Stages in the Normal Development of the Common Garter Snake, *Thamnophis sirtalis sirtalis*

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Multiple caesarian technique was used for obtaining embryos of different stages from individual females of *Thamnophis sirtalis sirtalis*. Ether and artificial respiration worked effectively.

Embryos were removed from 104 snakes and litter size averaged 12.9. Nine additional snakes were permitted to give birth naturally and had an average of 15.1 young.

The relative duration of the 37 pre-natal stages described is given.

Environmental temperature influences the rate of development. Embryos in four snakes held at 15°C passed from Stage 32 to Stage 33 in three weeks. In the same interval, embryos of three snakes held at 35°C moved from Stage 32 to Stage 37.

A SERIES of stages in terms of external anatomy is necessary if one is to study the normal development of a species adequately. The classic Keibel series of Normentafeln serves this function for a number of vertebrates, but only one reptile, *Lacerta,* is included among them. In recent years other such series have been described for species in common use by experimental embryologists, for instance, the stages of *Ambystoma* by Harrison (*in* Hamburger, 1942), and those of the chick by Hamburger and Hamilton (1951).

It is the purpose of this paper to define a series of stages for the development of the common garter snake, *Thamnophis sirtalis sirtalis.* A review of the literature shows that such a series does not exist. Ballowitz (1901) described gastrulation in *Natrix natrix,* and later his students Krull (1906) and Viefhaus (1907) added stages between neural fold formation and amnion closure. Ballowitz published part I of his great monograph on the development of the viper in 1903, but it only goes as far as the closure of the amnion, and part II was not completed before his death in 1936. Little has been published on older stages. Parker (1878) noted six stages of skull formation, and Berchelt (1936) described six other stages in the differentiation of the hemipenis. Franklin (1945) recorded the ages and lengths of 25 stages in the development of *Natrix taxispilota,* but these stages are not defined anatomically.

Few complete series of snake embryos are known to exist in research collections. It is felt that the relative ease of collecting garter snake embryos should be more widely appreciated and that the description of a series of stages in normal development will serve to encourage and coordinate the growth of knowledge about the embryology of this little studied group.

Materials and Methods

Specimens of the common eastern garter snake, *Thamnophis sirtalis sirtalis,* were obtained during early summer from amateur snake collectors in the Hanover, New Hampshire area. Pregnant females of this species are conspicuously larger than males, but sex can always be determined by probing for a hemipenis sac at the posterolateral edge of the cloaca. The probe finds no sac in the female, but readily enters the inverted hemipenis of the male.

Different stages of development were obtained from each female by performing caesarian sections (Clark, 1937) at one- to seven-day intervals under ether. The snake was usually anesthetized within fifteen minutes, and the position of the eggs could be felt by running a finger down her relaxed belly. An incision was then made at the posterior limit of the egg zone and a portion of the uterus was removed. The cut through the outer body wall was then sutured and the embryos were separated from the excised uterine tissue and fixed in Bouin's. All work was done under clean but not aseptic conditions.

When a snake was over-etherized, respiratory movements generally stopped. If breathing did not resume by the end of the operation, artificial respiration was applied at five- or ten-minute intervals by blowing air into the snake's lungs and then forcing it out by pressing on her inflated trunk. Breathing was usually normal within one or two hours and out of 242 operations, only 15 were fatal.

