

Early Behavior of Embryos of the Turtle, *Terrapene carolina* (L).

HIDEOMI TUGE. (Introduced by G. E. Coghill.)

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Male and female specimens of *Terrapene carolina* (L) were collected early in the breeding season and kept in pens. The times of egg-laying were observed, and the eggs were removed from the nest within a few hours after they were laid. Each clutch of eggs was planted separately and records kept of the time of laying. From these eggs 40 living embryos were procured, upon the basis of which this report is made.

It was found that the age of the embryo as calculated from the time of egg-laying could not be depended on as an index of the degree of development. Embryos of the same clutch in some cases differed considerably in this respect; and great difference appeared between clutches of the same age. Obviously eggs develop in the oviduct of this species for various periods. It was largely a matter of chance, therefore, in selecting eggs for study with reference to particular phases of development; although an embryoscope improvised upon a 6-volt, 108-watt, ribbon filament Mazda lamp was of considerable assistance in approximating the age desired.

Spontaneous movements occur, apparently for at least several hours, before movement can be elicited in response to touch. Embryos with carapace-length of 6 mm. respond to touch on the snout, but not to touch on other parts, except possibly on the shoulder on very rare occasions (probably spontaneous movement in these instances). The movements at this time always involve the head and neck, and usually, also, the trunk, tail, and fore and hind limbs. The limbs never move in this phase of development without movement of the trunk. These reactions are total reactions, inasmuch as a more caudal or distal part does not move without movement of the more anterior or proximal part. Since the limbs are at this time insensitive to touch there are no limb reflexes. In embryos with carapace-length of 7 to 7.5 mm., movements of the limbs, (fore and hind) occur in response to touch on the limbs, without perceptible movement of the trunk. These movements are local reflexes. While there were suggestions of total reactions only in response to touch on the appendages in some embryos, this pattern has not yet been definitely established as representing a definite

phase in the development of the behavior pattern. But it is demonstrated that in the turtle the limbs move as a part of a total behavior pattern before they move discretely in response to local stimulation. This is of particular interest because in the adult of this species the trunk, being anchored to the carapace, is not an active factor in locomotion, which is effected wholly by the appendages.

The specimens were photographed and preserved for correlated anatomical study.

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Reticulocytes.

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In an effort to adapt the reticulocyte stain for use with oxalated venous blood and incorporate it in our uniform system of hematologic methods,¹ 7 methods of reticulocyte staining in current use were tried. None proved wholly satisfactory. Experiment showed that increasing the time of exposure or increasing the concentration of the dye increased the number of reticulocytes found. It was also found that the presence of 2 mg. of potassium oxalate per cc. of blood did not alter the reticulocyte count.

The following procedure was found to give uniformly good stains with the maximum number of reticulocytes in a given blood:

Mix equal parts (5 drops) of oxalated venous blood¹ (or fresh blood) and 1% brilliant cresyl blue in 0.85% NaCl solution in a small test tube and allow to stand one minute or more. Mix and make a thin smear. This may be counted when dry, or counterstained with Wright's stain. Count all the red cells (preferably with a hand tally) in an oil immersion field and then count all the reticulocytes in that field. Move to an adjacent field and repeat until 1000 red cells have been counted. If the count is more than 5%, only 500 cells need be counted. The counterstain is necessary if the slide is to be kept for more than 48 hours.

This method has many advantages. It is very simple and convenient and consistently gives a higher reticulocyte count than the

¹ Osgood, E. E., Haskins, H. D., and Trotman, F. E., *J. Lab. and Clin. Med.*, 1931, **16**, 476.