentiate.]*
Beyond stage 14 the number of somites becomes increasingly difficult to determine with accuracy.

**Stage 15.** ×12 (c. 50–55 h).
Lateral body folds extend to level of somites 15–17.
Limb primordia: inconspicuous condensations of mesoderm for the wing bud. 24–27 pairs of somites.
Amnion extends to level of somites 7–14.
Flexures and rotation: cranial flexure – axes of forebrain and hindbrain form an acute angle. Cervical flexure is a broad curve. Rotation extends to level of somites 11–13.
Optic cup is completely formed.
Visceral (pharyngeal) arches: arch 3 and cleft 1 are distinct.
*[Lung primordia differentiate from pharynx.]*

**Stage 16.** ×12 (c. 51–56 h).
Lateral body folds extend to level of somites 17–20, between levels of wings and legs.
Limbs: wings are lifted off the blastoderm. Primordia of the legs are flat.
Somites: 26–28 pairs.
Amnion: extends to level of somites 10–18.
Flexures and rotation: all flexures are more accentuated.
Rotation extends to somites 14–15.
Tail bud: a short, straight cone.
*[Epiphysis develops.]*

**Stage 17.** ×12 (c. 52–64 h).
Lateral body folds extend around entire circumference of the body.
Limb buds: both wing and leg buds are lifted off the blastoderm.
Somites: 29–32 pairs.
Amnion: variable in extent.
Rotation: extends to level of somites 17–18.
Tail bud: bent ventrally. Its mesoderm is unsegmented.
Pineal: a distinct knob.
Allantois: not yet formed.

**Stage 18.** ×12 (c. 65–69 h).
Limb buds enlarged (see *HH Plates 4* and *5*).
Somites: 30–36 pairs.
Amnion: usually closed.
Rotation: now extends to posterior part of the body.
Visceral *(pharyngeal)* arches: maxillary process are absent or inconspicuous. Fourth cleft (groove) is indistinct or absent.
Tail bud: turned to right.

Allantois: short, thick-walled pocket.
*[First passive movements can be detected.]*

**Stage 19.** ×12 (c. 68–72 h).
Limb buds: the leg buds are slightly larger than the wing buds (see *HH Plate 5*).
Somites: 37–40 pairs, extend into the tail.
The contour of the posterior part of the trunk is straight to the tail.
Tail bud is curved, the tip pointing forward *(anteriorly).*
Visceral *(pharyngeal)* arches: maxillary process about the same length as mandibular process. The second *(hyoid)* projects slightly over the surface.
Allantois: small pocket of variable size.
Eyes: unpigmented.
*[Aortic arches form, the left soon to degenerate; nephric ducts unite with cloaca.]*

**Stage 20.** ×12 (c. 70–72 h).
Limb buds: the wing buds are approximately symmetrical, the leg buds slightly asymmetrical (see *HH Plate 5*).
Somites: 40–43 pairs.
Rotation: completed.
Visceral *(pharyngeal)* arches: maxillary equals or exceeds the mandibular in length. Second *(hyoid)* arch projects over surface. Fourth arch is smaller than the third arch.
Allantois: vesicular, variable size.
Eye pigment: faint grey.
*[Pulmonary arch forms; ductus arteriosus; embryo is surrounded by amnion.]*

**Stage 21.** × 12 (*c.* 3.5 days).
Limbs: both wing and leg buds are slightly asymmetrical with proximo-distal axes directed caudally.
Somites: 43–44 pairs.
Visceral *(pharyngeal)* arches: maxillary process is longer than the mandibular. Second *(hyoid)* overlaps the third arch ventrally. Fourth arch is distinct.
Allantois: variable in size, may extend to head.
Eye pigmentation: faint.
*[First active movements of the head and neck; earliest motor fibres contact the anterior trunk muscles.]*

**Stage 22.** ×8 (*c.* 3.5 days).
Eye pigmentation: distinct.
Somites: extend to tip of the tail. *(This is incorrect since the mesoderm at the tip of the tail never becomes fully segmented.)*
*[Adrenal cortical and medullary cells differentiate; insulin synthesis begins; gonadal anlagen differentiate; amniotic contractions begin; crop differentiates; erythropoiesis in yolk sac begins; oestrogen and oestrodiol-17β synthesis begin.]*

**Stage 23.** ×8 (*c.* 4 days).
Limbs: both wing buds and leg buds are approximately as long as they are wide.
Visceral *(pharyngeal)* arches: see *HH Plates 7* and *8*.
*[Pronephros disappears]*

**Stage 24.** ×8 (*c.* 4.5 days).
Limbs: both wing and leg buds are longer than they are wide.
Toe plate in the leg bud is distinct.
Visceral *(pharyngeal)* arches: see *HH Plates 7* and *8*.
*[Chorion and allantois fuse to give chorio-allantois; metanephros begins to differentiate; cochlear nucleus becomes visible.]*

**Stage 25.** ×8 (*c.* 4.5–5 days).
Limbs: elbow and knee joints are distinct. Digital plate in the wing is distinct, but no demarcation of the digits.
Visceral *(pharyngeal)* arches: see *HH Plates 7* and *8*.
*[Spleen differentiates.]*

**Stage 26.** ×8 (*c.* 5 days).
Limbs: longer. Contour of the digital plate is rounded. Demarcation of the first three toes is distinct.
Visceral *(pharyngeal)* arches: see *HH Plates 8* and *9*.
*[Mesonephros becomes functional; production of definitive erythrocytes begins; adult haemoglobin is synthesized; first active movements of trunk; corticosteroid synthesis begins; duodenum begins to differentiate; first mouth movements; bursa of Fabricius begins to differentiate; four-chambered heart is formed; nerves from retina reach the optic lobe; thymus anlagen differentiate.]*

**Stage 27.** ×5 (*c.* 5–5.5 days).
Limbs: grooves between the first, second and third digits of the wing buds are indicated; distinct grooves between the toes.
Visceral *(pharyngeal)* arches: see *HH Plates 8* and *9*.
*[Beak is forming; amnion begins to contract rhythmically.]*

**Stage 28.** ×5 (*c.* 5.5–6 days).
Limbs: second digit of wing bud and third toe are longer than the others; three digits and four toes are distinct.

Visceral *(pharyngeal)* arches: see *HH Plates 8* and *9*.
Beak: a distinct outgrowth is visible in profile.
*[First reflexes are established.]*

**Stage 29.** ×5 (*c.* 6–6.5 days).
Limbs: wing is bent at the elbow. Shallow grooves between first, second and third digits. Second to fourth toes stand out as ridges separated by grooves and with indications of webs. Rudiments of fifth toes are visible.
Visceral *(pharyngeal)* arches: mandibular process and second arch are broadly fused. Auditory meatus is distinct at the dorsal end of fusion.
Neck has lengthened: see *HH plate 10*.
*[Parathyroids differentiate; air sacs begin to differentiate; first eyelid and independent limb movements; ductus venosus is lost.]*

**Stage 30.** ×5 (*c.* 6.5–7 days).
Limbs: the three major segments of limbs are well demarcated. Wing is bent at the elbow and leg is bent at the knee. Distinct grooves between first and second digits.
Neck: lengthened further.
Feather germs: two dorsal rows on either side of the spinal cord at brachial level, three rows at level of the legs.
Scleral papillae: one on either side of the choroid fissure.
Egg tooth: distinct.
*[Thyroid concentrates iodine; sexual differentiation begins; testosterone production begins in the male.]*

**Stage 31.** ×4 (*c.* 7–7.5 days).
Limbs: rudiment of the fifth toe is still distinct.
Feather germs: on the dorsal surface, continuous from brachial to lumbo-sacral levels. Approximately seven rows at the lumbo-sacral level. Distinct feather papillae on the thigh.
Scleral papillae: usually six, four on the dorsal side, two on the ventral side.
*[Left allantoic blood vessel is lost; first eyeball movements begin; thyroid is able to synthesize monoiodotyrosine; ACTH secretion begins.]*

**Stage 32.** ×4 (*c.* 7.5 days).
Limbs: all digits and four toes have lengthened conspicuously. Webs between digits and toes are thin and their contours concave. Rudiment of the fifth toe has disappeared. Differences in size of individual digits and toes are conspicuous.
Feather germs: eleven rows or more on the dorsal surface at level of the legs. One row on the tail distinct.
Scleral papillae: 6–8 in groups.

