

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.

other methods tried. The stain keeps indefinitely and need not be filtered before using. The oxalated blood may stand for as long as 48 hours before the count is made. Overstaining does not occur even though the smears are not made until 2 hours after the stain and blood are mixed. The reticulocytes are clearly and deeply stained and the red cells are neither crenated nor distorted. The smears keep indefinitely if counterstained with Wright's stain.

A reticulocyte stain on oxalated blood which had stood for 144 hours and was badly hemolyzed showed the same percentage of reticulocytes as did the fresh blood. This suggested to us that perhaps this is not a vital stain as is generally stated, but is merely a dye which will not stain fixed cells. To investigate this point further, equal parts of blood and 1% sodium cyanide solution were mixed and allowed to stand from 20 minutes to 1 hour and reticulocyte counts were done. These gave practically the same results as counts on the original blood. Cells fixed by various methods failed to show any reticulocytes.

Normal values for reticulocytes will have to be established by this method. Preliminary studies suggest that bloods of healthy adults will show about 2% reticulocytes.

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The Phosphatase Content of Fractured Bone.*

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In a recent paper Kay¹ states that he observed an increase in plasma phosphatase following fracture, and indirectly he suggests a similar rise in bone phosphatase. In view of our findings on the breaking strength of fractured fibulae of rats,² we were of the opinion that a positive correlation existed between the breaking strength and bone phosphatase. This we sought to demonstrate. It was also believed that if the activity of phosphatase in the healing process of fractures is antagonized, after the primary callus has formed on the fifteenth

*The expenses of this investigation were defrayed by Davis and Geck, Inc.

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¹ Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 249.

² McKeown, R. M., Lindsay, M. K., Harvey, S. C., and Lumsden, R. W., *Arch. Surg.*, in press.