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A SERIES OF NORMAL STAGES IN THE DEVELOPMENT OF THE CHICK EMBRYO

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FORTY-FIVE FIGURES

The preparation of a series of normal stages of the chick embryo does not need justification at a time when chick embryos are not only widely used in descriptive and experimental embryology but are proving to be increasingly valuable in medical research, as in work on viruses and cancer. The present series was planned in connection with the preparation of a new edition of Lillie's *Development of the Chick* by the junior author. It is being published separately to make it accessible immediately to a large group of workers.

Ever since Aristotle "discovered" the chick embryo as the ideal object for embryological studies, the embryos have been described in terms of the length of time of incubation, and this arbitrary method is still in general use, except for the first three days of incubation during which more detailed characteristics such as the numbers of somites are applied. The shortcomings of a classification based on chronological age are obvious to every worker in this field, for enormous variations may occur in embryos even though all eggs in a setting are placed in the incubator at the same time. Many factors are responsible for the lack of correlation between chronological and structural age. Among these are: genetic differences in the rate of development of different breeds (e.g., the embryo of the White Leghorn breed develops more

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rapidly than that of the Barred Plymouth Rock and hatches approximately a day earlier); seasonal differences in the viability and vigor of embryos; differences in the stage of development when incubation is started; differences in the "freshness" of eggs, i.e., the lapse of time between laying and incubation; differences in the temperature of eggs when placed in the incubator, and in the size of individual eggs; differences in the temperature of incubation, and in type and size of incubator.

The wide variations in external form which occur at any given chronological age are clearly seen in tables 1 and 2 which show the distribution of 296 embryos from the 4th day until hatching when classified according to our series of stages. For example, a "6-day" embryo may range anywhere from stage 27 + to stage 31 (table 1). It will also be noted that the data in table 1 are based on an incubation-temperature of 103°F. (*ca.* 39.4°C.) whereas those in table 2 are based on a temperature of 37.5°C. This difference has resulted in the skipping of the "9-day" embryo altogether! It is not surprising, therefore, that the use of chronology with its lack of precision in the designation of embryos has actually led to misunderstandings and controversies which could readily have been avoided by the use of an adequate series of morphological stages.

Keibel and Abraham (1900) worked out a series of stages of the chick embryo based on morphological characters. This series never became popular and it has been rarely used and quoted. Among its shortcomings are its inadequate illustrations which often make the identification of an embryo difficult, the incomplete coverage of older stages, and perhaps also the format and relative inaccessibility of the *Normentafeln*. M. Duval's masterful *Atlas d'Embryologie* (1889) with its artistically perfect drawings is unfortunately incomplete in that it does not go beyond the 8th day of incubation.

Our own work covers the entire period of incubation. Its aim is to serve the practical purpose of identifying and designating embryos on the basis of external characters. The un-

TABLE 1

Distribution of chick embryos showing the relationship between stages of development and days of incubation at 103°F. (39.4°C.)

	STAGES OF DEVELOPMENT																							
DAYS OF INCUBATION	22	23	24	24 +	25	25 +	26	26 +	27	27 +	28	28 +	29	29 +	30	30 +	31	31 +	32	32 +	33	33 +	34	35
3½	5	5	2																					
4		1	5	4	2																			
4½				3	5	4	7	1	1															
5							3	2	8	6	4													
5½							1		3	2	5													
6										1	3	7	2	4	2	3	2							
6½											2	1	4	2	2	1								
7															4	2	5		1					
7½																			4	3	3	1		
8																						1	8	8
8½[1]																								8

[1] The omission of the 9th day is due to a shift in incubation-temperature between stages 35 and 36 (see p. 50).

TABLE 2

Distribution of chick embryos showing the relationship between stages of development and days of incubation at 37.5°C.

	STAGES OF DEVELOPMENT																								
DAYS OF INCUBATION	36	36 +	37 −	37	37 +	38 −	38	38 +	39 −	39	39 +	40 −	40	40 +	41 −	41	41 +	42 −	42	43	43 +	44 −	44	45	46
10	9	3	1																						
11				8	5	1																			
12							8	5		2															
13								1	3	8	1	1													
14												2	9												
15												1	1	1	1	7									
16																	1	3	6						
17																			1	10					
18																			1		2	1	6		
19																								12	
20																								7	5

excelled series of stages of *Amblystoma* by Harrison has served as a model. Our series is independent of chronological age and of size of embryos, as is the *Amblystoma* series. The photographs and drawings show most of the diagnostic criteria; this, we hope, will facilitate a rapid identification. A brief text is added, in which the distinguishing criteria are listed for each stage.

We are aware of the complications which derive from the independent variations of different characters. For instance, the progress of differentiation in the visceral arches may lag behind that in the limb-buds, when compared with an average sequence. For this reason, the amnion and allantois, and the number of pairs of somites beyond 22 are of no diagnostic value. We have tried to establish average or "standard" types by comparing a considerable number of embryos in each stage, and we have selected for illustrations those embryos which appeared typical.

During the different phases of development, different characters become prominent, and therefore particularly useful for the diagnosis. For the second day of incubation we have adopted the conventional designation of embryos according to numbers of pairs of somites. We have chosen intervals of three somites as "stages"; this makes it possible to designate embryos with intermediate numbers of somites by a + or — sign. Somites were not counted unless fully formed and completely separated by clefts from the adjacent mesoderm. The first somite was not included in the counts beyond stage 10 when it begins to dwindle away.

During the third day of incubation, or, more precisely, from the stage of 22 somites onward (stage 14), the rapid progress in development of the limbs provides the most convenient diagnostic criteria. Preliminary work on these stages has been done by Hamburger ('38, '42) and by Saunders ('48). Our stages 15 to 21 are identical with stages 1 to 7 of these authors. The original work was carefully rechecked and detailed descriptions of all characters were added. Stages 8 and 9 of Saunders are combined in our stage 22;

stage 10 of Saunders is identical with our stage 23. The developmental phase between 4 and 9 days of incubation is characterized by rapid changes in the wings, legs, and visceral arches. From the 8th to the 12th days, feather-germs and eyelids provide the most useful criteria. The designation of stages during the last phase of incubation is difficult because practically no new structures are formed and there is mainly just growth of what already exists. Hence, we have had to make use of measurements of the lengths of the beak and of the toes.

The senior author is responsible for stages 14 to 35 and the junior author for all the others.

All illustrations and descriptions are based on material fixed in Bouin's solution or formalin. It is possible that minor distortions have occurred due to differential shrinkage, for instance in the amnion. The embryos used for stages 14 to 35 came from a flock of White Leghorns at St. Louis. They were incubated in a small size Buckeye incubator (for 350 eggs) without forced draft, at a temperature of 103°F. (*ca.* 39.4°C.). The embryos used for the other stages were of several breeds (White Leghorn, Barred Plymouth Rock, and Rhode Island Red) from the Iowa State College Poultry Farm, and were incubated in a forced-draft incubator at a constant temperature of 37.5°C. During the course of this work several hundred embryos have been examined and classified from the second day of incubation until hatching.

We wish to express our great appreciation of the expert advice and help which Dr. Mary E. Rawles, Johns Hopkins University, and Dr. Nelson T. Spratt, University of Minnesota, have given us in the difficult matter of selecting stages 1 to 6. Dr. Rawles has generously supplied data on the range of time within which a given stage may usually be obtained, based on records of 700 embryos incubated at 38°C. Her data are included in the text for stages 5–14 and 22. Dr. Spratt has supplied photographs and slides for illustrating the pre-somitic stages and has given estimates of incubation-time for stages 2–4.

The photographic work for stages 22 to 35 was done by Mr. L. Pinkers and Mr. D. Bucklin at Washington University, and that for the remaining stages by Mr. John Staby of the Iowa State College Experiment Station. All drawings were made by Mrs. Elsie Herbold Froeschner of Ames, Iowa. Additional assistance was given by Miss Thelma Dunnebacke and Miss Mary Lee Winkler, both of Washington University. We wish to thank all our helpers for their efficient and untiring coöperation. The work was supported, in part, by a Research Grant of the Rockefeller Foundation to the Department of Zoölogy of Washington University, and by the Industrial Science Research Institute of Iowa State College.

The description which follows should be used in conjunction with the illustrations (plates 1–14) which are numbered according to stages.

Stage 1. Pre-Streak: Prior to the appearance of the primitive streak. An "embryonic shield" may be visible, due to the accumulation of cells toward the posterior half of the blastoderm. (See Spratt, '42, pp. 71–72.)

Stage 2. Initial Streak: ("Short-broad beginning-streak" of Spratt, '42). A rather transitory stage in which the primitive streak first appears as a short, conical thickening, almost as broad as long (0.3–0.5 mm in length), at the posterior border of the pellucid area. Usually obtained after 6–7 hours of incubation.

Stage 3. Intermediate Streak: (12–13 hrs.). The primitive streak extends from the posterior margin to approximately the center of the pellucid area. The streak is relatively broad throughout its length, and is flared out where it touches the opaque area. No primitive groove.

Stage 4. Definitive Streak: (18–19 hrs.). The primitive streak has reached its maximal length (average length = 1.88 mm, Spratt, '46). The primitive groove, primitive pit, and Hensen's node are present. The area pellucida has become pear-shaped and the streak extends over two-thirds to three-fourths of its length.

Stage 5. Head-Process: (19–22 hrs.). The notochord or head-process is visible as a rod of condensed mesoderm extending forward from the anterior edge of Hensen's node. The head-fold has not yet appeared. Since the length of the notochord increases during this stage, it is suggested that the length of the notochord in millimeters be appended to the number of the stage for further precision (e.g., "Stage 5—0.2" would designate a notochordal blastoderm with notochord 0.2 mm in length).

Stage 6. Head-Fold: (23–25 hrs.). A definite fold of the blastoderm anterior to the notochord now marks the anterior end of the embryo proper. No somites have yet appeared in the mesoderm lateral to the notochord. This is a transitory stage, since the head-fold and the first pair of somites develop rather closely in time.

Stages 7 to 14 are based primarily on the numbers of pairs of somites which are clearly visible. The number of somites appears to be the simplest criterion for staging this phase of development, and it is sufficiently accurate for practical purposes. A stage is assigned to every third pair of somites which is added; embryos with in-between numbers of somites are designated by adding a + or − sign to the appropriate stage. Thus, stage 7 designates an embryo with one pair of somites; stage 7 + = two pairs; stage 8 − = three pairs; stage 8 = four pairs; etc. (See plates 2 and 3.)

Stage 7. One somite: (23–26 hrs.). This is actually the second somite of the series; number one is not yet clearly defined. Neural folds are visible in the region of the head.

Stage 8. Four somites: (26–29 hrs.). Neural folds meet at level of midbrain. Blood-islands are present in posterior half of blastoderm.

Stage 9. Seven somites: (29–33 hrs.). Primary optic vesicles are present. Paired primordia of heart begin to fuse.

Stage 10. Ten somites: (33–38 hrs.). The first somite is becoming dispersed; it is not included in the counts for subsequent stages.[1] First indication of cranial flexure. Three primary brain-vesicles are clearly visible. Optic vesicles not constricted at bases. Heart bent slightly to right.