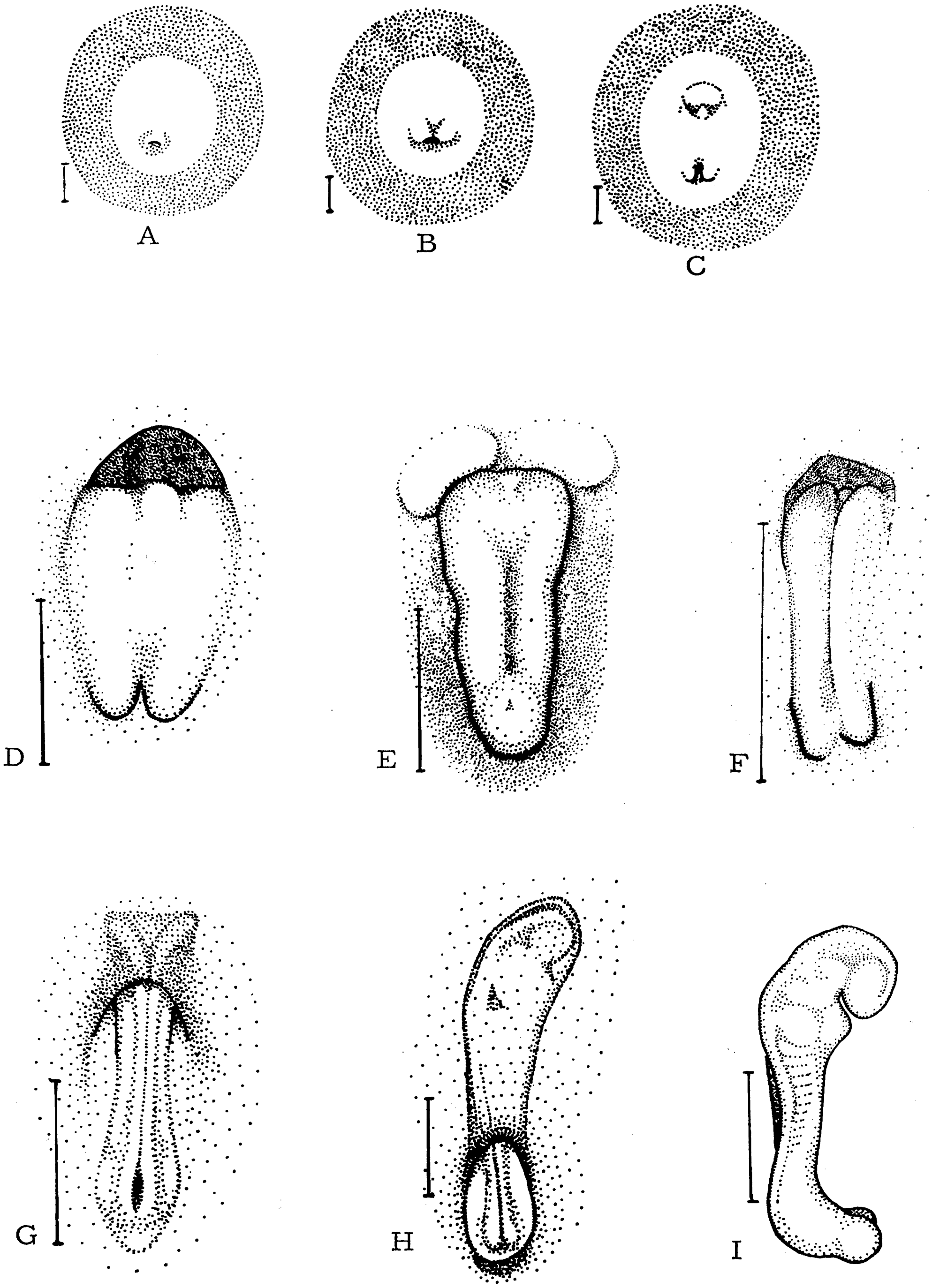

Fig. 1. Developmental stages of *Thamnophis sirtalis* (1). Bracket line for each drawing indicates 1 mm. A–C, early, middle and late Stage 7. D, Stage 8. E, Stage 9. F, Stage 10. G, Stage 11. H, Stage 13. I, Stage 15.