**Stage 33.** x4 (*c.* 7.5–8 days).
Limbs: web on radial margin of the arm and first digit.
Feather germs: three distinct rows in the tail, the middle one considerably larger than the others.
Scleral papillae: 13 present, forming almost a complete circle.
*[Mineralization of bone begins; development of the right Mullerian duct ceases in the female and of both left and right in the male]*

**Stage 34.** ×4 (*c.* 8 days).
Limbs: differential growth of the second digit. Third toe is conspicuous.
Feather germs: visible with good illumination over the scapula, ventral side of neck, procoracoid and posterior edge of wing. One row on the inner *(medial)* side of each eye. None around the umbilical cord.
Scleral papillae: 13 or 14.
Nictitating membrane: extends halfway between the outer rim of the eye and scleral papilla.

**Stage 35.** ×4 (*c.* 8–9 days).
Limbs: webs between the digits and toes are inconspicuous.
Beak: lengthened.
Feather germs: mid-dorsal line stands out distinctly in profile. At least four rows on the inner side of each eye. New feather germs near the mid-ventral line and extending to both sides of the umbilical cord.
Nictitating membrane: approaches the outer scleral papillae.
Eyelids (external to nictitating membrane) have extended towards the beak and begun to overgrow the eyeball.
*[Thyroid is able to synthesize diiodotyrosine; male Mullerian ducts regress; chorioallantois becomes fixed in relation to the shell; haemopoietic activity in the bone marrow; fibres of acoustic ganglion enter the cochlear nucleus.]*

**Stage 36.** ×l.2 (*c.* 10 days).
Limbs: tapering primordia of claws just visible on termini of the toes.
Comb: primordium visible as a prominent ridge with slightly serrated edge along the dorsal midline of the beak. Nostril has narrowed to a slit.
Feather germs: flight feathers conspicuous. Feather germs now cover the tibio-fibular portion of the leg. At least nine rows of feather germs between the upper eyelid and dorsal midline. Sternal tracts prominent.
Eyelids: lower lid has grown upward to level of the cornea.

*[Whole body movements become jerky and random; thyroid secretes thyroxine; TSH secretion begins; parathormone secretion begins; embryo's position is fixed at right angles to the long axis of the egg; first local proprioceptive muscle reflexes.]*

**Stage 37.** ×l.2 (*c.* 11 days).
Limbs: claws of toes are flattened laterally and curved ventrally. Pads on the plantar surface of the foot. Length of third toe = 7.4 ± 0.3 mm.
Comb: more prominent and clearly serrated.
Feather germs: elongated into long, tapering cones along the back and tail.
Eyelids: lower lid covers one-third to one half of the cornea. Upper lid has reached the dorsal edge of the cornea.
*[Metanephros begins to function; first auditory evoked responses from cochlear nuclei.]*

**Stage 38.** ×l.2 (*c.* 12 days).
Limbs: primordia of scales visible as ridges over entire surface of the leg. Length of third toe = 8.4 ± 0.3 mm.
Feather germs: coverts of web of the wing are becoming conical. Sternum is covered with feather germs except along the midline. Upper eyelid is covered with newly formed feather germs.
Lower eyelid covers two-thirds to three-quarters of the cornea.
*[Absorption of albumen begins; mesonephros degenerates; Mullerian ducts are lost in the male; calcium absorption from the shell begins; inbibition of amniotic fluid begins.]*

**Stage 39.** ×l.2 (*c.* 13 days).
Limbs: scales overlapping on the superior surface of the leg. Major pads of phalanges are covered with papillae. Length of third toe = 9.8 ± 0.3 mm.
Mandible and maxillae are cornified (opaque) as far back as the proximal level of the egg tooth.
Feather germs: coverts of web of the wing are long, tapering cones.
Eyelids: opening between the lids is reduced to a thin crescent.
*[Increased transport of lipids by yolk sac; amniotic contractions cease; sero-amniotic connection ruptures; pituitary–gonad axis is definitely established; neurohypophysis becomes active; gland cells of proventriculus begin secretion.]*
Stages 40–44 are based mainly on the length of the beak and on the length of the third (longest) toe, since other external features have lost their diagnostic value.

**Stage 40.** ×l (*c.* 14 days).
Length of the beak from anterior edge of nostril to tip of the bill = 4.0 mm.
Length of third toe = 12.7 ± 0.5 mm.
*[Exocrine pancreas begins maturation; oestriol synthesis begins in the female; maximum sensitivity to sound at 400 Hz; sporadic electrical activity in the cerebrum.]*

**Stage 41.** ×l (*c.* 15 days).
Length of the beak from anterior angle of nostril to tip of the upper bill = 4.5 mm.
Length of third toe = 14.9 ± 0.8 mm.
*[Stomach begins to contract; electrical activity in the optic lobes.]*

**Stage 42.** ×l (*c.* 16 days).
Length of the beak from anterior angle of nostril to tip of the upper bill = 4.8 mm.
Length of third toe = 16.7 ± 0.8 mm.
*[Embryo capable of respiratory movements; first electrical activity in the cerebellum.]*

**Stage 43.** ×l (*c.* 17 days).
Length of the beak from anterior angle of nostril to tip of the upper bill = 5.0 mm.
Length of third toe = 18.6 ± 0.8 mm.
*[Coordinated and stereotyped movements begin.]*

**Stage 44.** ×l (*c.* 18 days).
Length of the beak from anterior angle of nostril to tip of the upper bill = 5.7 mm.
Length of third toe = 20.4 ± 0.8 mm.
*[Beak becomes tucked under right wing; maximum sensitivity to sound rises to 800 Hz. Duodenum begins maturation; calcitonin secretion begins; first behavioural responses to light.]*

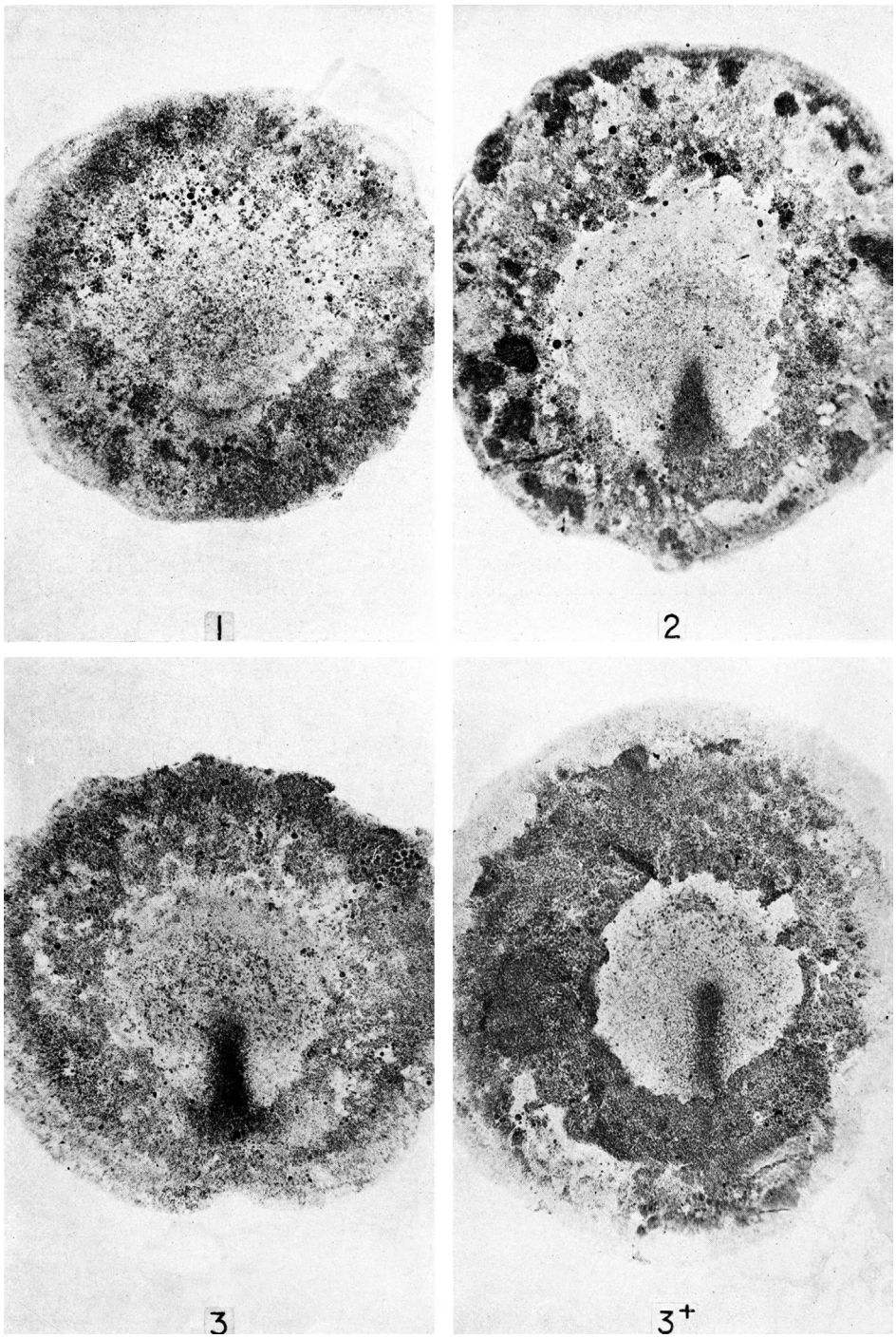
**Stage 45.** ×l (*c.* 19–20 days).
Beak length is no longer diagnostic because of reduction in length due to sloughing off of the periderm, but it is now shiny.
Length of third toe unchanged (except for certain specialized breeds with a slightly longer incubation period).
Extra-embryonic membranes: yolk sac is half enclosed in the body.
Chorioallantoic membrane is 'sticky' in the living embryo.
*[Absorption of the allantoic fluid is completed; hatching muscle matures; hepatic glycogen stores are mobilized; postural reflexes are fully developed; yolk sac withdrawal begins; cerebral auditory*