Stage 11. Thirteen somites: (40–45 hrs.). Slight cranial flexure. Five neuromeres of hindbrain are distinct. Anterior neuropore is closing. Optic vesicles are constricted at bases. Heart bent to right.

[1] It is suggested that embryos which have gained one somite beyond Stage 10, but have lost s. 1 in the meantime, be designated as Stage 10 ±; Stage 10 + would then have 11 s., not counting the rudimentary one; stage 11 − = 12 s., not counting the rudimentary one, etc.

Stage 12. Sixteen somites: (45–49 hrs.). Head is turning onto left side. Anterior neuropore closed. Telencephalon indicated. Primary optic vesicles and optic stalk well established. Auditory pit is deep, but wide open. Heart is slightly S-shaped. Head-fold of amnion covers entire region of forebrain.

Stage 13. Nineteen somites: (48–52 hrs.). Head is partly to fully turned to the left. Cranial and cervical flexures make broad curves. Distinct enlargement of telencephalon. Slight narrowing of opening to deep auditory pit. No indication of hypophysis. Atrio-ventricular canal indicated by constriction. Head-fold of amnion covers forebrain, midbrain, and anterior part of hindbrain.

Stage 14. Twenty-two somites: (50–53 hrs.).

Flexures and rotation. Cranial flexure: axes of forebrain and hindbrain form about a right angle. Cervical flexure a broad curve. Rotation of body back as far as somites 7–9. Behind this level, a slight flexure makes its appearance which will be referred to as "trunk-flexure."

Visceral arches 1 and 2, and clefts 1 and 2 are distinct. Posterior arches not distinct.

Primary *optic vesicle* begins to invaginate; lens-placode is formed. Opening of *auditory pit* constricted. *Rathke's pouch* can be recognized. Ventricular loop of *heart* now ventral to atrio-ventricular canal. *Amnion* extends to somites 7–10.

Beyond stage 14 the number of somites becomes increasingly difficult to determine with accuracy. This is due in part to the dispersal of the mesoderm of the anteriormost somites, and, in later stages, to the curvature of the tail. Total somite-counts given for the following stages are typical, but sufficiently variable so as not to be diagnostic. For these reasons, the limb-buds, visceral arches, and other externally visible structures are used as identifying criteria from stage 15 onward.

Stage 15. (Hamburger, '38; Saunders, '48, stage 1; *ca.* 50–55 hrs.).

1. *Lateral body-folds* extend to anterior end of wing-level (somites 15–17).
2. *Limb-primordia:* prospective limb-areas flat, not yet demarcated. Inconspicuous condensation of mesoderm in wing-level.
3. *Somites:* 24–27.
4. *Amnion* extends to somites 7–14.
5. *Flexures and rotation.* Cranial flexure: axes of forebrain and hindbrain form an acute angle. The ventral contours of forebrain and hindbrain are nearly parallel. Cervical flexure

a broad curve. The trunk is distinct. Rotation extends to somites 11 to 13.

6. *Visceral arches:* Visceral arch 3 and cleft 3 are distinct. The latter is shorter than cleft 2 and usually oval in shape.
7. *Eye:* Optic cup is completely formed; double contour distinct in region of iris.

Stage 16. (Hamburger-Saunders stage 2; *ca.* 51–56 hrs.).

1. *Lateral body-folds* extend to somites 17–20, between levels of wings and legs.
2. *Limbs.* Wing is lifted off blastoderm by infolding of lateral body-fold. It is represented by a thickened ridge. Primordium of leg is still flat; represented by a condensation of mesoderm.
3. *Somites:* 26–28.
4. *Amnion* extends to somites 10–18.
5. *Flexures and rotation:* All flexures are more accentuated than in stage 15. Rotation extends to somites 14–15.
6. *Tail-bud* a short, straight cone, delimited from blastoderm.
7. *Visceral arches:* Third cleft still oval in shape.
8. *Forebrain* lengthened; constrictions between brain-parts are deepened. *Epiphysis* indistinct or not yet formed.

Stage 17. (Hamburger-Saunders stage 3; *ca.* 52–64 hrs.).

1. *Lateral body-folds* extend around the entire circumference of the body.
2. *Limb-buds:* both wing- and leg-buds lifted off blastoderm by infolding of the body-folds. Both are distinct swellings of approximately equal size (see plate 5).
3. *Somites:* 29–32.
4. *Amnion:* Considerable variability, ranging from a condition in which posterior trunk and tail, from approximately somite 26, are uncovered, to complete closure except for an oval hole over somites 28–36. Intermediate stages with an anterior fold covering as far back as somite 25 and a posterior fold covering part of the tail are common.
5. *Flexures and rotation:* Cranial flexure is unchanged. Cervical flexure is more sharply bent than in preceding stages, but its angle is still larger than 90°. Trunk-flexure is distinct in brachial level. Rotation extends to somites 17–18.
6. *Tail-bud* bent ventrad. Its mesoderm unsegmented.
7. *Epiphysis:* a distinct knob. Indication of *nasal pits.*
8. *Allantois:* not yet formed.

Stage 18. (Hamburger-Saunders stage 4; *ca.* 65–69 hrs.).

1. *Limb-buds* enlarged; leg-buds slightly larger than wing-buds (see plates 4 and 5). L/W of wing = 6 or < 6 (L = length = anterior-posterior dimension as measured along the body-wall; W = width = distance from body-wall to apex; see stage 20, plate 5).
2. *Somites:* 30–36; extend beyond level of leg-bud.
3. *Amnion:* Usually closed; occasionally an oval hole in lumbar region.
4. *Flexures and rotation:* At the cervical flexure, the axis of the medulla forms approximately a right angle to the axis of the posterior trunk. The trunk-flexure has shifted to the lumbar region. The rotation extends now to the posterior part of the body; hence, the leg-buds are no longer in the horizontal plane.
5. The *tail-bud* is turned to the right, at about an angle of 90° to the axis of the posterior trunk.
6. *Visceral arches:* Maxillary process absent or inconspicuous. Fourth visceral cleft indistinct or absent.
7. *Allantois:* A short, thick-walled pocket; not yet vesicular.

Stage 19. (Hamburger-Saunders stage 5; *ca.* 68–72 hrs.).

1. *Limb-buds:* Enlarged, symmetrical. Leg-buds slightly larger and bulkier than wing-buds (see plate 5). L/W of wing-buds = 4–6.
2. *Somites:* 37–40; extend into tail; but the end of the tail which is directed forward is unsegmented.
3. *Flexures and rotation:* In the cervical flexure the axis of the medulla forms an acute angle with the axis of the trunk. The trunk-flexure has nearly or entirely disappeared due to the rotation of the entire body. The contour of the posterior part of the trunk is straight to the base of the tail.
4. *Tail-bud* curved, its tip pointing forward.
5. *Visceral arches:* The maxillary process is a distinct swelling of approximately the same length as the mandibular process. The first visceral cleft is an open narrow slit at its dorsal part. It continues into a shallow furrow. The second arch projects slightly over the surface. The 4th cleft is a fairly distinct slit at its dorsal part and continues ventrally as a shallow groove. It does not perforate into the pharynx as a true (open) cleft, but is, nevertheless, homologous to the other three clefts.
6. *Allantois:* A small pocket of variable size; not yet vesicular.
7. *Eyes* unpigmented.

Stage 20. (Hamburger-Saunders stage 6; *ca.* 70 – 72 hrs.).

1. *Limb-buds* enlarged; leg-buds are distinctly larger from now on than wing-buds. The wing-buds are still approximately symmetrical; the leg-buds are slightly asymmetrical (see plate 5). L/W of wing = 3–3.9; L/W of leg = 3–2.3.
2. *Somites:* 40–43; tip of tail still unsegmented.
3. *Flexures and rotation:* Cervical flexure more accentuated than in stage 19. The bend in the tail-region begins to extend forward into the lumbo-sacral region. Contour of mid-trunk a straight line. Rotation completed.
4. *Visceral arches:* Maxillary process distinct, equals or exceeds the mandibular process in length. Second arch projects over surface. Fourth arch less prominent and smaller than third arch. Fourth cleft shorter than third cleft; a narrow slit at its dorsal part, continuing into a shallow groove.
5. *Allantois:* Vesicular, variable in size; on the average of the size of the midbrain.
6. *Eye-pigment.* A faint grayish hue.

Stage 21. (Saunders stage 7; *ca.* 3½ days).

1. *Limbs:* Enlarged; both wing- and leg-buds are slightly asymmetrical; their proximo-distal axes are directed caudad, and the apex of the bud lies posterior to the midline bisecting the base of the bud. The posterior contours of wing- and leg-buds are steeper than the anterior contours; they meet the baseline at an angle of approximately 90°. L/W of wing = 2.3–2.7; L/W of leg = 2.0–2.5.
2. *Somites:* 43–44; extreme tip of tail unsegmented.
3. *Flexures:* The posterior curvature includes the lumbo-sacral region. The dorsal contour of the trunk is straight or slightly bent.
4. *Visceral arches:* Maxillary process is definitely longer than mandibular process, extending approximately to the middle of the eye. The second arch extends distinctly over the surface and overlaps the third arch ventrally. Fourth arch distinct; 4th cleft visible as a slit.
5. *Allantois:* Variable, usually larger than in stage 20; may extend to head.
6. *Eye-pigmentation:* Faint.

Stage 22. (Saunders stages 8 and 9 combined; *ca.* 3½ days).

1. *Limbs:* Elongated buds, pointing caudad. The anterior and posterior contours are nearly parallel at their bases (see plate 7). L/W of wing = 1.5–2; L/W of leg = 1.3–1.8.

2. *Somites:* Extend to tip of tail.
3. *Flexures:* Little change. The dorsal contour of the trunk is a straight line or curved.
4. *Visceral arches:* Little change compared with stage 21. Maxillary process enlarged; 4th cleft distinct as a slit.
5. *Allantois:* Variable in size; extends to head and may overlap the forebrain.
6. *Eye-pigmentation:* Distinct.

Stage 23. (Saunders stage 10; *ca.* 3½–4 days).

1. *Limbs:* Longer than in stage 22; particularly the proximal parts in which anterior and posterior contours run parallel are lengthened; otherwise, little change in shape. Both wing- and leg-buds approximately as long as they are wide.
2. *Visceral arches* (see plates 7 and 8): Maxillary process is lengthened further. The first visceral cleft is represented by a broken line. Its dorsal part is a distinct slit. A slight protuberance ("a") is noticeable anterior to the dorsal slit. The caudal part of the second arch is distinctly elevated over the surface. Arches 3 and 4 are still completely exposed. Visceral cleft 3 is a distinct groove, and cleft 4 is reduced to a narrow oval pit at its dorsal end.
3. *Flexures:* The dorsal contour from hindbrain to tail is a curved line.

Stage 24. (*ca.* 4 days).