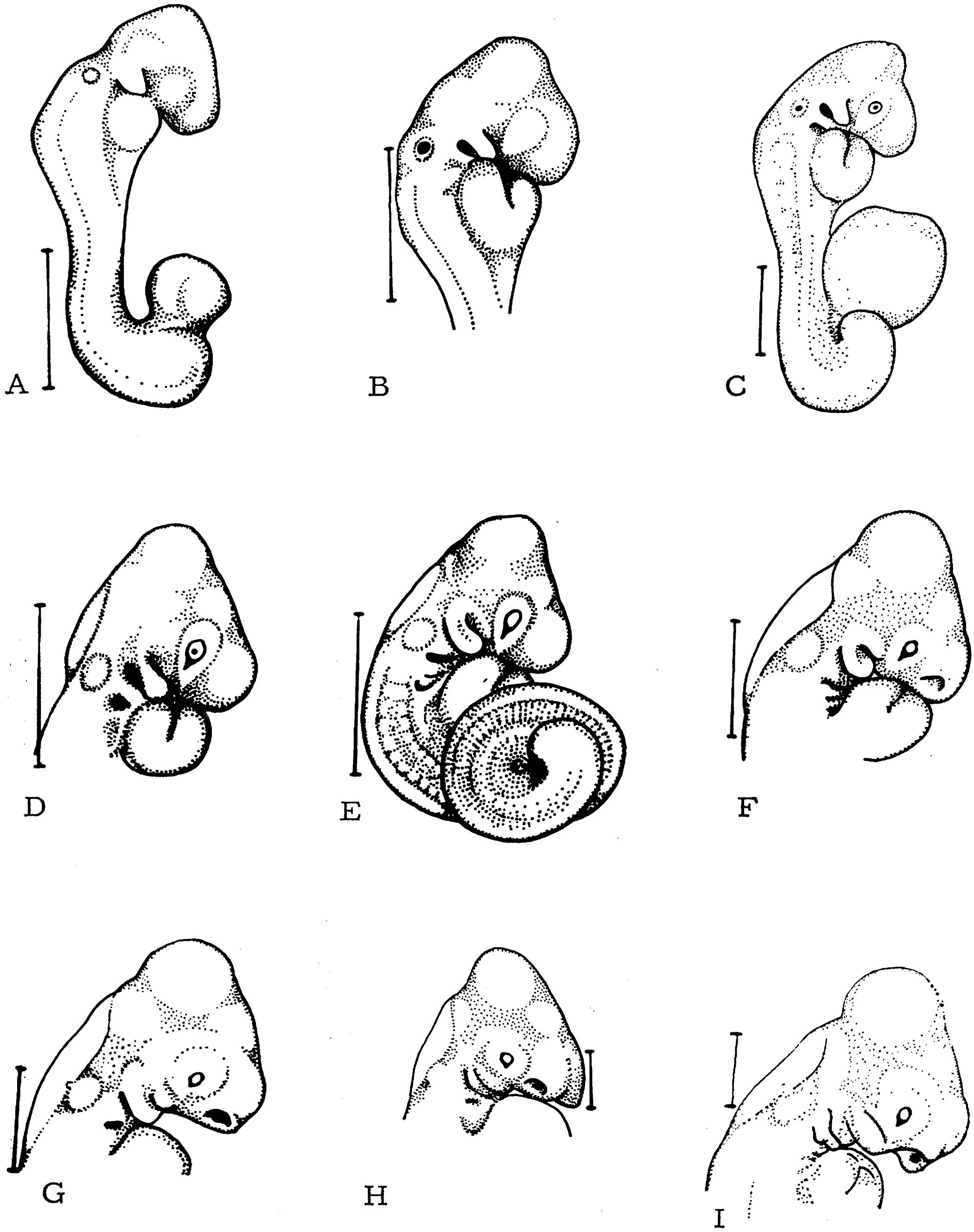

Fig. 2. Developmental stages of *Thamnophis sirtalis* (2). Bracket line for each drawing indicates 1 mm. A, Stage 16. B, Stage 17. C, Stage 18. D, Stage 19. E, Stage 20. F, Stage 21. G, Stage 22. H, Stage 23. I, Stage 25.

By using the multiple caesarian technique, 1,225 embryos were collected. Litter size averaged 12.9 embryos with a S. D. of 6.1. Nine additional snakes were permitted to give birth naturally and had from 9 to 27 young with an average of 15.1.

The stages presented in this paper were established by examining each embryo and noting the development of about twenty different structures. Similar specimens were then brought together and when any of these collections had embryos that were distinctly different, new characteristics were looked for that would make the description of stages more precise.

Definition of Stages in the Normal Development of the Common Garter Snake

(The stages are described in correct age sequence, but without designation of their actual ages from fertilization. See Correlation with Time, below.)