*waves attain regularity of those of the hatched chick.]*

Day 20: *[Begins to breathe and vocalize; inner shell membrane is pierced; shell is 'pipped'; ductus arteriosus is closed; interatrial foramina are closed; chorioallantoic ectoderm degenerates; chorioallantoic circulation is reduced; withdrawal of the yolk sac is completed.]*

**Stage 46.** ×l (*c.* 20–21 days). Newly hatched chick.

## Appendix II Plates 1



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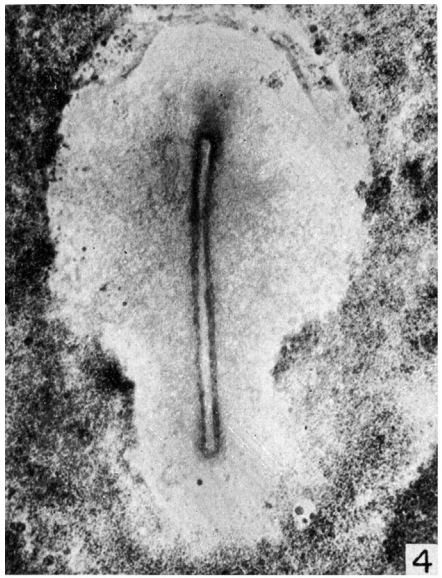