1. *Limbs:* Wing- and leg-buds distinctly longer than wide. Digital plate in wing not yet demarcated. Toe-plate in leg-bud distinct. Toes not yet demarcated.
2. *Visceral arches* (see plates 7 and 8): First visceral cleft a distinct curved line. Slight indication of two protuberances ("a," "b") on mandibular process and of three protuberances ("d," "e," "f") on second arch. Part "c" of mandibular process is receding. Second arch longer ventrally (at "f") and much wider than mandibular process. Third arch reduced and partly overgrown by second arch; 4th arch flattened. Both are sunk beneath the surface. Third visceral cleft is an elongated groove. Fourth visceral cleft reduced to a small pit.

Stage 25. (*ca.* 4½ days).

1. *Limbs:* Elbow and knee-joints distinct (in dorsal or ventral view). Digital plate in wing distinct, but no demarcation of digits. Indication of faint grooves demarcating the third toe on leg.
2. *Visceral arches* (see plates 7 and 8): Maxillary process lengthened; it meets the wall of the nasal groove (notice the notch at

point of fusion). Three protuberances on each side of first visceral cleft ("a" to "f"). In dorsal view, "a," "b," and "d" appear as round knobs, and "c" as a flat ridge. Part "f" is conspicuous and projects distinctly over the surface. It will be referred to as the "collar." Dorsal part of third arch still visible. Third and 4th visceral clefts reduced to small circular pits.

Stage 26. (*ca.* 4½-5 days).

1. *Limbs:* Considerably lengthened. Contour of digital plate rounded. Indication of faint groove between second and third digit. Demarcation of the first three toes distinct.
2. *Visceral arches* (see plates 8 and 9): Contour of maxillary process a broken line. Mandibular process lengthened ventrally. Protuberances "a" and "b" project over the surface. The middle protuberance ("b") is subdivided by a shallow groove. A small knob is distinct at the dorsal edge of "c." On the second arch, protuberances "d" and "e" are only slightly elevated over the surface. The "collar" ("f") has broadened and overgrown visceral arches III and IV. A deep groove separates "f" from "c." The two pits representing the 3rd and 4th visceral clefts are no longer visible.

Stage 27. (*ca.* 5 days).

1. *Limbs:* Contour of digital plate angular in region of first digit. Grooves between first, second, and third digits indicated. Grooves between toes are distinct on outer and inner surfaces of toe-plate. First toe projects over the tibial part at an obtuse angle. Tip of third toe not yet pointed.
2. *Visceral arches* (see plates 8 and 9): Contour of maxillary process is a curved, broken line. Mandibular process has broadened ventrally (at "c") and grown forward. Protuberances "a" and "b" project over the surface. Parts "d" and "e" are flat. Protuberances "b" and "e" are close to fusion, but a separating line is still distinct. The "collar" ("f") has broadened and continued its growth backward. It rises conspicuously above the surface. The groove between "c" and "f" has widened.
3. *Beak:* Barely recognizable.

Stage 28. (*ca.* 5½ days).

1. *Limbs:* Second digit and third toe longer than others, which gives the digital and toe-plates a pointed contour. Three digits and 4 toes distinct. No indication of 5th toe.
2. *Visceral arches* (see plates 8 and 9): Protuberance "a" still projects over the surface. Mandibular process has lengthened

and grown forward. Parts "b" and "e" have fused; a fine suture line is occasionally still visible. Parts "b," "d," and "e" no longer project above the surface. External auditory opening is now very distinct between "a," "b," and "d." "Collar" ("f") projects distinctly over the surface. The neck between "collar" and mandible has lengthened.

3. *Beak:* A distinct outgrowth is visible in profile.

Stage 29. (*ca.* 6 days).

1. *Limbs:* Wing bent in elbow. Second digit distinctly longer than the others. Shallow grooves between first, second, and third digits. Second to 4th toes stand out as ridges separated by distinct grooves, and with indications of webs between them. Distal contours of webs are straight lines, occasionally with indication of convexity. Rudiment of 5th toe visible.
2. *Visceral arches:* Mandibular process lengthened (compare with stage 28). Mandibular process and second arch are broadly fused. Auditory meatus distinct at dorsal end of fusion. All protuberances have flattened. Neck between "collar" and mandibular process has lengthened. "Collar" stands out conspicuously.
3. *Beak:* More prominent than in stage 28. No egg-tooth visible as yet.

Stage 30. (*ca.* 6½ days).

1. *Limbs:* The three major segments of wing and leg are clearly demarcated. Wing bent in elbow-joint. Leg bent in knee-joint. Distinct grooves between first and second digits. Contours of webs between first two digits and between all toes are slightly curved concave lines.
2. *Visceral arches:* The mandibular process approaches the beak, but the gap between the two is still conspicuous. Lengthening of neck between "collar" and mandible is very conspicuous. "Collar" begins to flatten.
3. *Feather-germs:* Two dorsal rows to either side of the spinal cord at the brachial level. Three rows at the level of the legs; they are rather indistinct at thoracic level. None on thigh.
4. *Scleral papillae:* One on either side of choroid fissure; sometimes indistinct but never more than two.
5. *Egg-tooth* distinct, slightly protruding. Beak more pronounced than in previous stage.

Stage 31. (*ca.* 7 days).

1. *Limbs:* Indication of a web between first and second digits. Rudiment of 5th toe still distinct.

2. *Visceral arches:* The gap between mandible and beak has narrowed to a small notch. "Collar" inconspicuous or absent.
3. *Feather-germs:* On dorsal surface, continuous from brachial to lumbo-sacral level. Approximately 7 rows at lumbo-sacral level. Distinct feather papillae on thigh. One indistinct row on each lateral edge of the tail.
4. *Scleral papillae:* Usually 6; 4 on the dorsal side near the choroid fissure, and two on the opposite side.

Stage 32. (*ca.* 7½ days).

1. *Limbs:* All digits and 4 toes have lengthened conspicuously. Rudiment of 5th toe has disappeared. Webs between digits and toes are thin and their contours are concave. Differences in size of individual digits and toes become conspicuous.
2. *Visceral arches:* Anterior tip of mandible has reached the beak. "Collar" has disappeared or is faintly recognizable.
3. *Feather-germs:* Eleven rows or more on dorsal surface at level of the legs. One row on tail distinct, second row indistinct. Scapular and flight feather-germs barely perceptible at optimal illumination or absent.
4. *Scleral papillae:* Six to 8, in two groups; one group on dorsal and one on ventral side. Circle not yet closed.

Stage 33. (*ca.* 7½–8 days).

1. *Limbs:* Web on radial margin of arm and first digit becomes discernible. All digits and toes lengthened.
2. *Visceral arches:* Mandible and neck have lengthened conspicuously. (Compare the ventral contour of body, from heart-region, along neck to tip of mandible, in this and the preceding stages.)
3. *Feather-germs:* Scapular and flight feather-germs not much advanced over stage 32. Tail: three rows distinct, the middle row considerably larger than the others.
4. *Scleral papillae:* Thirteen, forming an almost complete circle, with gap for one missing papilla at a ventral point near the middle of the jaw.

Stage 34. (*ca.* 8 days).

1. *Limbs:* Differential growth of second digit and third toe conspicuous. Contours of webs between digits and toes are concave and arched.
2. *Visceral arches:* Lengthening of mandible and of neck continues (see previous stage).
3. *Feather-germs:* On scapula, on ventral side of neck, on procoracoid, and posterior (flight) edge of wing, feather-germs are visible under good illumination. Feather-germs next to

dorsal midline, particularly at lumbo-sacral level, extend slightly over surface when viewed in profile. Feather-germs on thigh protrude conspicuously. One row on inner side of each eye. None around umbilical cord.

4. *Scleral papillae:* Thirteen or 14.
5. *Nictitating membrane* extends halfway between outer rim of eye (eyelid) and scleral papillae.

Stage 35. (*ca.* 8–9 days).

1. *Limbs:* Webs between digits and toes become inconspicuous. A transitory protuberance on the ulnar side of the second digit is probably a remnant of the web. Phalanges in toes are distinct.
2. *Visceral arches:* Lengthening of beak continues. Compare the distance between the eye and the tip of the beak, in this and the preceding stages.
3. *Feather-germs:* All are more conspicuous. Mid-dorsal line stands out distinctly in profile view. At least 4 rows on inner side of each eye. New appearance of feather-germs near mid-ventral line, close to sternum, and extending to both sides of umbilical cord.
4. *Nictitating membrane* has grown conspicuously and approaches the outer scleral papillae. Eyelids (external to nictitating membrane) have extended towards the beak and have begun to overgrow the eye-ball. The circumference of the eyelids has become ellipsoidal.

Stage 36. (*ca.* 10 days).

1. *Limbs:* Distal segments of both wing and leg are proportionately much longer. Length of third toe, from its tip to the middle of its metatarsal joint $= 5.4 \pm 0.3$ mm. Tapering primordia of claws are just visible on termini of the toes and on digit 1 of the wing. Protuberance on posterior side of digit 2 of wing is missing.
2. *Visceral arches:* Primordium of the comb appears as a prominent ridge with slightly serrated edge along the dorsal midline of the beak. A horizontal groove (the "labial groove") is clearly visible at the tip of the upper jaw, but is barely indicated on the tip of the mandible. Nostril has narrowed to a slit. Length of beak from anterior angle of nostril to tip of bill $= 2.5$ mm.
3. *Feather-germs:* Flight-feathers are conspicuous; coverts are just visible in web of wing. Feather-germs now cover the tibiofibular portion of the leg. At least 9–10 rows of feather-germs between each upper eyelid and the dorsal midline. Sternal tracts prominent, with 3–4 rows on each side of ventral mid-

line when counted in anterior part of sternum, merging into many rows around the umbilicus.

4. *Eyelids:* Nictitating membrane covers anteriormost scleral papillae and approaches cornea. Lower lid has grown upward to level of cornea. Circumference of lids is a narrowing ellipse with its ventral edge flattened.

Stage 37. (*ca.* 11 days).

1. *Limbs:* Claws of toes are flattened laterally and curved ventrally; dorsal tips are opaque, indicating onset of cornification. Tip of claw on wing is also opaque. Pads on plantar surface of foot are conspicuous. Transverse ridges along the superior surfaces of the metatarsus and phalanges are first indication of scales. Length of third toe $= 7.4 \pm 0.3$ mm.
2. *Visceral arches:* Labial groove on mandible is now clearly marked off. The comb is more prominent and clearly serrated. Length of beak from anterior angle of nostril to tip of bill $=$ 3.0 mm.
3. *Feather-germs:* Much more numerous, and in most-advanced tracts (e.g., along back and on tail) elongated into long, much-tapered cones. External auditory meatus is nearly surrounded by feather-germs. Circumference of eyelids is bordered by a single row of just-visible primordia; none on remainder of lids. Sternal tracts contain 5–6 prominent rows when counted at anterior end of sternum.
4. *Eyelids:* Nictitating membrane has reached anterior edge of cornea. Upper lid has reached dorsal edge of cornea. Lower lid has covered one-third to one-half of cornea. Circumference of lids now bounds a much-narrowed and ventrally-flattened biconvex area.

Stage 38. (*ca.* 12 days).