Stage 1 One cell.
Stage 2 Two cells.
Stage 3 Four cells.
Stage 4 Eight cells.
Stage 5 Small cell clump.
Stage 6 Blastodisc.
Stage 7 Gastrula (Fig. 1, A–C).
Stage 8 Early neurula—raised neural plate plus folds (Fig. 1D).
Stage 9 Middle neurula—brain folds flaring (Fig. 1E).
Stage 10 Late neurula—amniotic fold sharply raised anterior to head (Fig. 1F).
Stage 11 Amnion covers the anterior portion of the neural folds (Fig. 1G).
Stage 12 Amnion covers half the embryo; torsion of the head region is suggested.
Stage 13 Amnion encloses more than one-half the embryo; posterior amniotic fold is beginning to cover the tail bud; head lies on its left side (Fig. 1H).
Stage 14 Amnion encloses all but a small circular area.
Stage 15 Amnion closed; neither pharyngeal segments nor auditory pit visible; allantois bulge small, not inflated (Fig. 1I).
Stage 16 Mandibular segment visible; auditory pit visible; trunk coiled 180°; allantois beginning to inflate; anterior portion of neural folds still open (Fig. 2A).
Stage 17 Fourth ventricle inflated; lens not visible yet; hyomandibular cleft open; trunk coiled 270° (Fig. 2B).
Stage 18 Lens placode invaginating; auditory pit still open though only as pin-prick; allantoic sac inflated to size of head (Fig. 2C).
Stage 19 Auditory pit closed; maxillary process not visible; trunk coils—1½ (Fig. 2D).
Stage 20 Maxillary process visible as a mound of tissue posterior to ventral pole of eye; nasal pit not yet visible; auditory vesicle has formed but still lacks endolymphatic duct; choroid fissure prominent in eyeball; lens vesicle has closed; trunk coils—2 (Fig. 2E).
Stage 21 Nasal pit visible; endolymphatic duct not visible; fourth ventricle inflation extends posterior to ear; trunk coils—3¼ to 3¾ (Fig. 2F).
Stage 22 Endolymphatic duct visible; vomeronasal organ can be seen in nasal pit; no internasal depression; trunk coils—3½ to 3¾ (Fig. 2G).
Stage 23 Cerebral hemispheres expanded to the extent that an internasal depression is present; eyes lack pigmentation; cloacal mound can be seen at trunk coil level 3½ or 3 of larger specimens; trunk coils—3 to 4 (Fig. 2H).
Stage 24 (Except for eye pigmentation, resembles Stage 23.) Eye is lightly pigmented; maxillary process extends under eye as far as its middle; cloacal mound visible at the end of coil 3; trunk coils—3½ to 4.
Stage 25 Tip of maxillary process is now continuous with the lateral rim of the nasal depression; eye not yet surrounded by a mesenchyme bulge; trunk coils—3½ to 5 (Fig. 2I).
Stage 26 Eyeball margins stand out clearly from surrounding tissue; mesenchyme bulge surrounds eye; external naris not yet formed; lateral view of vomeronasal organ obscured by closing nasal fold; a trace of pharyngeal clefts remains as slight surface grooves; cloacal mound very distinct; anlagen of hemipenis visible; trunk coils—5½ to 6¼ (Fig. 3A).
Stage 27 Medial and lateral nasal processes have fused except anteriorly where a small pore remains as the external naris; internasal depression still present; hemipenes are distinct lateral mounds at trunk coil 3¼ to 3; trunk coils—5½ to 6½ (Fig. 3B).