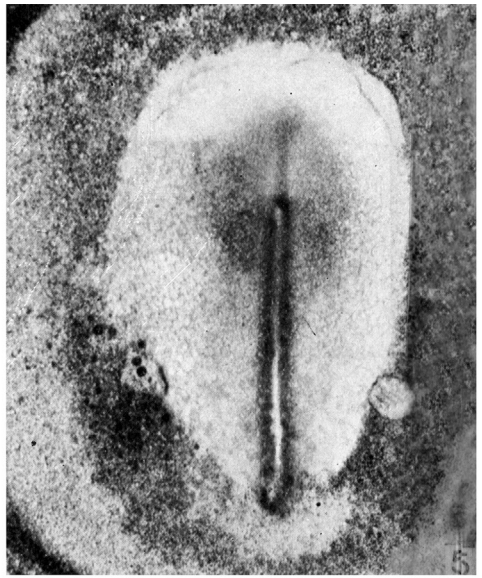


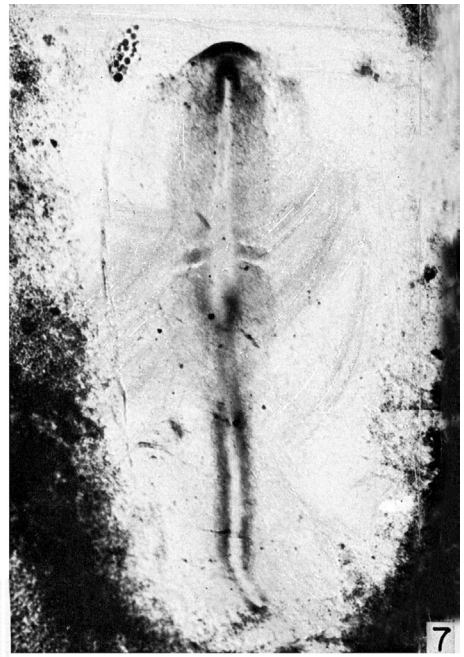


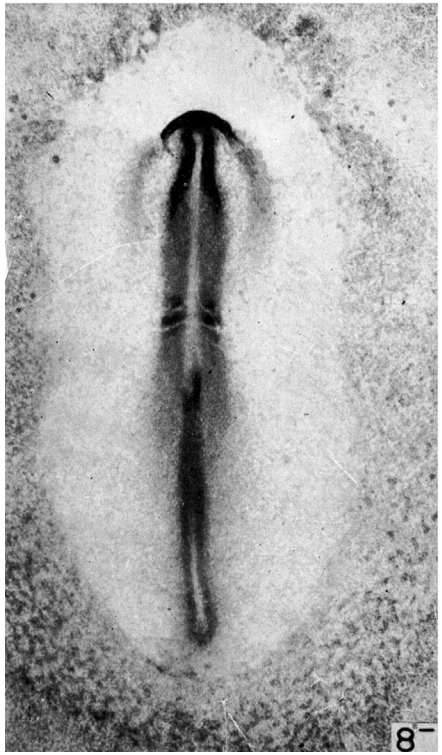


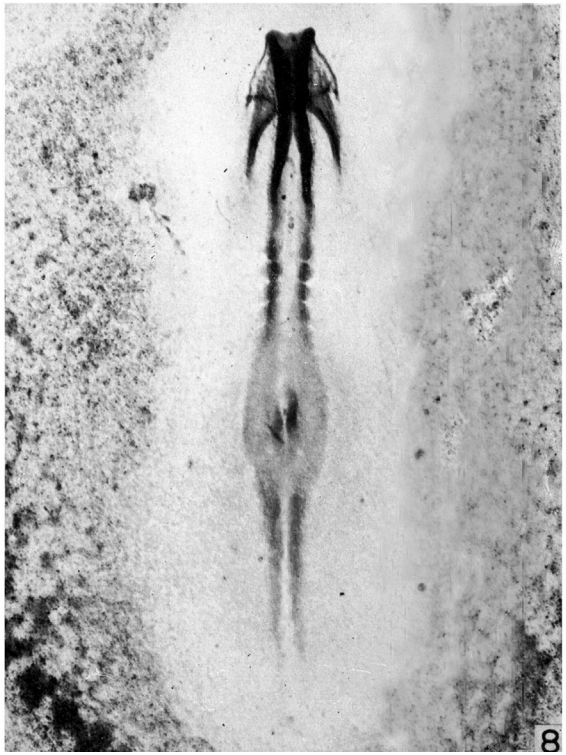


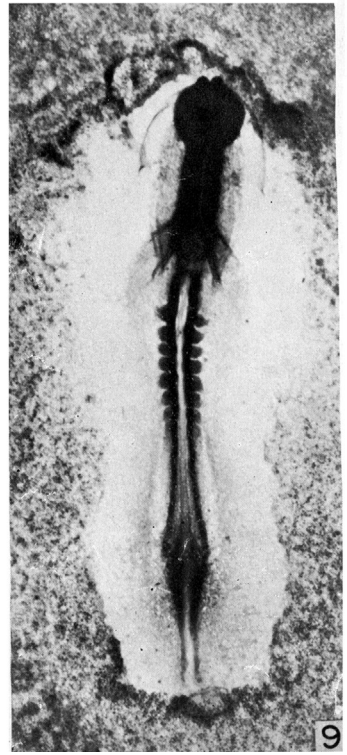


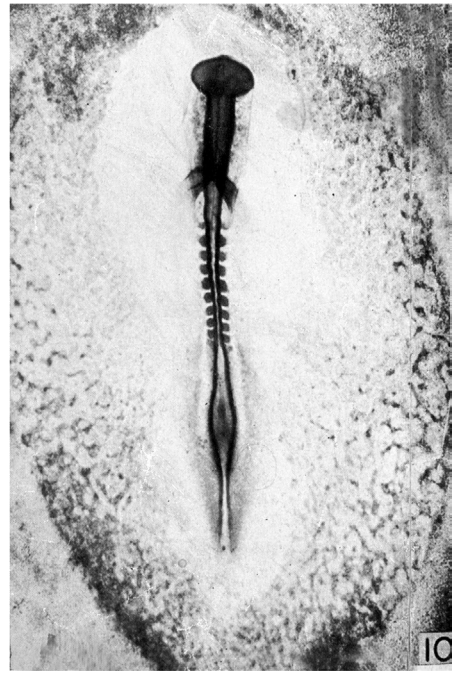

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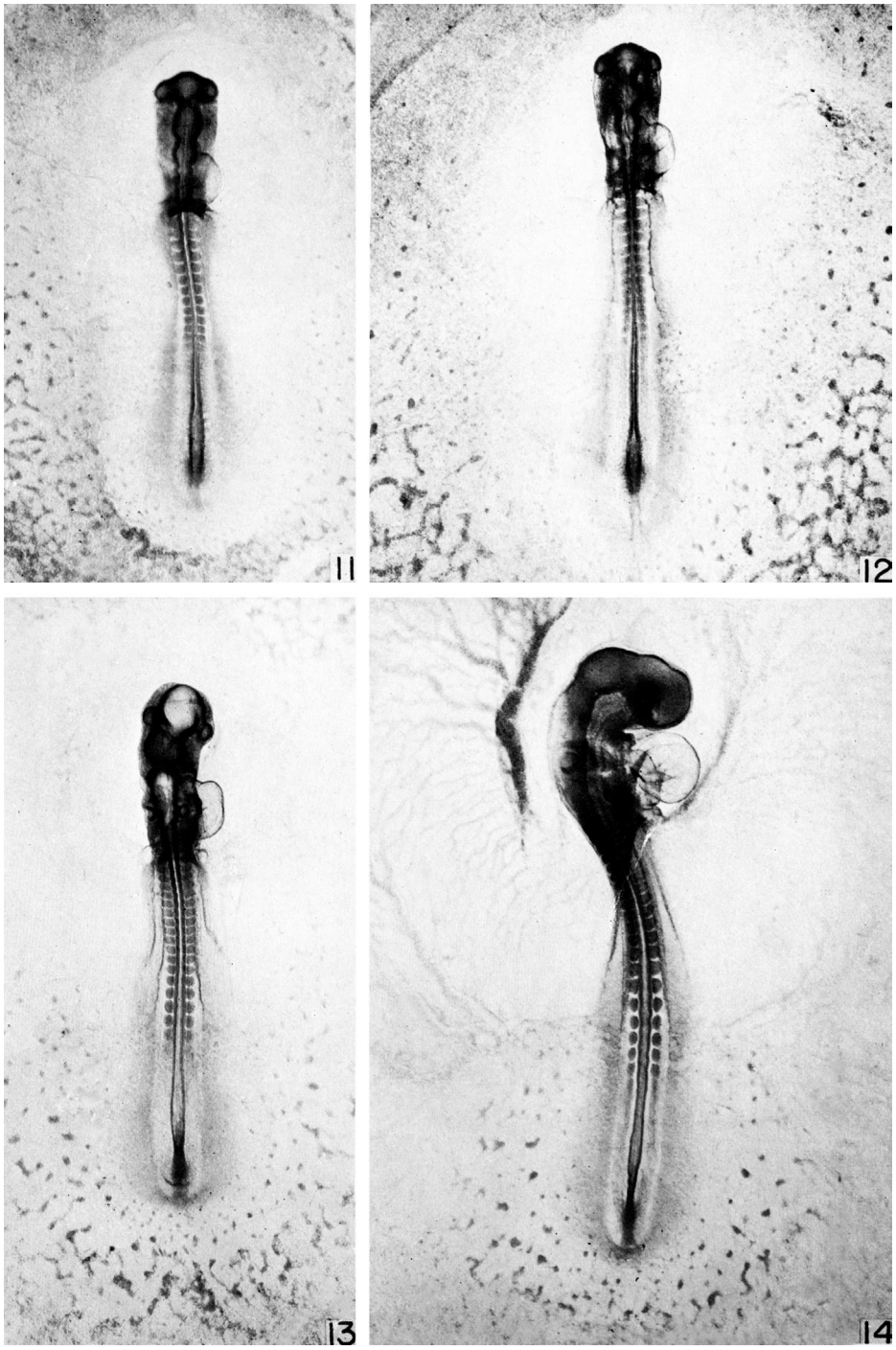

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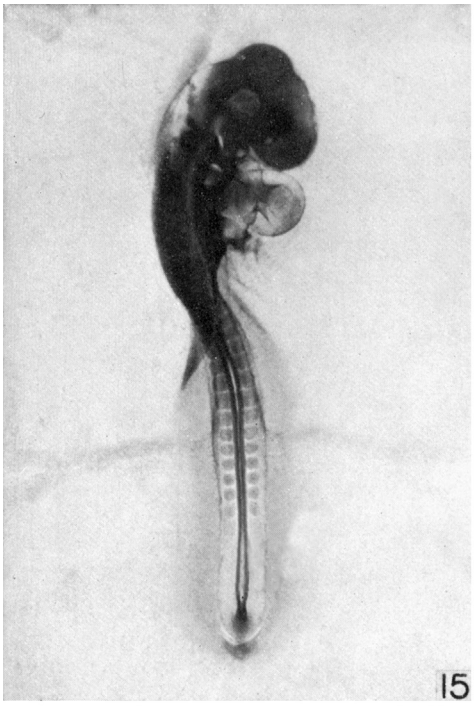