1. *Limbs:* Primordia of scales are marked off over entire surface of leg; ridges have not yet grown out to overlap surface. Tips of toes show a ventral center of cornification as well as the more extensive dorsal one. Main plantar pad is ridged when seen in profile. Length of third toe $= 8.4 \pm 0.3$ mm.
2. *Visceral arches:* Labial groove marked off by a deep furrow at the end of each jaw. Length of beak from anterior angle of nostril to tip of bill $= 3.1$ mm.
3. *Feather-germs:* Coverts of web of wing are becoming conical. External auditory meatus is surrounded by feather-germs. Sternum is covered with feather-germs except along midline. Upper eyelid is covered with newly-formed feather-germs; lower lid is naked except for 2–3 rows at its edge.

4. *Eyelids:* Lower lid covers two-thirds to three-fourths of cornea. Opening between lids is much reduced.

Stage 39. (*ca.* 13 days).

1. *Limbs:* Scales overlapping on superior surface of leg. Major pads of phalanges covered with papillae; minor pads are smooth. Length of third toe $= 9.8 \pm 0.3$ mm.
2. *Visceral arches:* Mandible and maxilla cornified (opaque) back as far as level of proximal edge of "egg-tooth." The channel of the auditory meatus can be seen only at the posterior edge of its shallow external opening. Length of beak from anterior angle of nostril to tip of bill $= 3.5$ mm.
3. *Feather-germs:* Coverts of web of wing are very long tapering cones. Note great increase in length of feather-germs in major tracts. Four to 5 rows of feather-germs at edge of lower eyelid.
4. *Eyelids:* Opening between lids reduced to a thin crescent.

Stages 40 to 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. Of these two criteria, the length of the beak is the better, because it is more easily and accurately measured (with calipers) and shows less variability.

Stage 40. (*ca.* 14 days).

1. *Visceral arches:* Length of beak from anterior edge of nostril to tip of bill $= 4.0$ mm. The main channel of the auditory meatus is not visible in strictly lateral view of its external chamber.
2. *Limbs:* Length of third toe $= 12.7 \pm 0.5$ mm. Scales overlapping on inferior as well as superior surfaces of leg. Dorsal and ventral loci of cornification extend to base of exposed portion of toe-nail. Entire plantar surface of phalanges is covered with well-developed papillae.

Stage 41. (*ca.* 15 days).

1. *Beak:* Length from anterior angle of nostril to tip of upper bill $= 4.5$ mm.
2. *Third toe:* Length $= 14.9 \pm 0.8$ mm.

Stage 42. (*ca.* 16 days).

1. *Beak:* Length from anterior angle of nostril to tip of upper bill $= 4.8$ mm.
2. *Third toe:* Length $= 16.7 \pm 0.8$ mm.

Stage 43. (*ca.* 17 days).

1. *Beak:* Length from anterior angle of nostril to tip of upper bill $= 5.0$ mm. "Labial grooves" are reduced to a white granular crust at the edge of each jaw; that of the lower jaw may be partially or completely sloughed off.

2. *Third toe:* Length $= 18.6 \pm 0.8$ mm.

Stage 44. (*ca.* 18 days).

1. *Beak:* Length from anterior angle of nostril to tip of upper bill $= 5.7$ mm. The translucent peridermal covering of the beak is starting to peel off proximally.
2. *Third toe:* Length $= 20.4 \pm 0.8$ mm.

Stage 45. (*ca.* 19–20 days).

1. *Beak:* Length is no longer diagnostic; in fact, the beak is usually shorter than in stage 44, due to a loss (by sloughing off) of its entire peridermal covering. As a consequence, the beak is now shiny all over and more blunt at its tip. Both labial grooves have disappeared with the periderm.
2. *Third toe:* Average length is essentially unchanged from that of stage 44, except in those breeds with a longer period of incubation (21 days) and a heavier build of body. For these latter, length of third toe $=$ *ca.* 21.4 ± 0.8 mm.
3. *Extra-embryonic membranes:* Yolk-sac is half-enclosed in body-cavity. Chorio-allantoic membrane contains less blood and is "sticky" in the living embryo.

Stage 46. Newly-hatched chick (20–21 days).

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EXPLANATION OF PLATES

All numbers in the following plates refer to the corresponding stage numbers in the text. The description of each stage should be consulted for a more complete explanation of the figures.

PLATE 1

EXPLANATION OF FIGURES

Stages 1–3^+, illustrated by photographs provided by Dr. Nelson T. Spratt, Jr. (Stages 1 and 2 are published in J. Exp. Zool., *103:* 265 and 274.) $\times$ 20.

NORMAL STAGES OF THE CHICK
V. HAMBURGER AND H. L. HAMILTON

PLATE 1

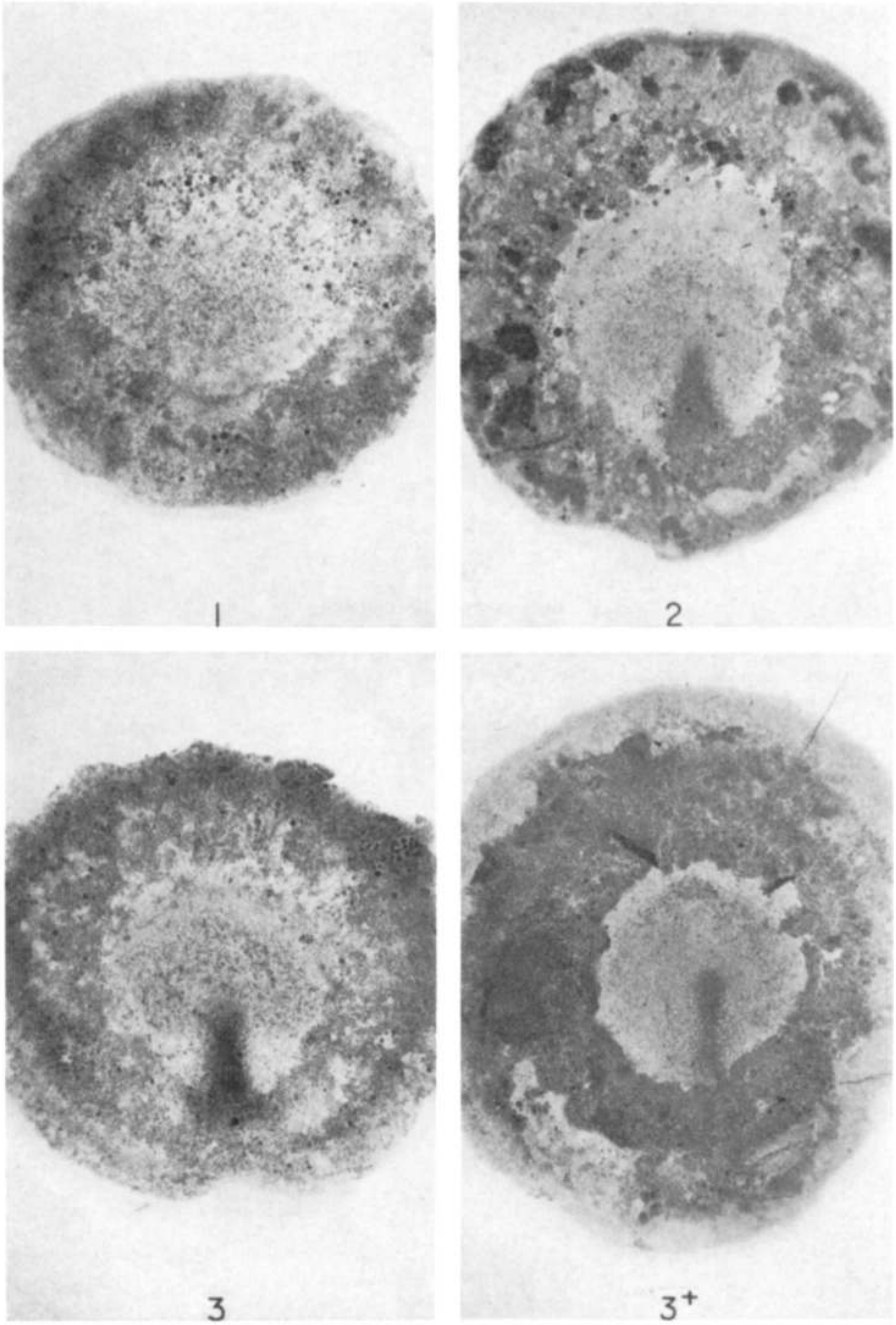


PLATE 2

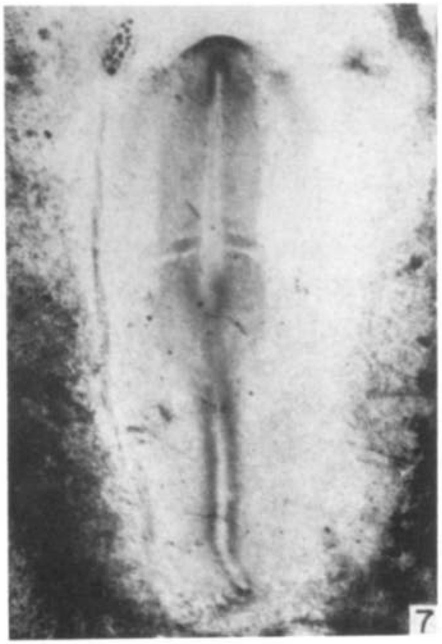
EXPLANATION OF FIGURES

Stages 4–9, × 20. Stage 10, × 12. (Stages 4, 5, and 8⁻ were photographed from slides provided by Dr. Nelson T. Spratt, Jr. All others are from the Iowa State College collection.)