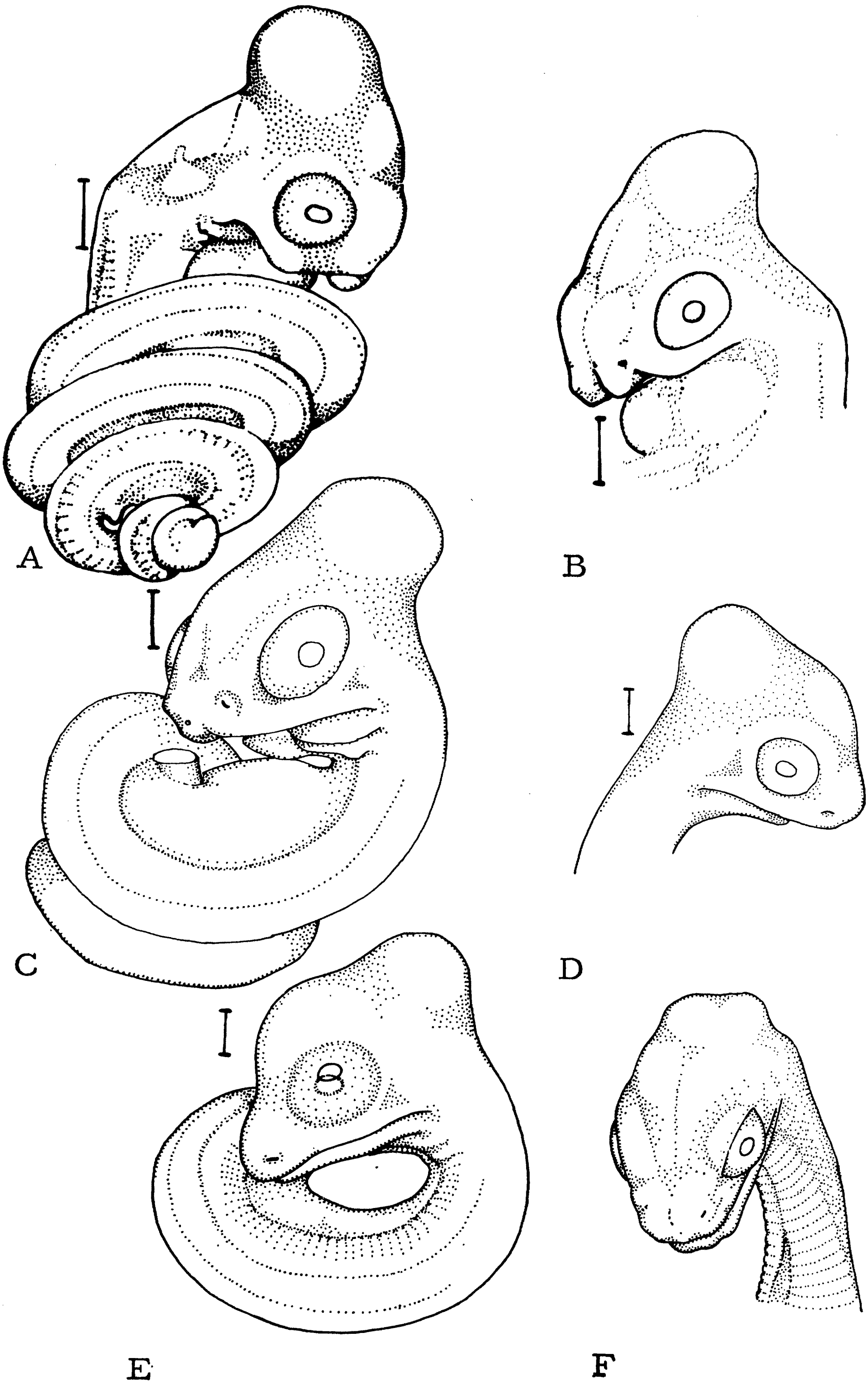

Fig. 3. Developmental stages of *Thamnophis sirtalis* (3). Bracket line for each drawing indicates 1 mm. A, Stage 26. B, Stage 27. C, Stage 28. D, Stage 30. E, Stage 31. F, late Stage 32.

Stage 28 Internasal depression now filled in; eyelid folds beginning to form; endolymphatic duct is easily seen, though the central mass of the vesicle is now indistinct; hemipenes are fingerlike projections located at trunk coil 2½ to 3; tongue not visible when mouth is pried open; trunk coils—5 to 6 (Fig. 3C).

Stage 29 Tongue visible; cervical flexure prominent; hemipenes are blunt projections on trunk coil 2 to 2½; trunk coils—4 to 5, loosely coiled.

Stage 30 Cervical flexure no longer prominent; scales not yet visible; hemipenes located at trunk coil 2; trunk coils—4, loosely coiled (Fig. 3D).

Stage 31 Scales are now visible on the trunk but not on the head; eyelid fold has spread over the eye, but has not quite closed; lateral flank muscles of the trunk are distinctly separated from the thin transparent ventral body wall; hemipenes inverted in some specimens (Fig. 3E).

Stage 32 Eyelid folds have completely covered the eyes; heart is still visible in lateral view through the thin ventral body wall (Figs. 3F, 4A).

Stage 33 In lateral view, the heart is no longer visible, but the lateral flanks have not met along the ventral midline in the heart region (Fig. 4B).

Stage 34 Lateral trunk muscle sheets have met along the ventral midline in at least the heart region; body pigmentation absent (Fig. 4C).