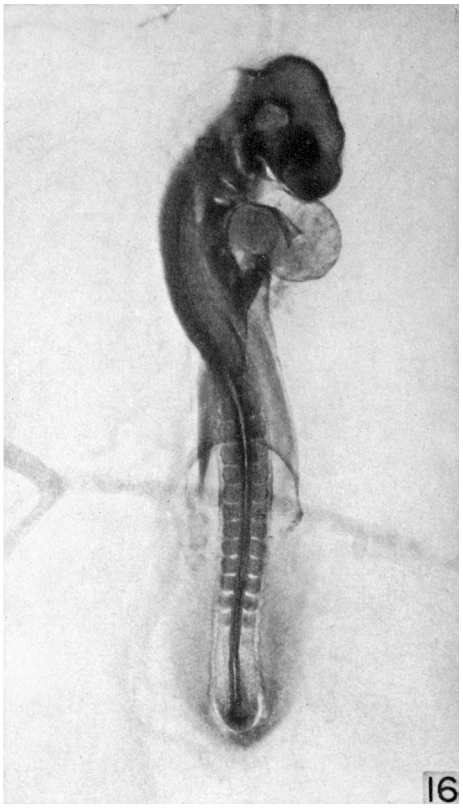


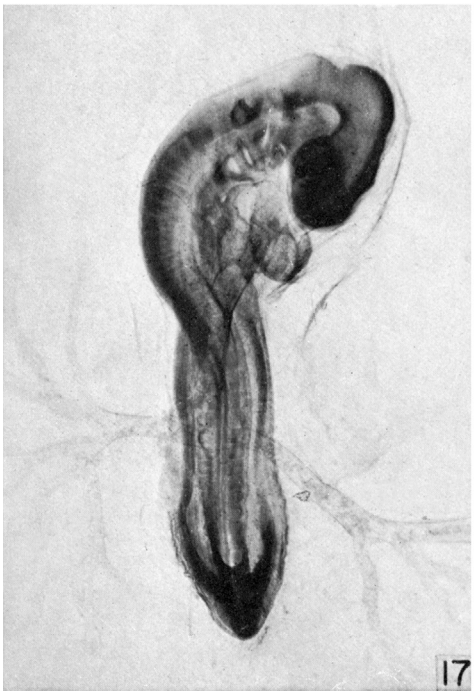


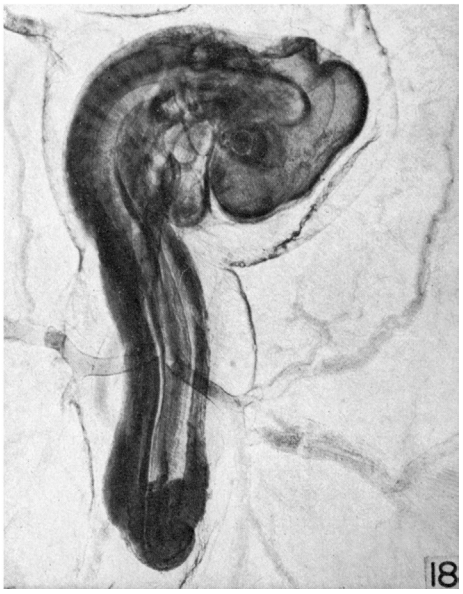

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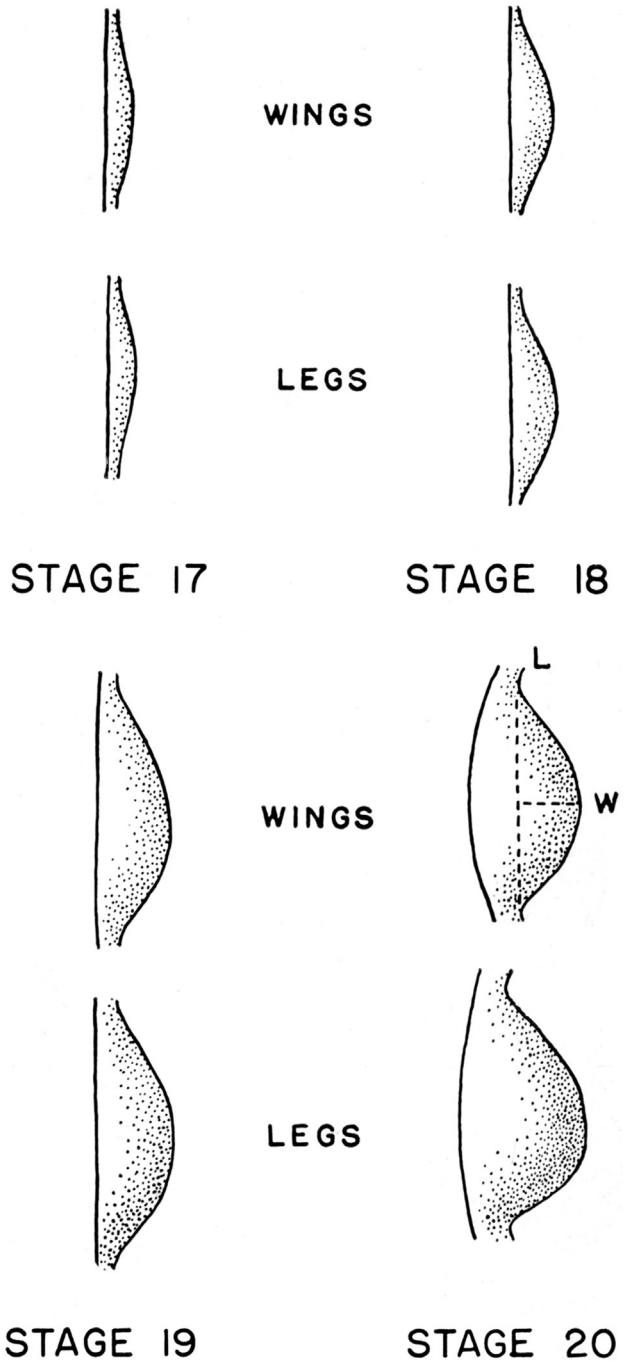

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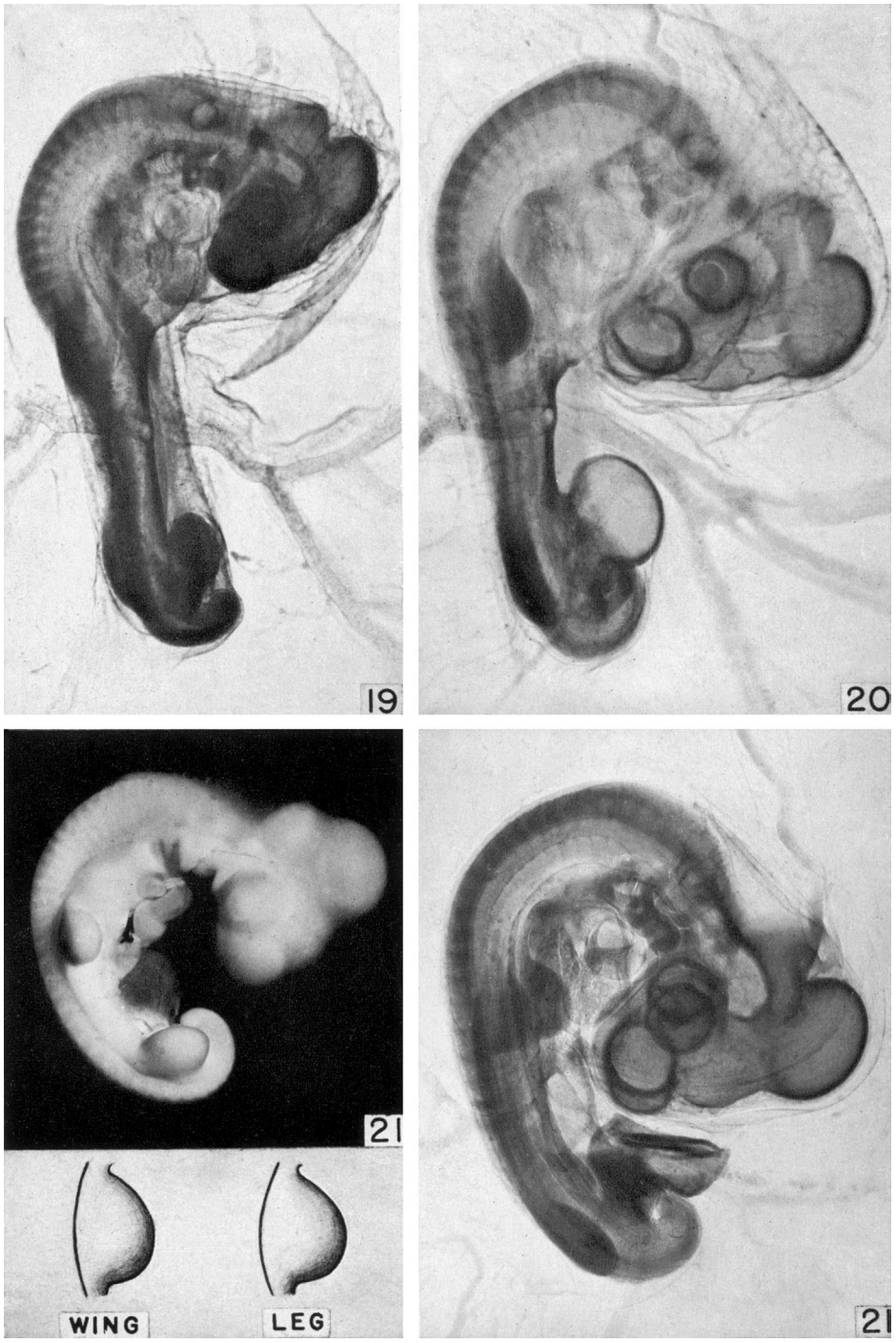

