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Return of Vision and Other Observations in Transplanted Amphibian Eyes.

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Return of vision and various other observations were reported by Stone and Ussher¹ on replanted eyes in *Amblystoma punctatum* larvae. The present paper gives a report of similar observations based on 126 transplanted larval eyes in the same species. The methods of technique in operation were similar to those employed by the above mentioned authors. In nearly all cases right eyes were exchanged between pairs of larvae 22 mm. in length. Animals were killed at intervals of a few days from the time of operation until long after metamorphosis. Observations obtained of eyes in the animals while living have been correlated with those obtained from a microscopic examination in 62 cases.

Very little hemorrhage was encountered. Resorption of the eye, when it took place, could usually be correlated with known injuries to the bulb at the time of operation, or to prolonged use of anesthesia, or to too strong anesthesia. Slight reduction in the size of the transplanted eye was noted in most cases after operation, but usually the normal size appeared to be regained within 5 weeks. In fact the general macroscopic appearance of the transplanted eyes was so similar to that of the normal eyes in most cases that it was often difficult to differentiate between them. Shortly after operation the pupil of the transplanted eye showed some dilatation, but later approached normal size.

Return of circulation was first noted within the superficial vessels of the iris as early as 24 hours after operation in one case. The average time, however, was about the 4th day. Return of some ocu-

¹ Stone, L. S., and Ussher, N. T., *PROC. SOC. EXP. BIOL. AND MED.*, 1927, **xxv**, 213.

ar movement was observed as early as 7 days after operation, but in most cases this occurred much later. Pupillary and corneal reactions did not occur in any transplanted eyes until the animals had begun metamorphosis. This is always true of normal *Ambystoma punctatum* eyes.

To test for return of vision in the transplanted eyes care was taken to eliminate olfactory, gustatory, tactile and mechanical disturbances such as jarring or touching the aquarium. The lure for eliciting visual response was a piece of red rubber impaled on a wire and moved about a tightly covered glass aquarium. The normal eye was excised in animals so tested. If the animal snapped at the lure and followed it about the bowl on several successive days, when tested by disinterested observers as well as the author, vision was considered present. Vision was not considered to have returned if the animal never snapped at the lure after trials made over a period of several weeks. Vision was found to have returned in one case, a larva, as early as 48 days after operation. Many tested after metamorphosis showed visual response through the transplanted eye. In some animals return of vision was not tested, for it was necessary to preserve the normal eye in a sufficient number of cases to supply controls for histological studies. For comparison, controls were used, *viz.*, animals (a) with 2 normal eyes present, (b) with 2 eyes extirpated, and (c) with 1 normal eye present and 1 extirpated.

Microscopic studies rarely revealed any changes in the lens. The ganglion-cell layer of the retina showed a single row of cells—a contrast to the double row of these cells in normal eyes. Occasionally there were small patches devoid of cells and in rare instances small areas contained a second row of pale-staining vesicular cells. This irregularity in the ganglion-cell layer was not noticed in the larval eyes before the first month after operation and it was not a constant finding until well after the second month. In a few cases of adult animals there were noted undulations and irregularities in contour involving the entire retina—a condition not observed in normal eyes. The stratum opticum and the rod and cone layers of the transplanted eyes in both adult and larval stages seemed to reveal no striking deviation from that of the normal eye. The optic nerve, in nearly all cases, showed an enlargement at its exit from the bulb where the severed ends of the nerve had come together. Proliferation of cells at the cut ends of the nerve could be seen as early as the 4th day after operation. After the 25th day, in nearly all of the transplanted larval eyes, the enlargement was present and the nerve itself extended to the chiasma and brain without a break in its continuity.

In a majority of cases studied that portion of the optic nerve extending from the enlargement to the brain seemed to be about normal in size, but tortuous and irregular. In no case after metamorphosis was the optic nerve lacking.

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Effect of Drying on Certain of the Virus Group.*

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It has long been known that certain of the virus group such as vaccine and rabies virus will withstand drying but as a rule there is considerable loss in virulence during the process. Murphy¹ showed that the chicken tumor agent was less damaged if the material was kept frozen during desiccation. Harris and Shackell² demonstrated that rabies virus frozen and dried lost little of its virulence and, furthermore, the deterioration was less rapid than when the tissues were dried by the usual methods.

The present study deals with the resistance of vaccine virus, virus III and *Herpes febrilis* virus to freezing and desiccation.†

The various tissues containing the viruses were mixed finely and spread in a thin layer in a sterile dish. This was placed in a vacuum jar over concentrated sulphuric acid and the pressure reduced to 5.0 mm. of mercury. The jar was immediately put in a freezing room at a temperature of -5.0° C. and left for 2 days. The tissues, thoroughly desiccated by this time, were removed, rubbed to a powder in a mortar and sifted through a fine meshed sieve to remove the larger particles. For the infectivity test the powder was taken up in Locke's solution and injected in the usual manner for the individual virus.

Vaccine virus, after the above treatment, when tested dermally and intradermally in normal rabbits, showed that the activity of the virus was practically unimpaired. The lesions produced arose as

* This investigation was carried out by means of funds from the Rutherford Donation.

¹ Murphy, James B., *J. Exp. Med.*, 1911, *xiii*, 889.

² Harris, D. L., and Shackell, L. F., *J. Am. Pub. Health Assn.*, 1911, *i*, 52.

† We wish to acknowledge the assistance of Dr. Thomas Rivers, who supplied the viruses used and tested their infectivity after desiccation.