4


5


6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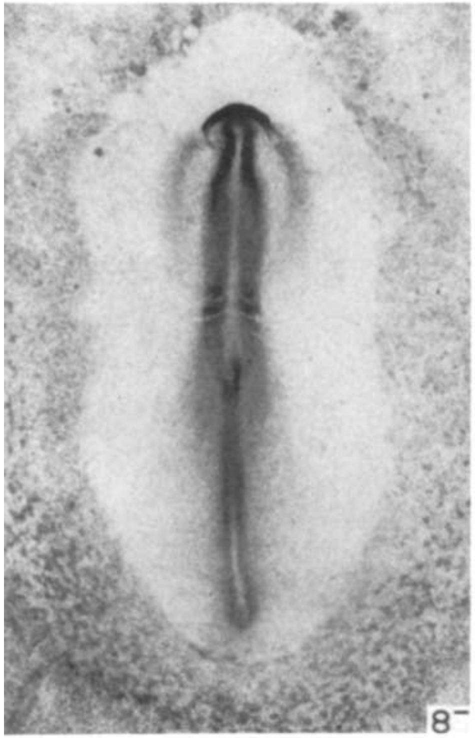
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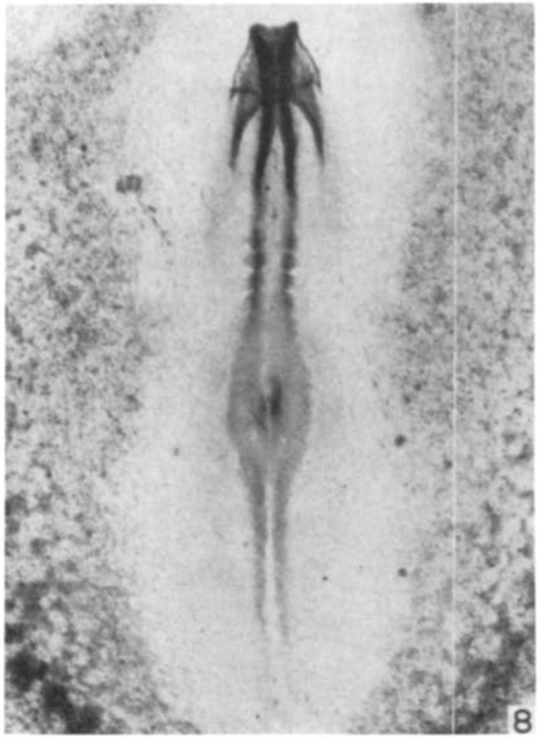
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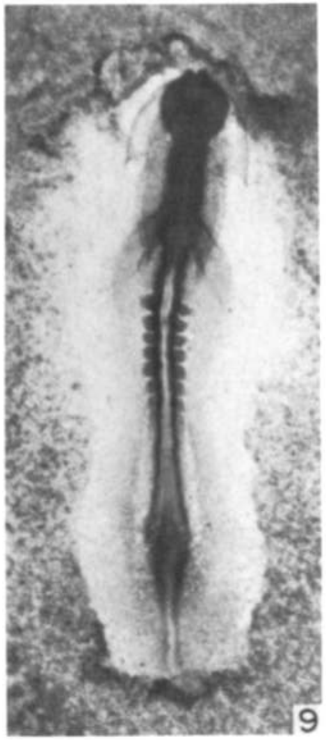
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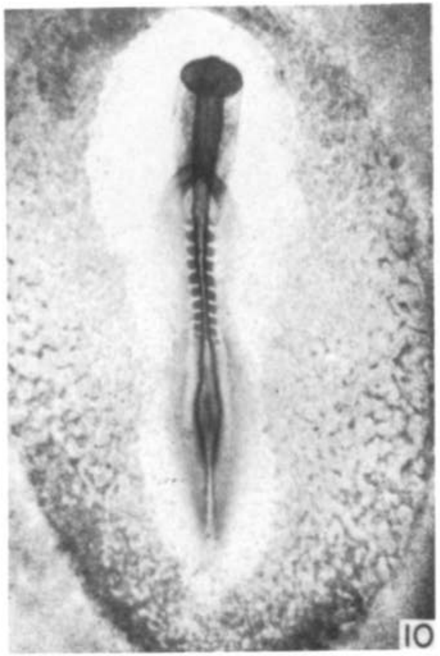
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PLATE 3

EXPLANATION OF FIGURES

Stages 11–14, × 12.

PLATE 3

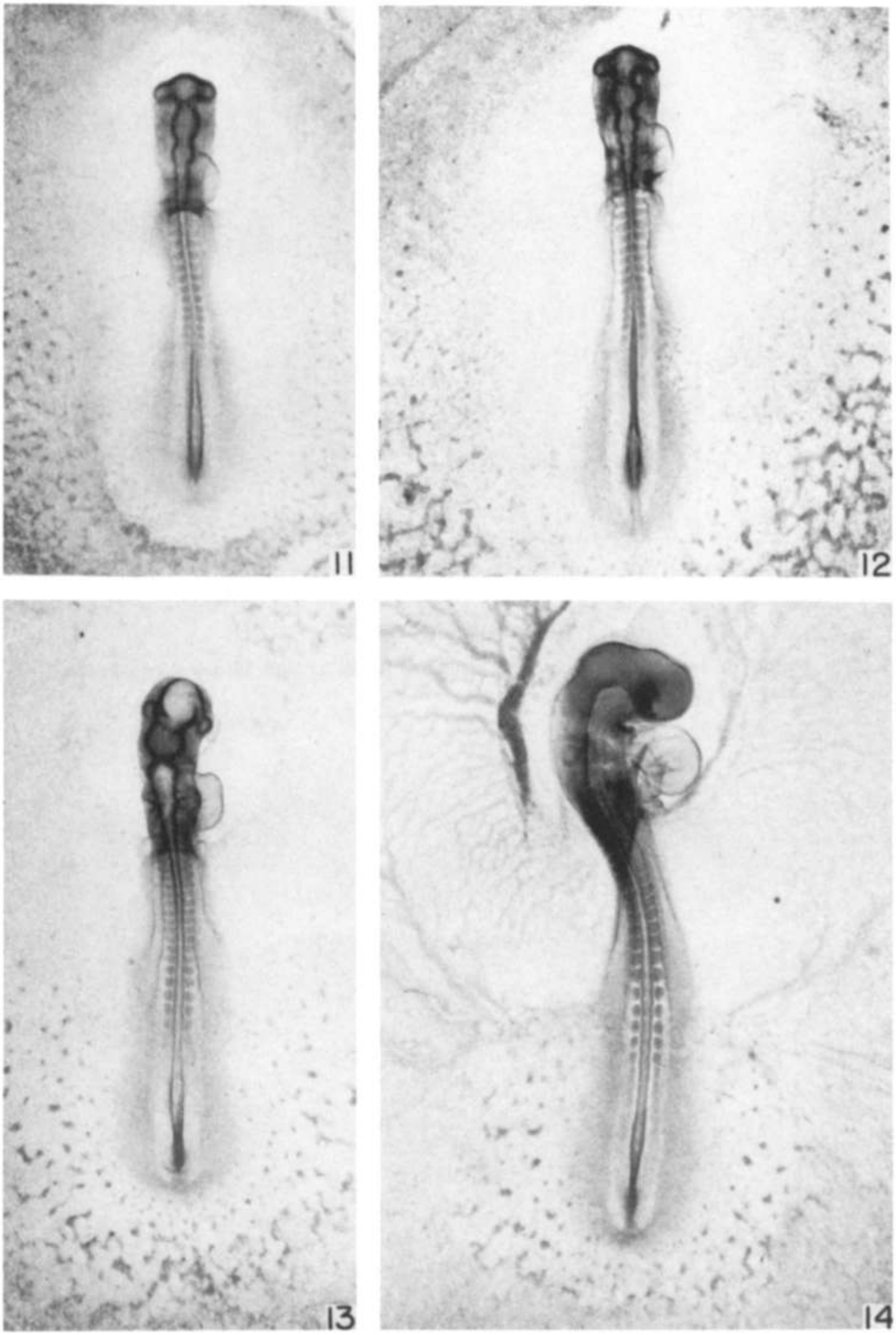
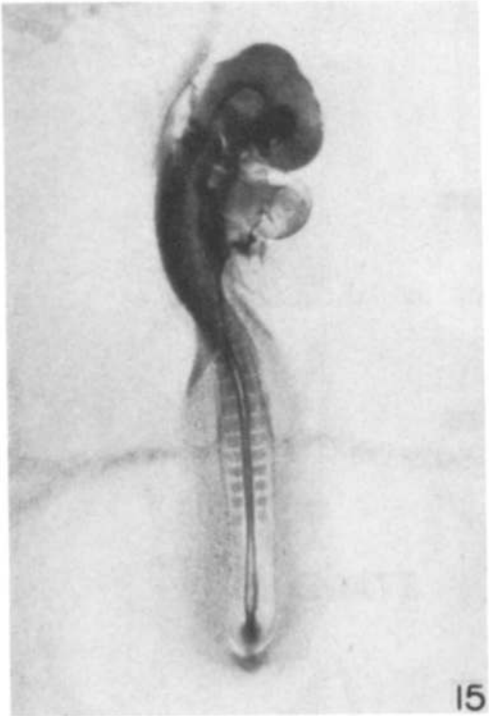


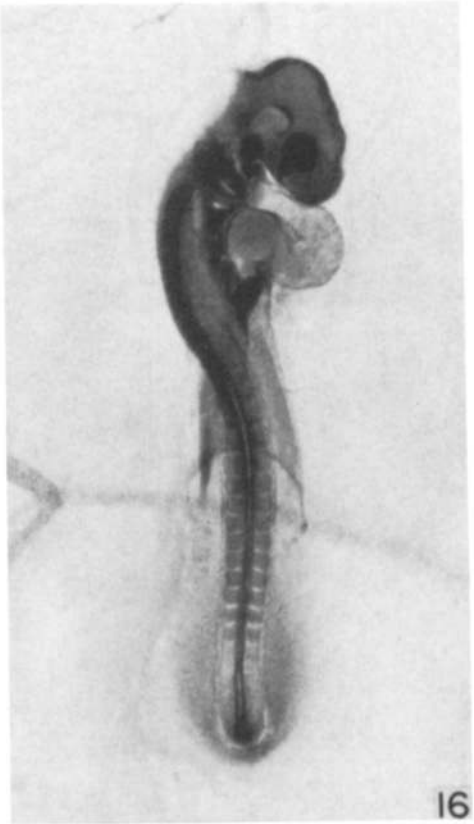
PLATE 4

EXPLANATION OF FIGURES

Stages 15–18, × 12. Contours of limbs for stages 17 and 18 are shown in the drawings on plate 5.