Stage 35 Body pigmentation visible but pattern is not well developed; scales are visible around the mouth and eyes; except for a short length near the umbilical cord, the trunk muscles have completely fused at the ventral midline.

Stage 36 Scales cover the entire head; outlines of the brain still visible; hemipenes still everted in some specimens; pigment pattern well developed (Fig. 4D).

Stage 37 Brain can no longer be seen through cranial scales due to dense pigmentation; pigment pattern

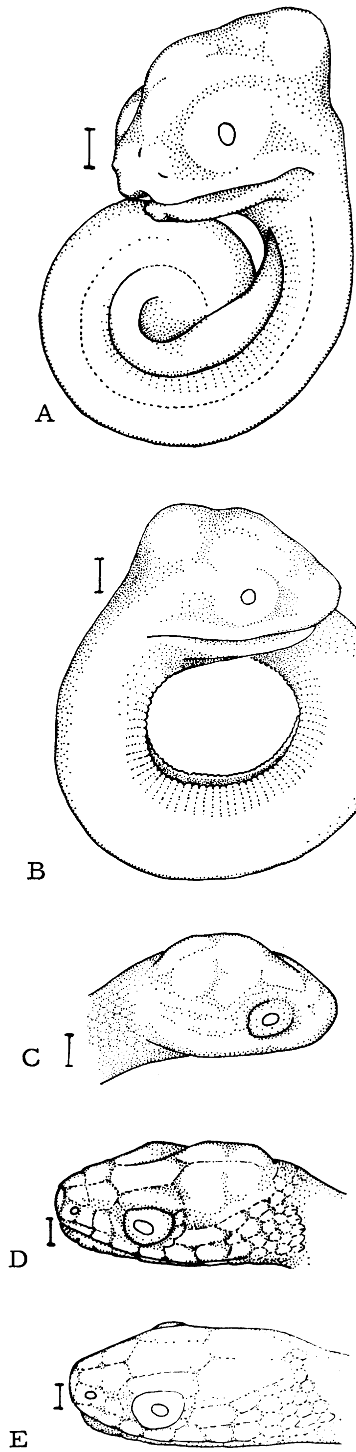

Fig. 4. Developmental stages of *Thamnophis sirtalis* (4). Bracket line for each drawing indicates 1 mm. A, Stage 32. B, Stage 33. C, Stage 34. D, Stage 36. E, Stage 37.

TABLE 1. REPRESENTATIVE SAMPLE OF STAGES FOUND AT SUCCESSIVE CAESARIAN SECTIONS ARRANGED SO THE RELATIVE DURATION OF STAGES MAY BE SEEN. SNAKES SUMMARIZED ABOVE WERE OPERATED ON SOON AFTER CAPTURE AND THEN CAGED IN THE LABORATORY AT TEMPERATURES BETWEEN 23 AND 27°C

Snake 2	61-4	8	J	61-1	Z-1	Z-3	61-20	61-7	Z-10	Z-11	61-22	61-13
Date of First Operation												
8 June 1948	10 June 1961	23 June 1948	28 June 1947	8 June 1961	13 June 1959	14 June 1959	29 June 1961	24 June 1961	22 June 1959	22 June 1959	6 July 1961	26 June 1961
Stages found at successive operations (x represents a day between operations)												
6												
x												
x												
x	7	7										
7	x	7										
9	x	x										
	x	10										
	x	13										
	x	15	16									
	x	17	17									
	x		19									
	20		21	20								
	x		21	x	22							
	x			x	x	22						
	x			x	x	x						
	x			x	23	x						
	x			x	23	24	23					
	25			x	24	25	x	25	25			
	x			x		25	x	x	25			
	x			26			x	x	25	26		
	x			x			x	x		27		
	x			x			x	x		27		
	x			x			x	x		27		
	x			x			28	x				
	28			x			x	28				
	x			x			x	x			29	
	x			x			x	x			x	
	x			29			x	x			x	
	x			x			x	x			x	
	x			x			x	x			29	29
	x			x			30	x			x	x
	x			x			x	31			x	x
	31			x			x				x	x
	x			x			x				32	x
	x			32			x				x	x
	x			x			x				x	x
	x			x			x				x	32
	x			x			32				33	x
	33			x							x	x
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fully developed; hemipenes inverted in all specimens. (Stage 37 resembles the newly hatched snake. Average length of 70 snakes from four mothers at birth was 159.8 mm with a S. D. of 14 mm.) (Fig. 4E)

Correlation of Stages with Time

The date of fertilization and, consequently, absolute age are not known; however, the relative duration of stages may be determined by studying the embryos removed from one female at successive operations. Several females that readily demonstrate this are summarized in Table 1. A more precise correlation of stages with time is not possible at this time since embryos were never obtained at intervals less than one day.

