The characteristic cytopathic effect first appeared in the 2nd passage and became more rapid and destructive with increasing passage. With undiluted inoculum of adapted virus, the lesions were widespread by the third day and consisted of granular rounding up and fragmentation of the cells (Fig. 1). The cells remained adherent to the glass, but eventually many were shed into the supernatant fluid. There was no evidence of formation of giant cells or syncytia. With smaller virus inocula, the lesions appeared later, were focal, and remained localized for as long as 2 weeks after initial appearance. These focal lesions could be counted with the unaided eye and the number was proportionate to the virus input. Under agar overlay, minute plaques, 0.5 to 1.0 mm in diameter, were present, which were best counted under oblique light, where they appeared as gray-blue opalescent centers.

The titer at the 10th and 20th passages was determined by the appearance of lesions on the chorioallantoic membrane of the chick embryo and also by focal lesion count in the fibroblast monolayer under fluid medium and by use of the agar overlay technique (Table 1). The three methods showed essentially similar titers.

The identity of the agent responsible for the cytopathic effect was determined after 10 and 20 passages in tissue culture. When the tissue culture virus was back passaged on the chorioallantoic membrane, typical punctate and linear lesions were observed as described previously for the Onderstepoort strain (*5*). The chorioallantoic membrane and tissue culture lesions were inhibited by antiserum obtained from a dog following experimental infection with the virulent Snyder Hill strain of CDV, and were not inhibited by the preinfection serum (*4*). Neutralization of chorioallantoic membrane and tissue culture lesions was also demonstrated by using chicken hyperimmune serum that had been prepared from the original parent chick-embryo-adapted Onderstepoort strain.

Rockborn (*6*) reported the growth of a virulent "street" strain of CDV in dog kidney tissue culture with production of multinucleated giant cells, similar to that seen with measles virus. The difference in cytopathology of CDV in dog kidney and chick embryo as described above is notable.

An egg-adapted CDV strain (Lederle) has recently been reported to multiply without cytopathic effect in chick embryo tissue culture (7). The tissue-culture-adapted Lederle strain (35th passage) (*4*) readily produced typical cytopathic effect in chick tissue culture prepared as described above, but failed to cause cytopathic effect in cultures grown in Eagle's solution containing 10 percent horse serum and maintained in mixture 199, as described by Cabasso (*7*). Tissue-culture-adapted Onderstepoort strain, however, was rapidly cytopathic in cells handled with either technique. The above results indicate that differences in both virus strain and cultural conditions may be important in the manifestation of tissue culture cytopathology by CDV.

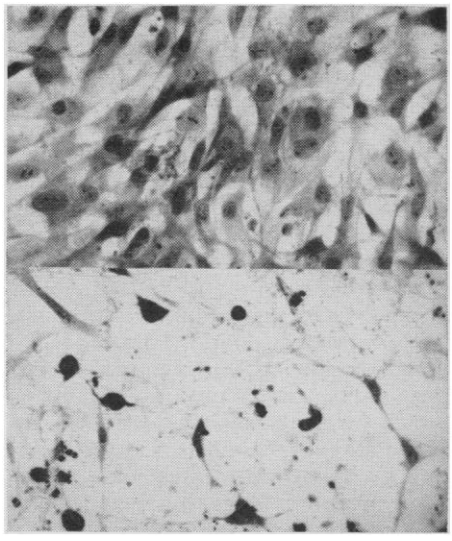

Fig. 1. (Top) Normal chick fibroblast monolayer tissue culture, day 5, hematoxylin and eosin stain; (bottom) same, infected on day 0 with canine distemper virus (× 285).

The adaptation of CDV to chick fibroblast culture with the rapid formation of specific lesions provides a tool for the further development of quantitative methods of virus titration and serologic study (*8*).

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Evidence That Cut Optic Nerve Fibers in a Frog Regenerate to Their Proper Places in the Tectum

Abstract. The frog's retina projects into the superficial neuropil of the opposite tectum in four functionally different layers of terminals. Each layer displays a continuous map of the retina in terms of its particular function. The four maps are in register. The fourth-dimensional order is reconstituted after section and regeneration of the optic fibers.

Sperry (*1*) pointed out that the results of his experiments on optic nerve regeneration in adult frogs were consistent with specific reconnection of the optic fibers. He proposed that each individual neuron grew back to its original terminus in the tectum, for the behavior after visual recovery was as if the nerve had not been cut. In addition to the behavioral evidence, he produced scotomata in predicted quadrants by fairly large tectal lesions in frogs that had regrown their optic connections. The implications of his proposal are so odd that, while his elegant experiments were accepted, the interpretation was much disputed. Furthermore, the experiments with tectal lesions cannot be considered conclusive, since, by destroying part of the tectum, the ability of the animal to respond is also impaired. The purpose of this communication is to give electrophysiological evidence in support of Sperry's hypothesis.