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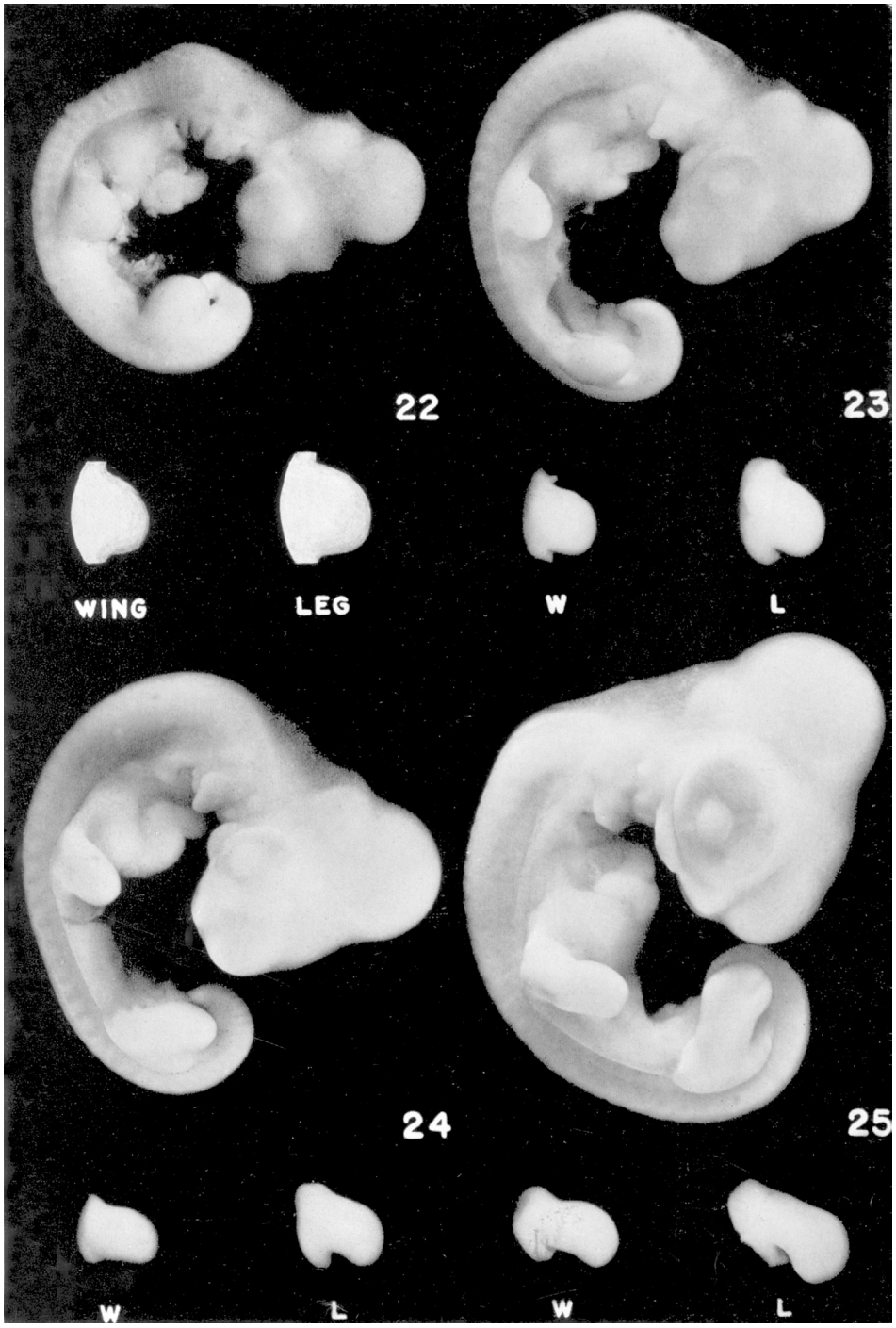

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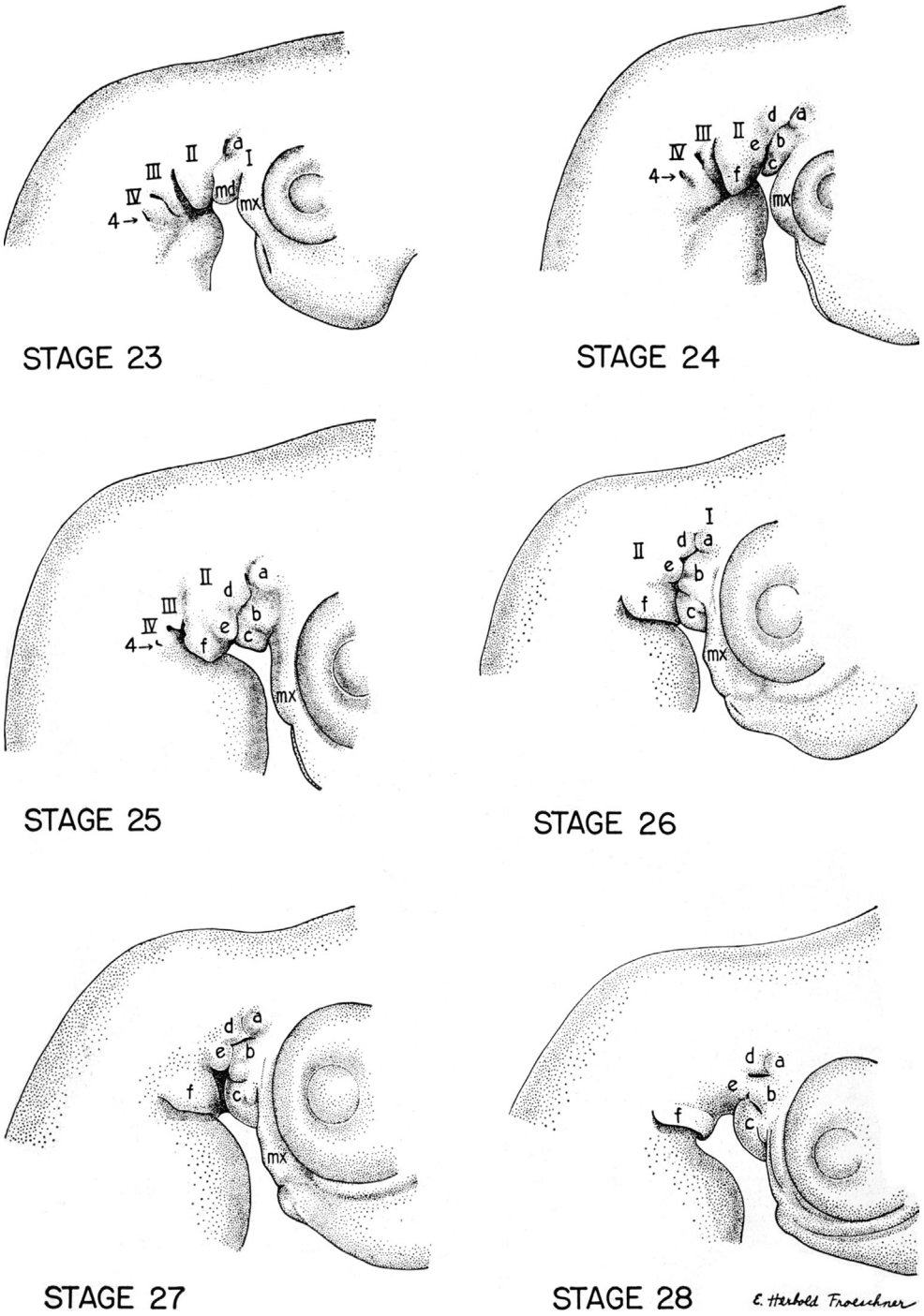