The calendar date on which a particular stage was found varied greatly. For example, Stage 7, gastrulation, was collected as early as 4 June, and as late as 3 July; Stage 29 was obtained as early as 15 June, and as late as 8 July. Normal birth of litters was recorded as early as 2 August, and as late as 26 August. The effect of environmental temperature on the rate of development will be discussed later.

Discussion

The multiple caesarian technique is easy to use, but past investigators have had trouble with anesthesia. Franklin (1945) and Clark (1937) advised against ether, but its use is justified by the speed of recovery when artificial respiration is applied. There is no evidence that ether anesthesia, or successive operations, interfere with the development of the embryos that remain in the mother.

The effect of temperature on the rate of development may account for variations in the gestation period. To demonstrate this, seven snakes found to contain Stage 32 embyros were divided into two groups; three were incubated at 35°C and four at 15°C, and their embryos were removed at successive intervals. At the end of three weeks, the embryos at 35° had reached Stage 37 while those at 15° had only reached Stage 33.

Though temperature clearly affects the rate of development, it is not known how great this factor might be in nature. In experiments now under way, snakes move toward warmer areas when given choices of temperatures between 0° and 40°C. If this behavior takes place in the wild, a snake may be able to maintain a fairly constant gestation period by heating up during warm weather so that the retarding effects of cold periods are not noticed in the over-all rate of development.

Although the data presented in this paper on the relative duration of stages were not gathered under controlled temperatures, they should stand as a rough guide until a more careful study is done. Regardless of variations that may be found, the existence of stages will greatly facilitate future work on this little studied creature.

Acknowledgments.—This study was started and completed under the direction of Dr. W. W. Ballard of Dartmouth College. Without his help, this paper would not have been written.

Literature Cited

Ballowitz, E. 1901. Die Gastrulation bei der Ringelnatter (*Tropidonotus natrix* Boie) bis zum auftreten der Falterform der Embryonalanlage. *Zeit. Wiss. Zool. 70:675–732.*

———. 1903. Die Entwickelungsgeschichte der Kreuzotter (*Pelias berus* Merr.). Teil I. Die Entwicklung vom Auftreten der ersten Furche bis zum Schlusse des Amnios. *Fischer, Jena.*

Berchelt, Hans. 1936. Bau, Funktion und Entwicklung der Begattungsorgane der männlichen Ringelnatter (*Natrix natrix* L.) und Kreutzotter (*Vipera berus* L.). *Morph. Jahr. 78:445–516.*

Clark, Hugh. 1937. Embryonic series in snakes. *Science 85:569–70.*

Franklin, Malcolm. 1945. The embryonic appearance of centers of ossification in the bones of snakes. *Copeia 1945:68–72.*

Hamburger, Viktor. 1942. *A Manual of Experimental Embryology. Univ. of Chicago Press xviii + 213 pp.*

——— and H. L. Hamilton. 1951. A series of normal stages in the development of the chick embryo. *J. Morph. 88:49–92.*

Keibel, Peter. 1904. Normentafel zur Entwicklungsgeschichte der Zauneidechse (*Lacerta agilis*). *Keibels Normentafeln, Heft 4, Jena.*

Krull, Joseph. 1906. Die Entwicklung der Ringelnatter (*Tropidonotus natrix* Boie) vom ersten Aufstreten des Proamnios bis zum Schlusse des Amnios. *Zeit. Wiss. Zool. 85: 107–55.*

Parker, W. K. 1878. On the structure and development of the skull in the common snake (*Tropidonotus natrix*). *Phil. Trans. Royal Soc. London 169:385–417.*

Viefhaus, Theodor. Die Entwicklung der Ringelnatter (*Tropidonotus natrix* Boie) nach Ausbildung der Falterform bis zur Erhebung des Proamnios (mit Taf. IV–VI u. 8 im Text). *Zeit. Wiss. Zool. 86:55–99.*

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