We have developed a technique for recording single fibers in the frog's optic nerve and single terminal bushes in the tectum (*2*). In this work we have found that normally the frog's tectum has the following organization. The fibers of each optic nerve cross completely in the optic chiasma and enter the opposite colliculus after dividing into two bundles. One is rostromedial; the other, caudolateral. They sweep over the surface and are distributed in several layers in the outer neuropil that forms the superficial half (250 μ) of the tectal cortex (Fig. 1). Most tectal cell bodies lie below this neuropil and send their main dendrites through it up to the pial surface. The axons of the majority of these cells form a narrow stratum that lies immediately above the compact layers of cell bodies. The optic fibers end in a systematic way both along the surface and in the depths of the superficial neuropil, mapping the retina in a pattern that is constant from animal to animal. There are four layers of these optic fiber terminals, which we have thus far identified only physiologically. Each displays a continuous map of the retina with respect to each of the four following operations on the image at the receptors. The four maps are in register with each other and

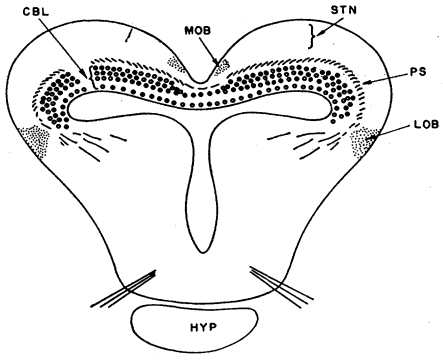

Fig. 1. Transverse section of the tectum of the frog at the level of the oculomotor nerves. CBL, cell-body layers; MOB, medial optic bundle; STN, superficial tectal neuropil; PS, palisade stratum; LOB, lateral optic bundle; HYP, hypophysis.

show position on the retina according to the cartography of Gaze (*3*).

The first layer of terminals is formed by those elements each of which is sensitive to moving or maintained contrast within its receptive field. The sharper the contrast, the better the response. These are equivalent to Hartline's (*4*) and Barlow's (*5*) "on" fibers. The second layer is made up of terminals of units each of which detects a moving or recently stopped boundary within its receptive field, provided there is a net positive curvature of the edge of the darker phase. Such a fiber will not respond, for example, to a straight-edge boundary moving across its receptive field or to a preestablished edge within that field. Both of these strata represent the endings of the unmyelinated fibers of the optic nerve.

The third layer is made up of terminal bushes from "on-off" fibers.

The fourth layer is composed of endings from "off" fibers.

The layers of endings are distinct in depth, and with the exception of the first and second layers they rarely merge at the transition zones. In this conspicuous order, both along the surface and in the depths, the area of the retina "seen" from any point in the superficial neuropil is, at most, 10° in radius. Most of the ganglion cells whose terminals appear at that point are crowded toward the middle of that area.

For the purpose of testing Sperry's hypothesis of the specific regrowth of the optic fibers after section of the optic nerve, we cut one optic nerve in several adult frogs *(Rana pipiens)*, ensuring the complete separation of the two stumps. At the end of 2 months the first signs of visual recovery were apparent, but full use of the eye did not occur for another month. When the visual recovery seemed complete, we exposed the colliculi and tested the initially deafferented colliculus for mapping of the retina. We found that the map had been regenerated along the surface, although the ganglion cells from whose terminals we were recording at any point were now spread over an area about two times as large as normal. The separation of operations in depth was also restored, and there was no sign of confusion between the operational layers.

The specific regrowth of the terminals to their proper stations cannot be explained by saying that an initial orderly array of fibers in the optic nerve crudely orders the fibers again at the time of regeneration. The fibers in the nerve simply are not in order *ab initio*. Any two contiguous fibers can come from the most widely separated points on the retina (*2, 6*).

This finding strongly supports Sperry's hypothesis that optic-nerve fibers grow back to their original destinations. They do so in an even more highly specific way than he proposed; the regrowth of the termini is also proper in depth (*7*).

Note added in proof. After this manuscript was prepared we noted that R. M. Gaze, of the University of Edinburgh, has presented to the Physiological Society similar findings in *Xenopus laevis* (*8*). He, however, has not studied the reconstitution of the distribution in depth of the optic fibers.

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Lunar and Solar Perturbations on Satellite Orbits

Abstract. Calculations of the solar and lunar effects on highly eccentric satellite orbits show that the sun and the moon may cause large changes in perigee height over extended periods of time. The amplitude and sign of the perigee height variations depend on the orbit parameters and the hour of launch; for a typical orbit and various choices of launch time, the perigee height will either rise or fall at the rate of 1 km/day over the course of several months. These results may be significant in deciding the launch conditions for future satellites with highly eccentric orbits.

A refinement of earlier computations on the orbit of the Vanguard I satellite has revealed the presence of a very slow variation in perigee height, with a period of 449 days and an amplitude of about 2 km. Kozai has suggested recently that a term of this period and amplitude will result from a combination of lunar and solar perturbations on that satellite (private communication). Kozai and Whitney have extended their calculations to the case of the paddle-wheel satellite, Explorer VI (Kozai, New York *Times*, 21 Aug. 1959). Explorer VI has an apogee of 48,700 km, a perigee of 6640 km, and an orbital inclination of 47.3° to the equator. Kozai and Whitney find that the highly eccentric orbit of this satellite produces substantial lunar and solar perturbations which decrease the perigee altitude rapidly, shortening its lifetime from several decades to a probable value of 2 years.

The very interesting work of Kozai and Whitney has encouraged us to explore further the possible lunar and solar effects on perigee height for satellite orbits of large eccentricity. We find that in general both the eccentricity and the perigee height vary with time as a result of these effects. The amplitudes, frequencies, and relative phases of the variations are determined by the orbit parameters and the hour of launch. For a special set of launch conditions, and for representative orbit parameters, the perigee height may be made to rise steadily over the course of several years at a rate of approximately 1 km/day. Thus the sun and the moon may provide a substantial perigee boost for the satellite under properly chosen circumstances. For other conditions the perturbations may be minimized to obtain a relatively stable orbit. These considerations may be of importance in deciding the launch programs for future satellites with highly eccentric orbits.

As a basis for our calculations we have used a convenient series development by Musen, which is equivalent to that of Kozai to our degree of accuracy.