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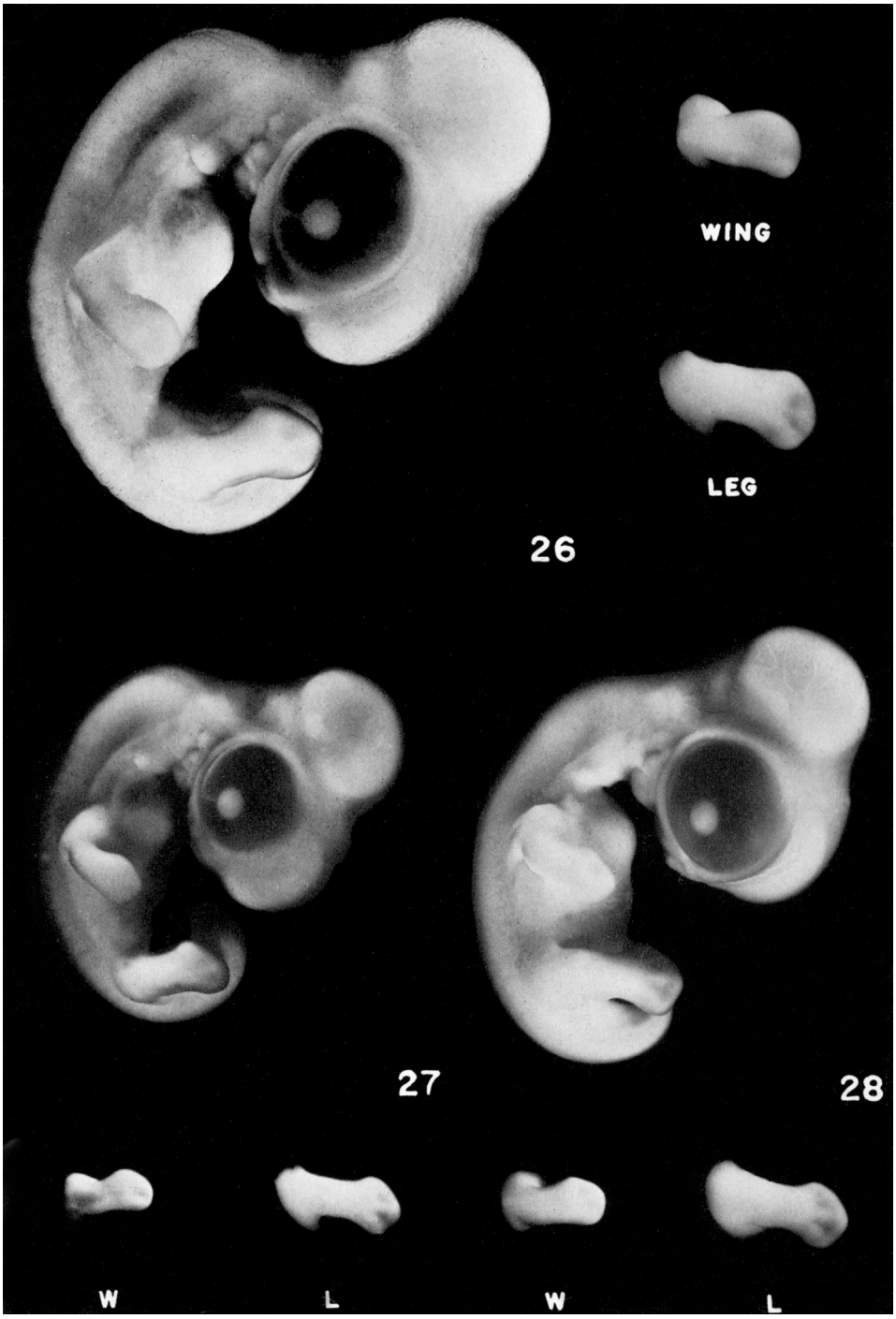

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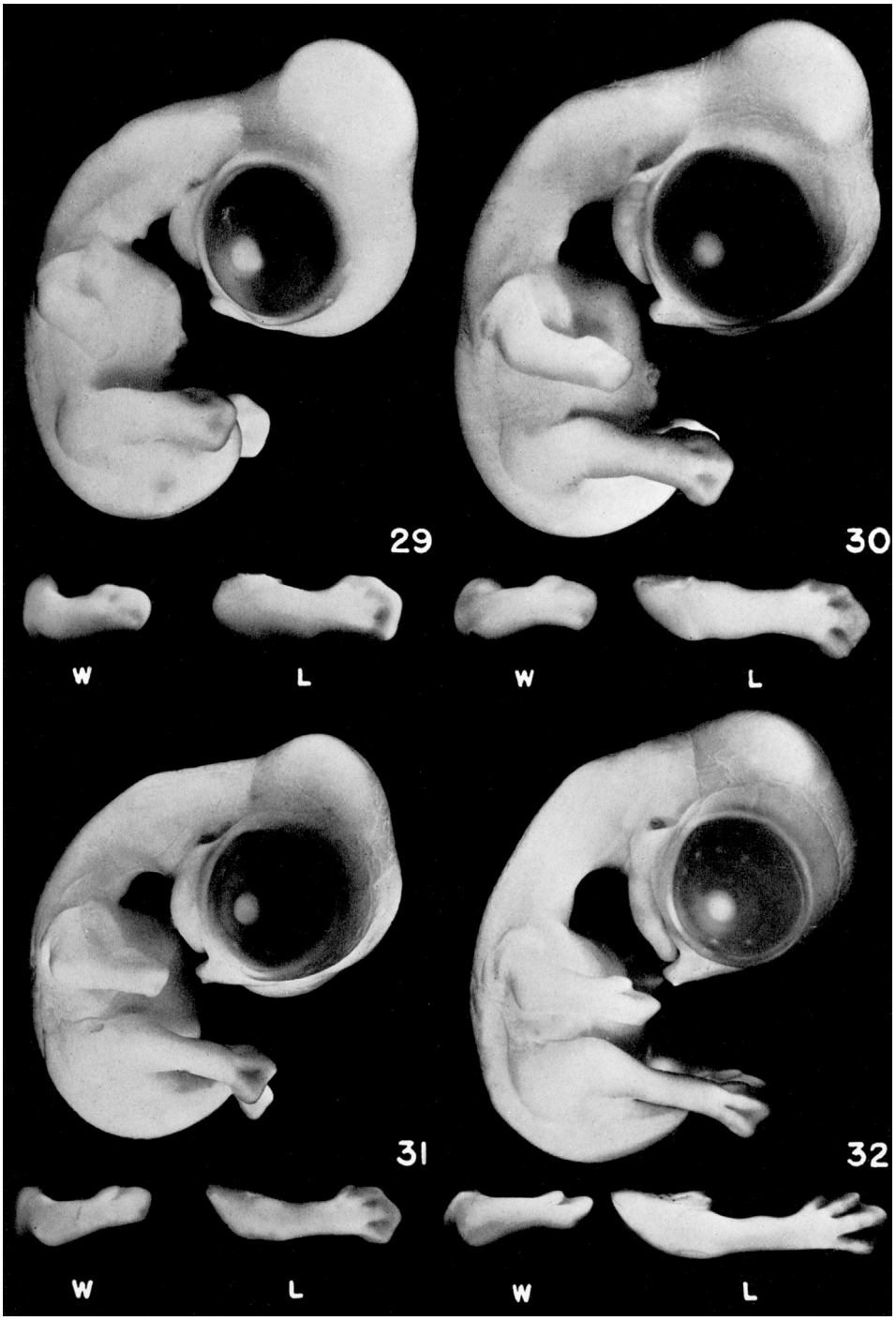
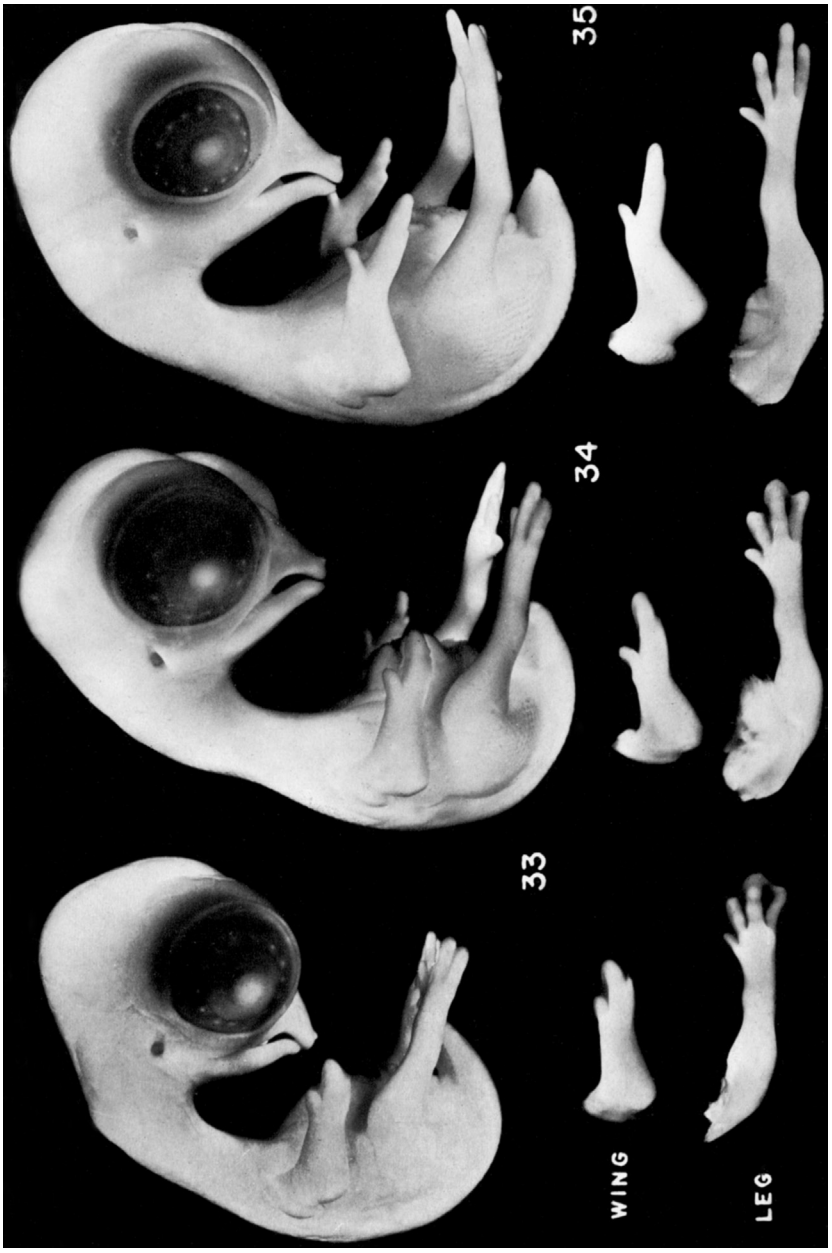
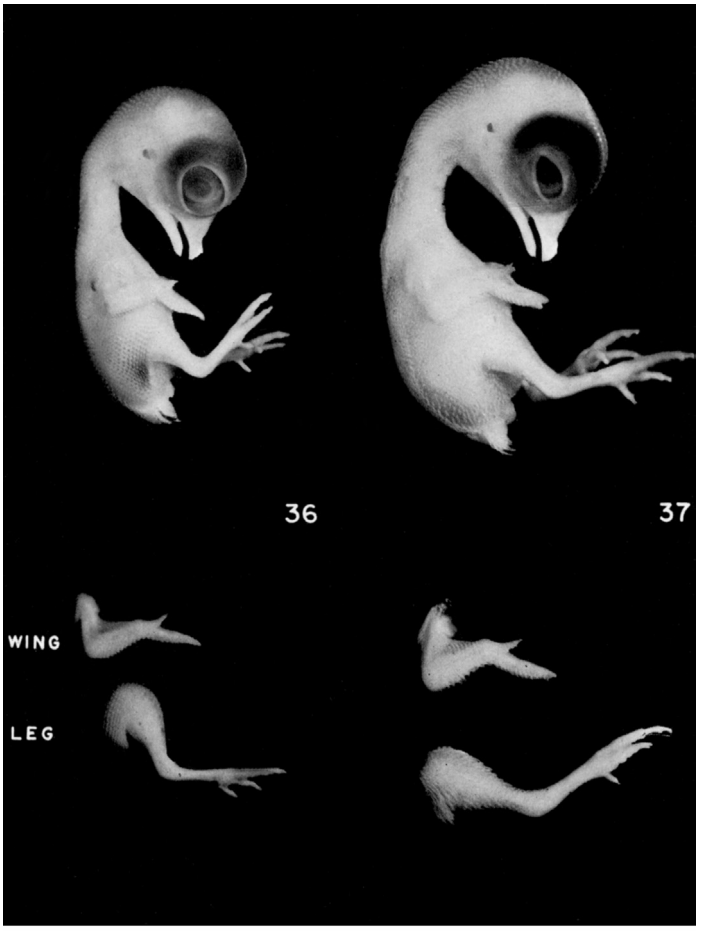