NORMAL STAGES OF THE CHICK PLATE 4
V. HAMBURGER AND H. L. HAMILTON

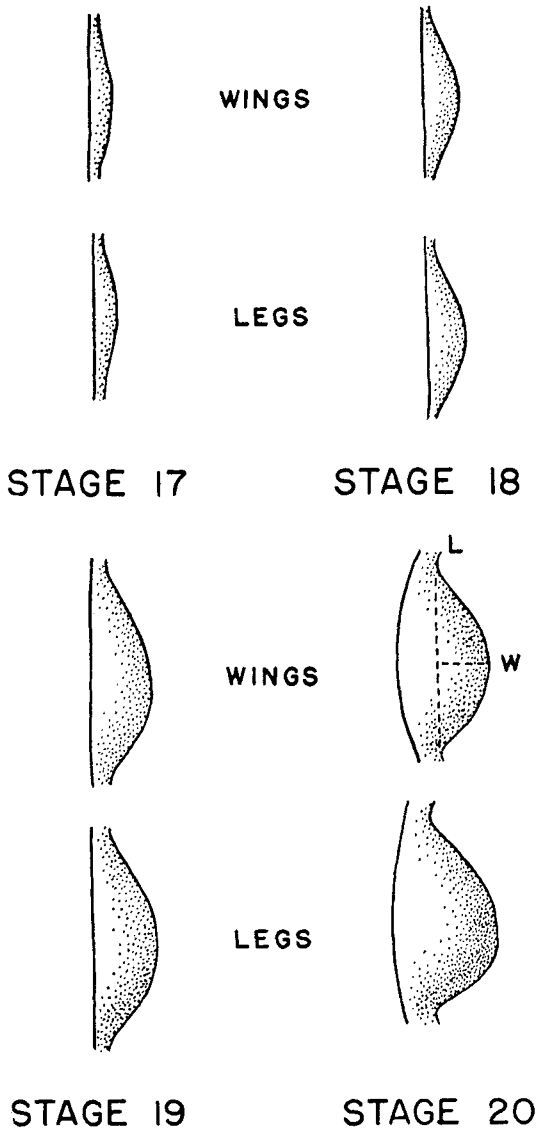




NORMAL STAGES OF THE CHICK

NORMAL STAGES OF THE CHICK
V. HAMBURGER AND H. L. HAMILTON

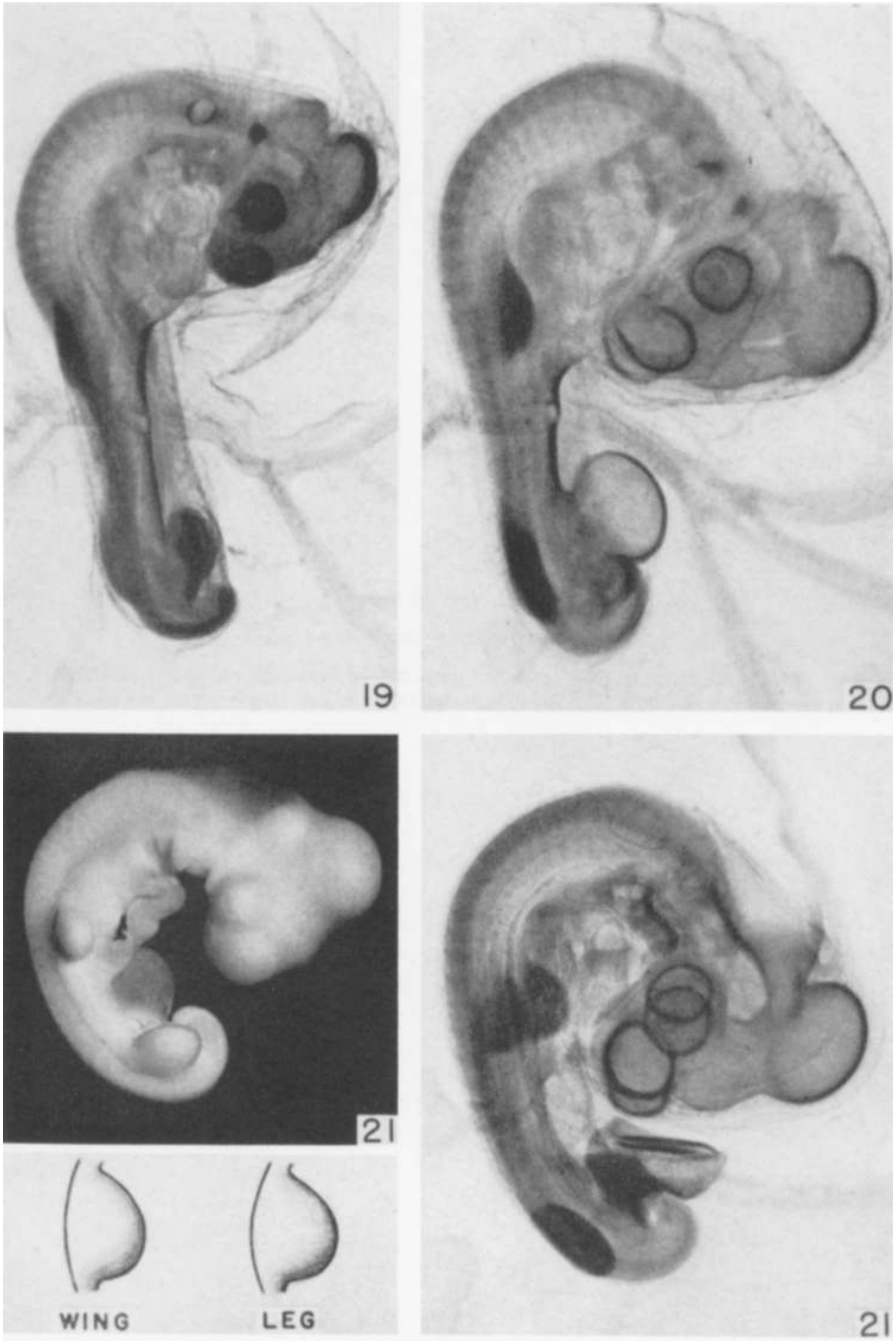
PLATE 5



Drawings of the contours of the right limbs of stages 17–20, *ca.* × 12. In stage 20 the dotted lines indicate the levels at which the length (L) and width (W) are measured (see text, stages 18–22).

NORMAL STAGES OF THE CHICK
V. HAMBURGER AND H. L. HAMILTON

PLATE 6

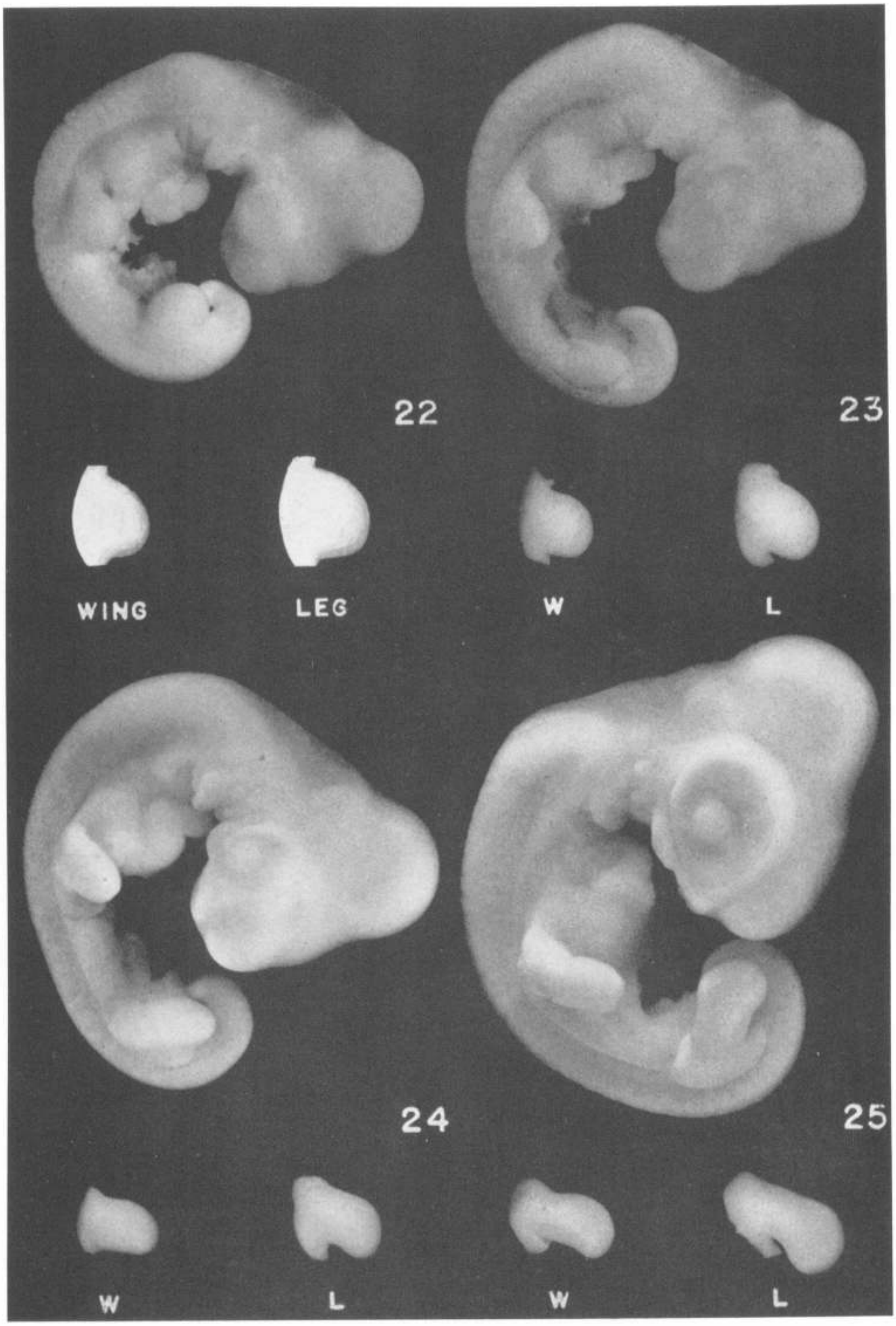


Stages 19–21 (cleared embryos), × 12. Stage 21 (opaque), × 8, with contours of limbs shown in the drawings below, *ca.* × 12.

