4. *Grafting.* Four days after injection, skin homografts (A/He), 1 cm square and of full thickness, were placed on the panniculus carnosus of each recipient and control, according to a modification of the technique devised by Billingham and Medawar (*5*).

5. *Histologic evaluation and scoring.* The method of scoring depends upon the observation that an initial skin homograft on a mouse is largely viable on the 6th day after grafting, while, in contrast, a homograft on a mouse which has received a prior antigenic stimulus in the form of either a skin homograft or of whole homologous cells introduced intraperitoneally is partially or totally necrotic by the 6th day (*6*).

Each homograft with its bed was therefore excised on the 6th day and fixed in neutral, buffered Formalin. Grafts were cut transversely into thirds, embedded in paraffin, and stained with hematoxylin and eosin. Sections were then examined microscopically. The percentage of necrotic epithelial cells of the surface and of the hair follicles of each homograft was estimated from the appearance of all three graft sections, and the results were scored with the following values: 0, 10, 25, 50, 75, 90, and 100 percent of surviving epithelial cells. For convenience in statistical analysis, these values were transformed to integers 0 through 6, respectively.

We have found that the scores of control skin homografts (first set) were statistically indistinguishable on the 5th and 6th days. The scores for homografts placed after a single maximal antigenic stimulus ($20 \times 10^6$ homologous whole spleen cells, injected intraperitoneally) and excised on the 6th day were significantly smaller than the scores for those excised on the 5th day ($0.005 < P < 0.01$).

6. *Control values.* The mean score for control homografts on the 6th day for seven different experiments was 4.75 units (standard error, $\pm$ 0.174, $N = 55$). (This is equivalent to between 75- and 90-percent survival of epithelial cells.)

7. *Reproducibility of scoring method.* Seventy-five slides, chosen at random, were reexamined, and scores of the first and second readings were compared. A statistical analysis of the differences in paired data showed that there were no significant differences between the original and the repeat readings.

In Fig. 1, the injected doses are indicated by arrowheads above the abscissa; the units of the abscissa are the logarithms (base 10) of the number of injected cells. The mean scores for the individual test groups are shown by the crosses. The dose-response, represented by the solid line *AB*, was obtained by the method of least squares from the observed points (crosses); all but the highest dose ($6.3 = \log_{10} 2 \times 10^6$ cells), of which the mean score and variance were zero, were used. The dotted lines *mm'* and *nn'* are the confidence limits at $P = 0.05$ for the regression line (*not* for data for the individual groups). The other symbols (squares and circles) indicate the observed dose-response in two subsequent experiments. By means of statistical analysis, the regression is found to have a very high order of probability ($P < 0.001$) (*7*).

It is evident that a significant response can be measured over a 20-fold range in dose, linear with the logarithm of the dose in the range of 50,000 to 1 million cells (*8*).

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## Cytopathic Effect of Canine Distemper Virus in Tissue Culture

*Abstract.* A characteristic cytopathic effect was obtained with canine distemper virus (Onderstepoort strain) propagated in chick embryo fibroblast monolayer tissue culture. In limiting dilutions the lesions were focal. The titer at the 20th tissue culture passage was approximately $10^5$ $TCD_{50}$/ml. Cytopathogenicity was specifically inhibited by distemper immune serum. Minute plaques were produced under agar overlay.

Studies of canine distemper virus (CDV) have been handicapped by the lack of a tissue culture system in which the virus produces visible lesions. In addition to the veterinary importance of distemper virus, interest has been stimulated by the recently observed serologic relationship between CDV and measles virus (*1*).

Table 1. Titration of tissue-culture-adapted CDV in the chick embryo and in chick fibroblast tissue culture.

| Chick embryo CAM* ($ID_{50}$) | Tissue culture fluid medium (focal lesions) | Tissue culture agar overlay (pfu)† |
|---|---|---|
| *Tissue culture passage level:* 10 | | |
| $2.1 \times 10^4$‡ | $2 \times 10^5$ | $4.2 \times 10^5$ |
| *Tissue culture passage level:* 20 | | |
| $2.9 \times 10^4$ | $1 \times 10^5$ | $6.2 \times 10^5$ |

* CAM, chorioallantoic membrane. † pfu, plaque-forming units. ‡ Titer/1.0 ml.

Tissue cultures were prepared by trypsinization of 11-day-old chick embryos (*2*). The cells were suspended in Earle's saline containing 75 mg of $NaHCO_3$ per 100 ml, 0.25 percent lactalbumin hydrolyzate, and 4 percent calf serum. Ten milliliters of the cell suspension (900,000 cells/ml) were added to 4-oz prescription bottles, and confluent monolayers were obtained within 24 hours. The virus employed was the egg-adapted Onderstepoort strain (*3*) of CDV (208th embryo passage) (*4*). Tissue culture passage was initiated by inoculation of 1.5 ml of a stock CDV chorioallantoic membrane suspension (20 percent) which had been stored at −70°C. A control series of tissue cultures was initiated with an inoculum of uninfected chorioallantoic membrane. The cultures were inoculated at 24 hours and maintained in a medium consisting of 50 percent bovine amniotic fluid, 2 percent calf serum, and 48 percent Earle's saline, containing 75 mg of $NaCHO_3$ per 100 ml. This medium maintained cells in good condition for 3 weeks without further exchange. The early passages were harvested between the fifth and ninth days, and beginning with the 14th passage, on the third or fourth day. The cultures were harvested by scraping. The cells plus supernatant fluid were passaged without storage or freezing. Beginning with the 15th passage, the cytopathic effect was sufficiently rapid that the fibroblast monolayer could be maintained adequately in the original growth medium and therefore no exchange with maintenance medium was necessary. The present passage method (32nd passage) consists of the inoculation of 0.1 ml of undiluted supernatant plus cells simultaneously with the plating of the trypsinized chick cell suspension in the growth medium. The fibroblast monolayer forms within 24 hours and cytopathic effect appears in the formed sheet at 48 to 72 hours. Two of three other attempts to establish the Onderstepoort strain of CDV in this system were successful.

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.

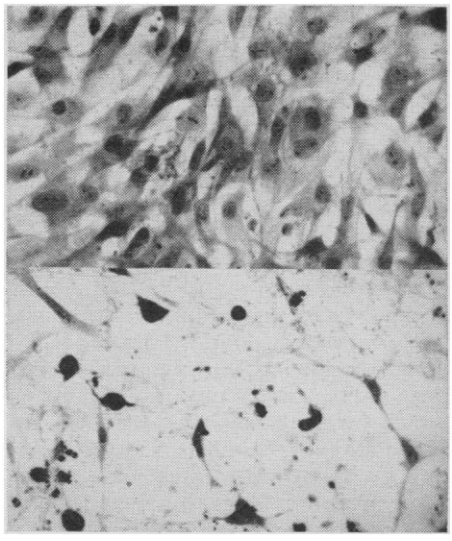

Fig. 1. (Top) Normal chick fibroblast monolayer tissue culture, day 5, hematoxylin and eosin stain; (bottom) same, infected on day 0 with canine distemper virus (× 285).

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 $\mu$) 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