and size of the images of the virus particles is not clear. There is the possibility that the calcium salt may have entered into a loose combination with the peripheral substance, which, by this means, became sufficiently dense for demonstration. The apparently greater size of the particles represented in Fig. 2, however, was not paralleled by change in the sedimentation rate which was not altered by treatment for 1 hr with $CaCl_2$. It is unlikely that the change was due wholly to drying effects occurring at the particle surface while on the film, since the appearance of the image was altered only when the virus was in contact with the $CaCl_2$ for some time in solution.

The present results indicate that the Eastern strain equine encephalomyelitis virus is probably spherical in shape, constituted peripherally of a substance the limits of which were ill-defined in the micrographs in the absence of special treatment. The average diameter of the particles of vague outline of Fig. 1 was 40.2 mμ. If the true particle limits are those portrayed after treatment with $CaCl_2$, the diameter was 47.5 mμ.

13963

Further Experiments Dealing with Embryonic Enucleation in Amblystoma.

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In a previous communication[1] we described the results of embryonic enucleation in *Amblystoma punctatum* upon growth, metamorphosis and pigmentary changes. Our results upon growth did not conform with those of Browman[2] who found that rats enucleated at birth, regardless of environment, had a growth rate consistently lower than their litter mate controls of the same sex.

Browman[3] found that rats enucleated at birth suffered retarded development of the genital tracts and the gonads. We investigated this matter in *Amblystoma punctatum,* but could find no evidence at metamorphosis because of insufficient development of the gonads. Because of the difficulties of raising *A. punctatum* through metamorphosis, we have repeated the work on *A. tigrinum* and *A. mexicanum* (axolotl).

Twenty embryos (tail bud stage) of *A. tigrinum* were enucleated. Ten eyeless and 5 normal controls were reared in darkness, and 10 eyeless with 5 controls were kept under ordinary daylight illumination. Ten unilateral and 10 bilateral enucleations were done on axolotl embryos (tail bud stage). These along with 10 normal embryos were reared in aquaria under normal conditions of illumination.

In *A. tigrinum* we dissected the gonads of 5 females without eyes and 2 with eyes which had been raised in the dark; also 3 males without eyes and 3 with eyes reared in the light. The animals studied ranged from 109 to 170 days of age. All had metamorphosed except one. We found considerable variation in the size of the ovaries and the testes irrespective of the presence or absence of eyes or the conditions of illumination. Although the gonads were well differentiated, they had not reached maturity in either the experimental or the control animals. Because of our failure to obtain evidence of any effects of eyelessness upon gonadal development in *A. tigrinum* up to 170 days of age, we reared the axolotls *(A. mexicanum)* for two years. At the end of this period, we dissected 2 control and 5 eyeless axolotls and found no significant size difference between the gonads of the two groups.

[1] Detwiler, S. R., and Copenhaver, W. M., *Anat. Rec.*, 1940, **76**, 241.

[2] Browman, L. G., *Anat. Rec.*, 1938, **72**, Suppl. 41.

[3] Browman, L. G., *Anat. Rec.*, 1938, **72**, Suppl. 122.

Our observations showed that there was no difference in the growth rate between eyeless and normal forms in either species studied. Throughout the entire period, eyeless axolotls, reared in the light, were pale; normals were decidedly dark. The one-eyed axolotls, with respect to color changes, behaved like the normal 2-eyed animals. This situation agrees with the findings of Parker[4] on Fundulus and is in contrast with his findings on Ameiurus.

Pale eyeless axolotls 9 months old when injected with intermedin,* became dark but never as dark as the normal 2-eyed controls. The effect wore off gradually and at the end of a week the original light color was restored.

Removal of the second eye from a one-eyed axolotl at 9 months, caused the animal to become pale, but not as pale as those rendered eyeless in the embryonic stage. Enucleation of a 12-year-old axolotl had no effect upon coloration. The animal remained in a permanently dark state.

Summary. Embryonic enucleation of *A. punctatum, A. tigrinum* and *A. mexicanum* (axolotl) has no significant effect upon the growth rate. The influence of the eyes upon color changes and possibly upon metamorphosis seems not to be related to any eye substance (hormone) developed within the eye itself.[1] No effect of embryonic enucleation upon the development of the gonads could be found in *A. tigrinum* up to 170 days after the embryonic stage nor in axolotls two years of age.

4 Parker, G. H., *Proc. Nat. Acad. Sci.*, 1939, **10**, 499.

* The preparation which we used was made from the anterior lobe of the whale, and was kindly supplied by Dr. Geiling of the University of Chicago.

13964

Serial Bone Marrow Studies in Pernicious Anemia. I. Fluctuation in Number and Volume of Nucleated Cells.

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A number of authors[1-14] who studied the bone marrow at intervals after the beginning of liver-induced remission emphasized the rapid disappearance of megaloblasts and the appearance of normoblasts. These authors obtained by biopsy[1,2,7] or aspiration technic[3-5, 7-14] specimens from patients with pernicious anemia before liver treatment and one or two specimens at varying intervals during liver therapy. Some authors aspirated the same patient 2 or 3 times at different intervals during liver therapy,[5,12,14] or in groups of from 4 to 10 patients, performing the aspiration in different phases of their re-

1 Zadek, I., *Klin. Wchnschr.*, 1924, **3**, 1483.

2 Peabody, F. W., *Am. J. Path.*, 1927, **3**, 179.

3 Tempka, T., and Braun, B., *Folia Haemat.*, 1932, **48**, 355.

4 Segerdahl, E., *Acta Med. Scand.*, Suppl., 1935, **64**, 1.

5 Nordenson, N. G., *Studies on Bone Marrow from Sternal Puncture*, Stockholm, Börtzells, Esselte, 1935.

6 Isaacs, R., *Am. J. Med. Sc.*, 1937, **193**, 181.

7 Dameshek, W., and Valentine, E. H., *Arch. Path.*, 1937, **23**, 159.

8 Storti, E., *Hematologica I Archivio*, 1937, **18**, 1.

9 Schartum-Hansen, H., *Folia Haemat.*, 1937, **58**, 145.

10 Koller, F., *Deutsches Arch. f. Klin. Med.*, 1939, **184**, 568.

11 Boek, H. E., and Malamos, B., *Folia Haemat.*, 1939, **62**, 408.

12 Rohr, K., *Das menschliche Knochenmark*, Georg Thieme, Leipzig, 1940.

13 Houghton, B. C., and Doan, C. A., *Am. J. Clin. Path.*, 1941, **11**, 144.

14 Davidson, L. S. P., Davis, L. J., and Innes, J., *Quart. J. Med.*, 1942, **11**, 19.