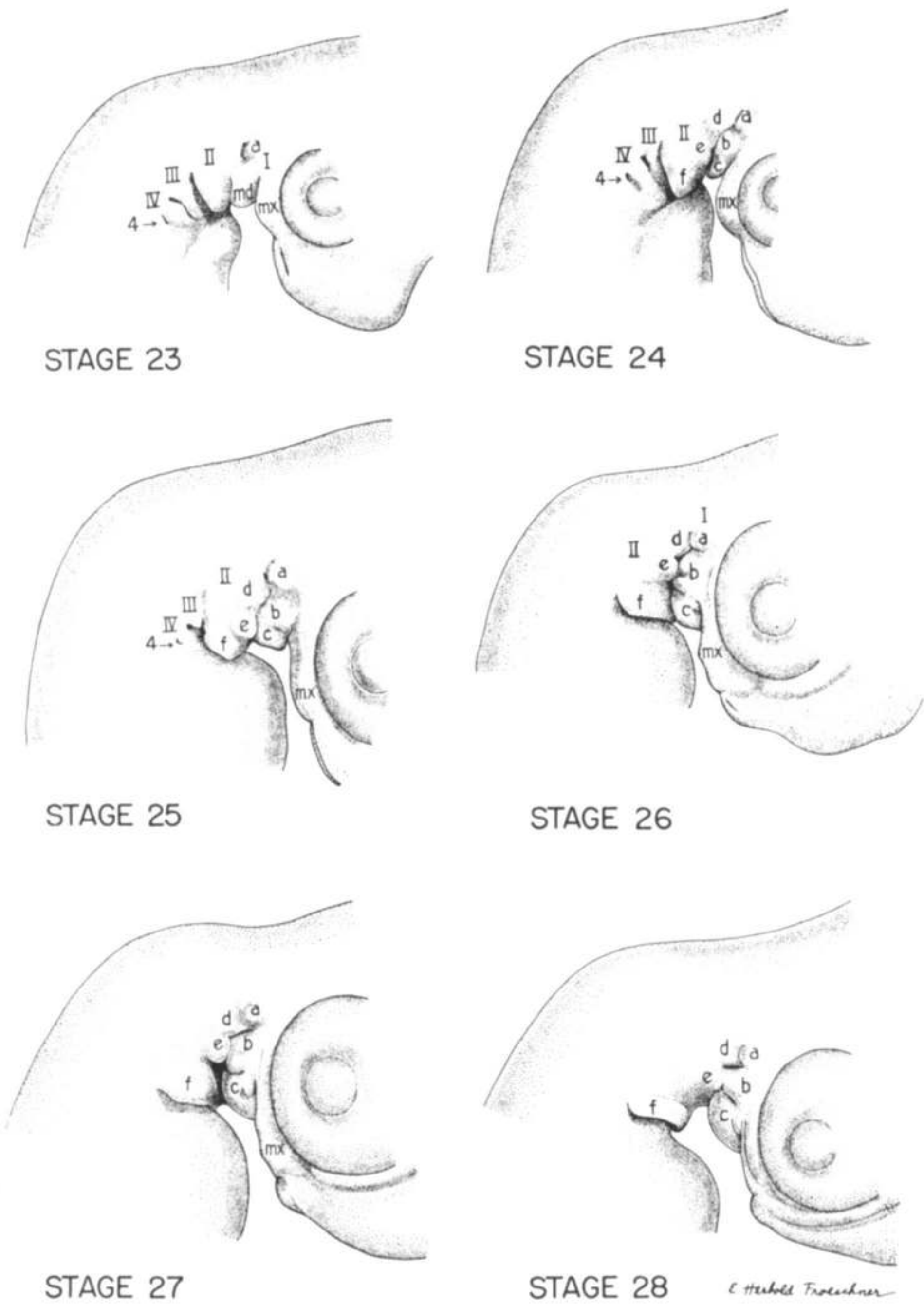
PLATE 7

EXPLANATION OF FIGURES

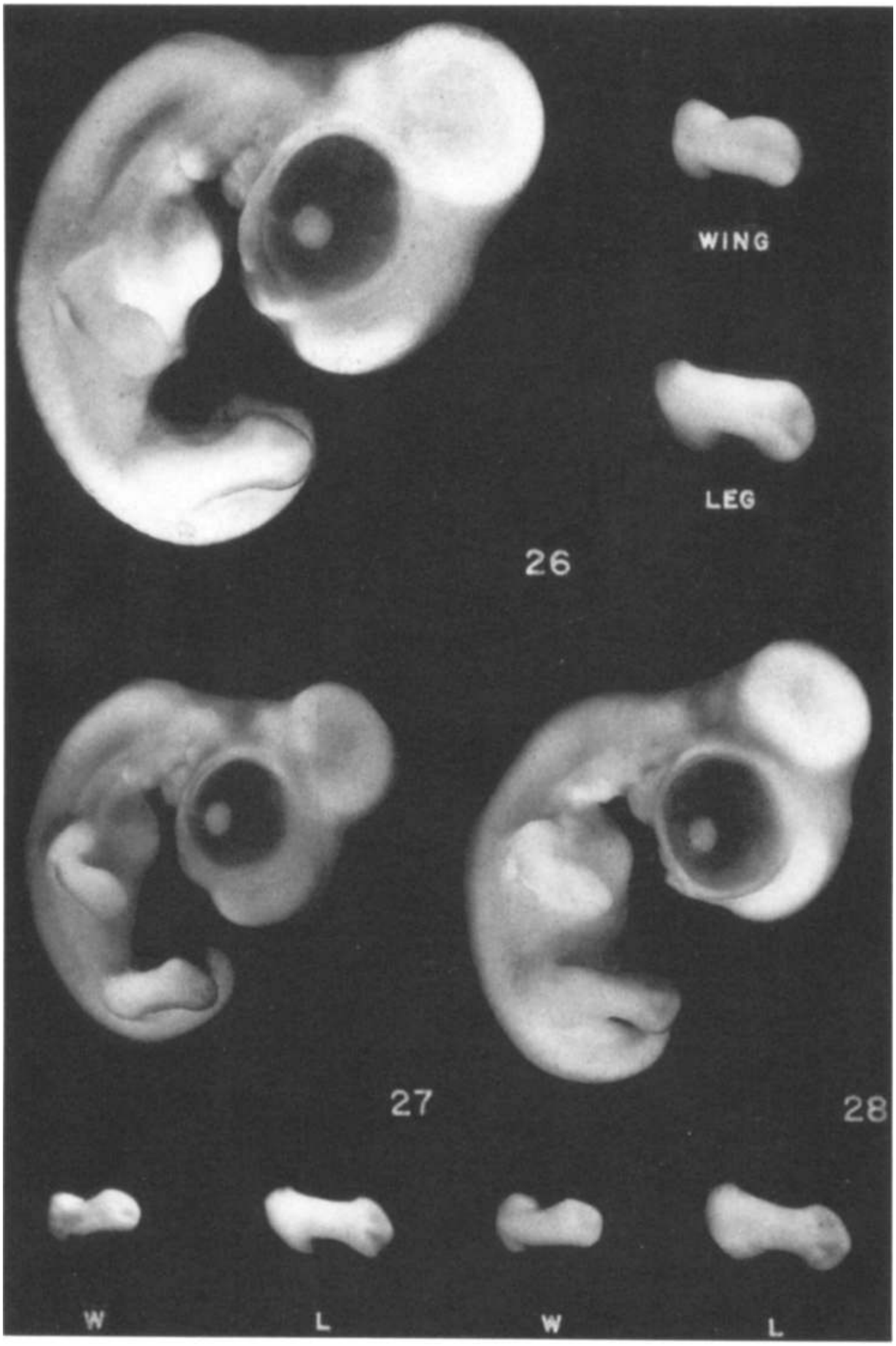
Stages 22–25, × 8. The limbs for stage 22 are drawings, *ca.* × 12; all others are photographs, × 8. For details of visceral arches of stages 23–25, see plate 8.


22
23
WING
LEG
W
L
24
25
W
L
W
L

NORMAL STAGES OF THE CHICK PLATE 8
V. HAMBURGER AND H. L. HAMILTON



Drawings of the region of the visceral arches, made from camera lucida tracings. Stages 23–25, × 7. Stages 26–28, × 4.2. I–IV = visceral arches; mx., md. = maxillary and mandibular processes of visceral arch I; 4 = 4th visceral cleft. See text for explanation of letters a–f.



Stage 26, embryo and limbs, × 8. Stages 27–28, × 5.

PLATE 10

EXPLANATION OF FIGURES

Stages 29–30, × 5. Stages 31–32, × 4.