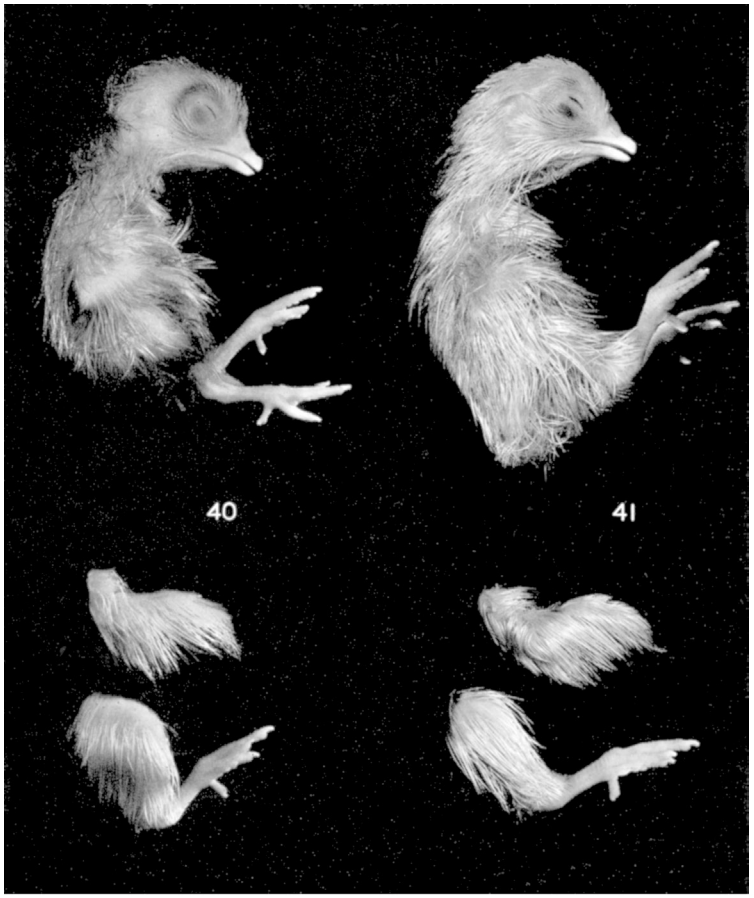
## APPENDIX II PLATES 11

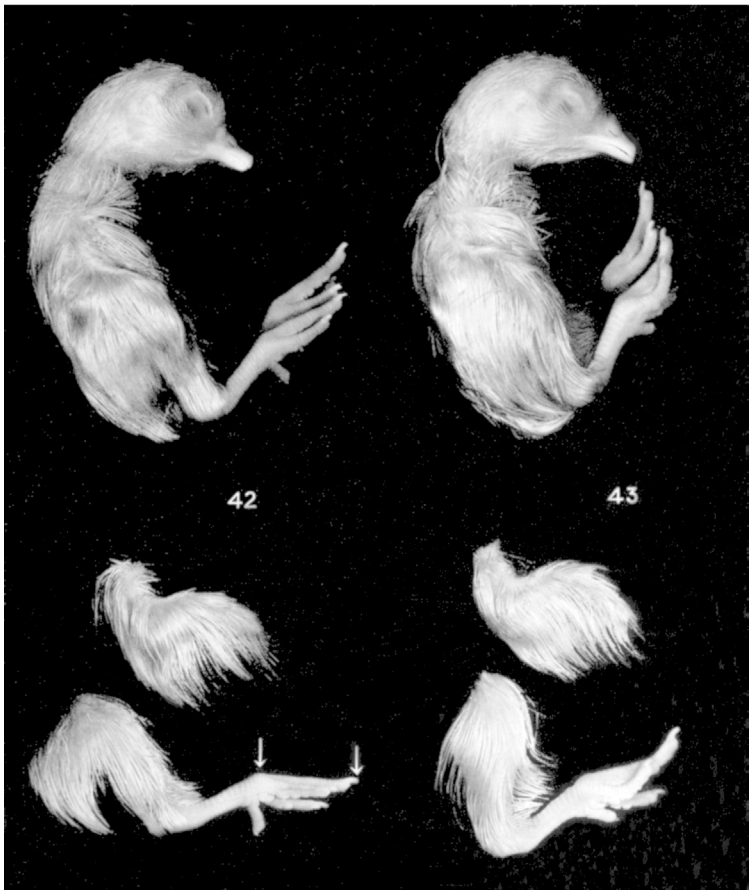


## Appendix II Plates 12




# Appendix II Plates 13



## APPENDIX II PLATES 14