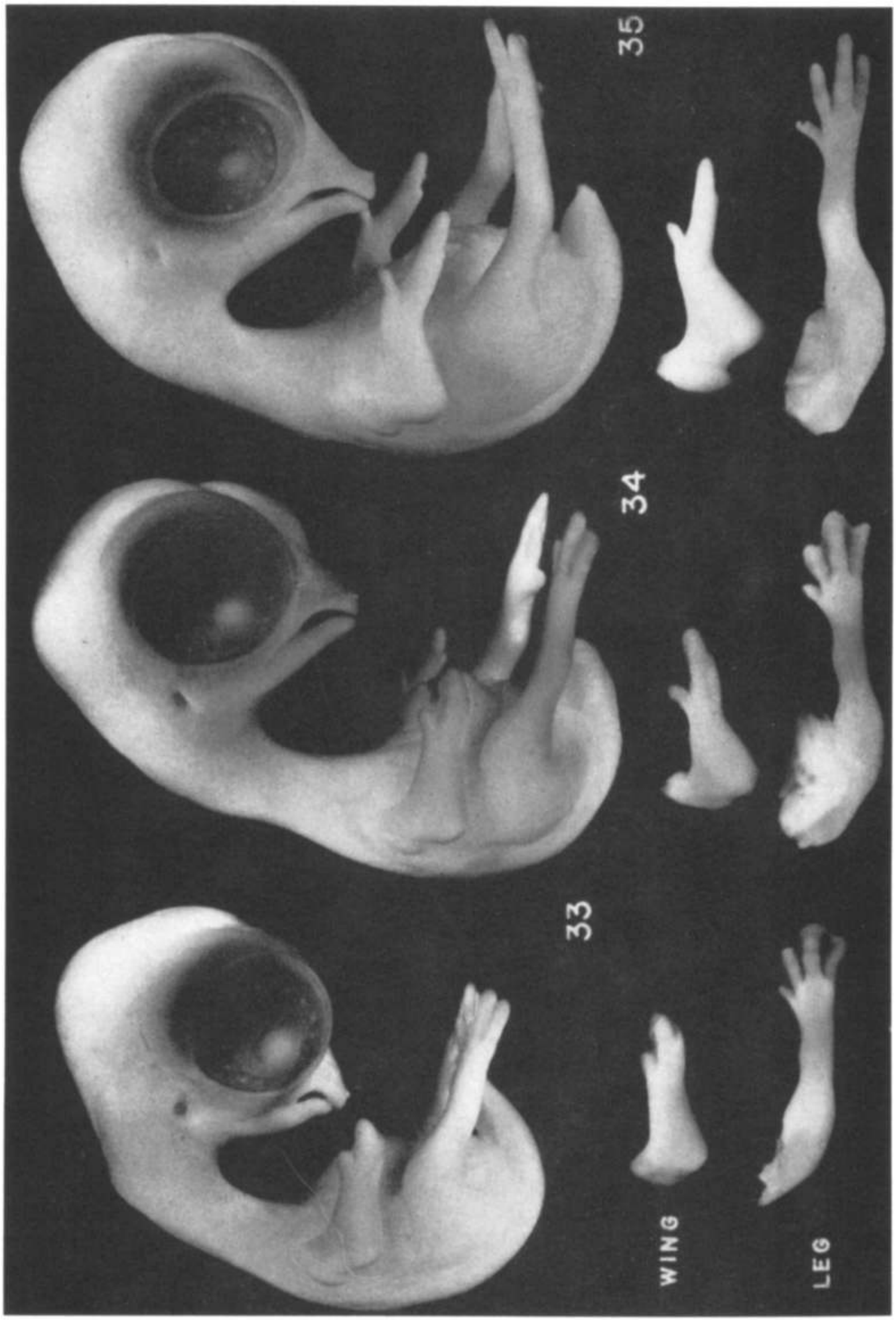
NORMAL STAGES OF THE CHICK PLATE 10
V. HAMBURGER AND H. L. HAMILTON

PLATE 11

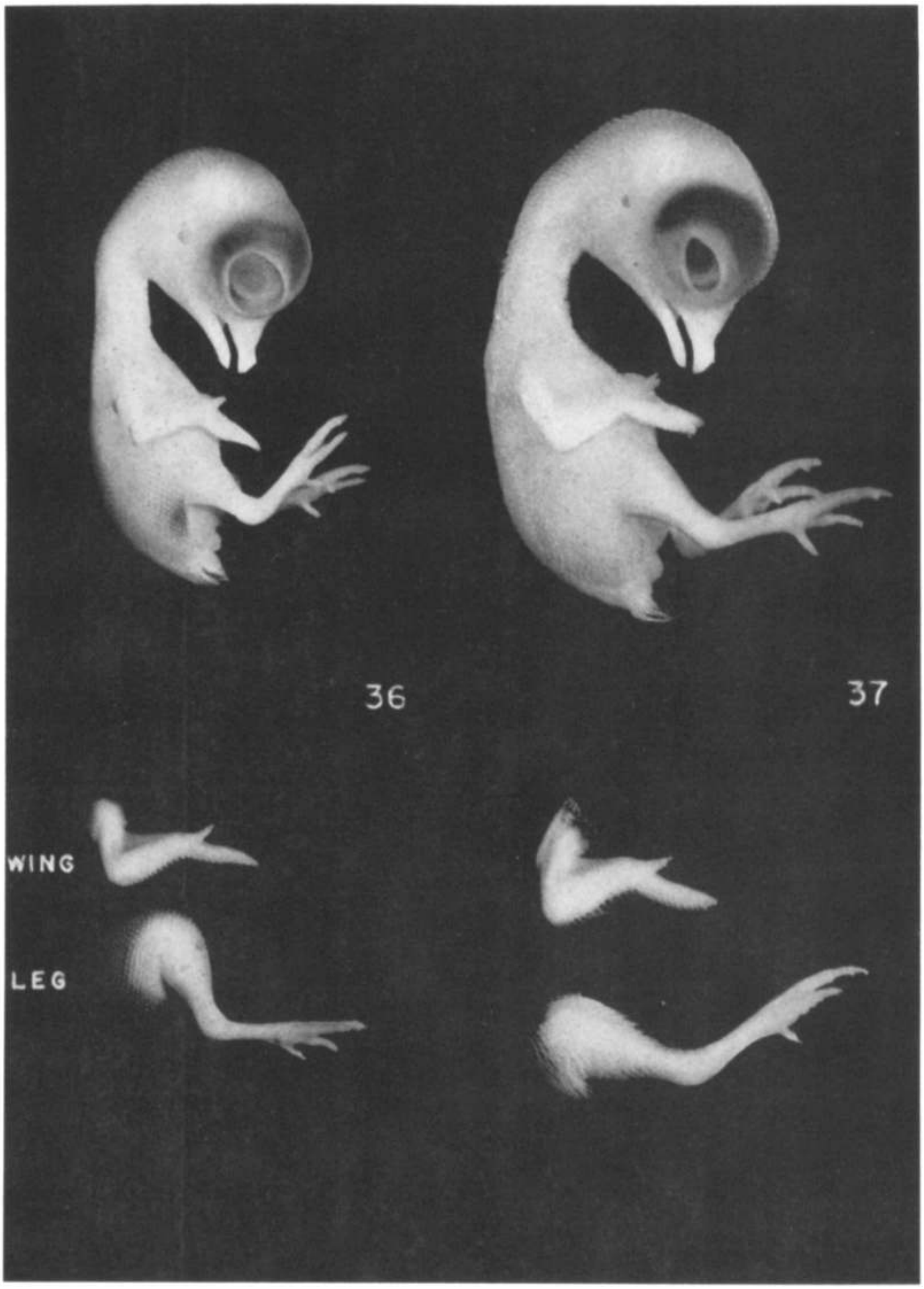
EXPLANATION OF FIGURES

Stages 33–35, $\times$ 4.

PLATE 11



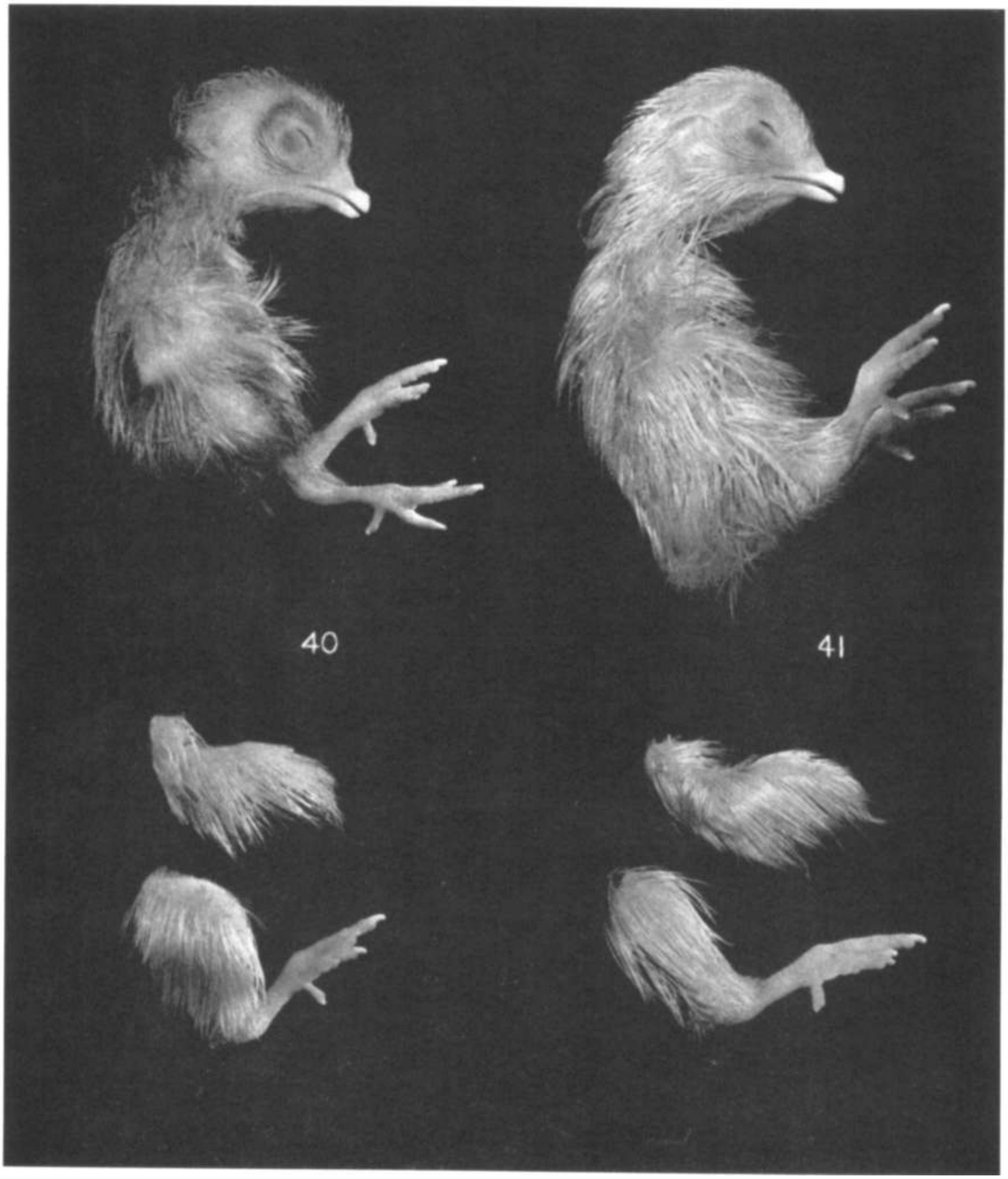
NORMAL STAGES OF THE CHICK
V. HAMBURGER AND H. L. HAMILTON

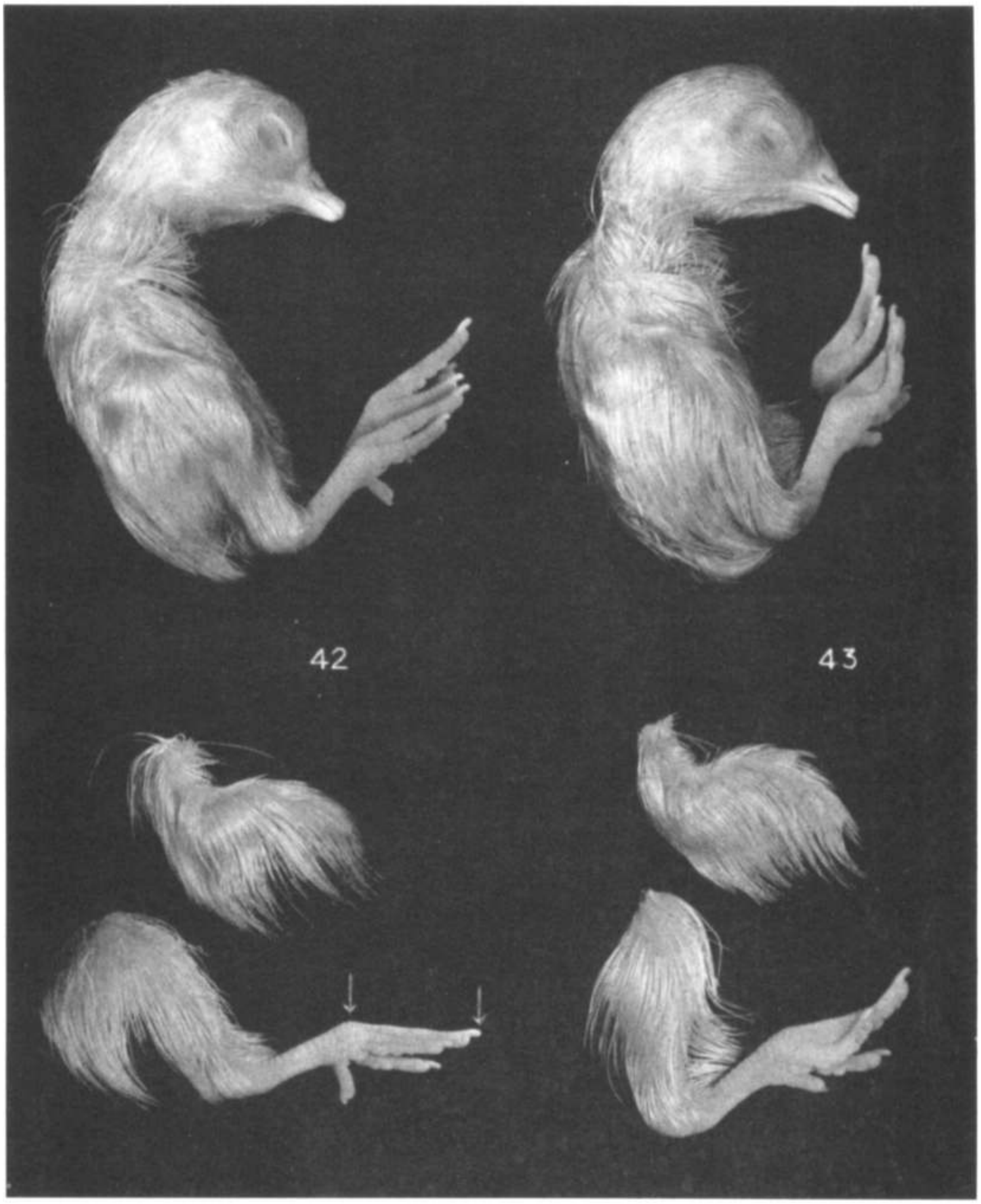

36
37
WING
LEG

Stages 36–39, × 2.

NORMAL STAGES OF THE CHICK
V. HAMBURGER AND H. L. HAMILTON

PLATE 13





Stages 40–43, × $1\frac{1}{8}$. The white arrows on the leg of stage 42 indicate the points between which measurements are made to determine the length of the third (longest) toe in stages 36–45.

NORMAL STAGES OF THE CHICK

PLATE 14

Stages 44–45, $\times 1\frac{1}{8}$.