Transplantation of Cornea to Chorio-Allantoic Membrane, with Observations on Virus Inoculation. (17720)

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It has been known for many years that a number of normal and tumor tissues, derived from various species, can be grafted upon the chorioallantoic membrane (CAM) of the developing chick embryo. Such tissue grafts may remain sufficiently viable to support multiplication of certain viruses which are adapted to propagation in the transplanted tissue, but not necessarily to growth in the chick embryo. Thus, Tenbroeck has demonstrated multiplication of hog cholera virus in minced hog testicle implanted onto the CAM, although the virus had not been adapted to the chick(1). Goodpasture, Douglas and Anderson(2), and later Blank, Coriell and Scott(3) reported that intact human skin could be successfully grafted onto the CAM and that the viruses of vaccinia, variola and herpes simplex, which are infectious for the chick embryo, readily produced typical inclusion bodies in the epithelial cells of the transplant. Similar results were obtained, on occasion, with the virus of herpes zoster which has not as yet been grown in the embryonated egg(3,4). Finally, grafts of fetal human membranes onto the CAM have been found to support the viruses of herpes simplex, variola, vaccinia and mare abortion(5).

It was thought that the transplant technic might prove to be of value in the study of viral agents affecting human ocular tissues, some of which have not as yet been adapted to propagation in host cells other than those of man. To explore this possibility attempts were made to graft corneal tissue of rabbit and man onto the CAM. The results are briefly summarized below.

Methods and materials. Embryonated hen's eggs of various ages were used. In the case of eggs 8-10 days of age the shell over the natural air sac was removed and a small slit was made in the shell membrane by means of a sterile hypodermic needle. Through this needle a drop of saline was deposited which seeped through the opening under the shell membrane, as a result of which the latter could easily be peeled off the underlying CAM. In embryos older than 10 days the CAM was exposed by means of a false air sac. Rabbits were sacrificed and their eyes enucleated as soon after death as possible. On occasion, however, the eyes were removed from rabbits stored at 4°C for as long as 48 hours after death. The enucleated eyes were kept for 1 - 14 hours in phosphate-buffered saline solution of pH 7.2 containing 500 units of penicillin and 0.1 mg of streptomycin per ml. The eyes of a few human stillborn infants were enucleated and kept in the same manner. For the transplanting the cornea was cut off at the limbus with corneal scissors and spread on a sterile cork disk with the epithelium facing downward. In most experiments the inner surface of the cornea was scraped with a fine scalpel in an attempt to remove Descemet's membrane. For infection with virus the epithelial side of the cornea was scarified either by means of a sterile scalpel or by multiple pin pricks with a sterile 24 gauge hypodermic needle. Virus was then rubbed onto the prepared epithelium with a cotton swab. The normal or infected corneae were then lifted off the cork with a sterile forceps and transferred onto the CAM with the endothelial side facing the membrane, preferably over a large

1. Tenbroeck, C., *J. Exp. Med.*, 1941, v74, 427.

2. Goodpasture, E. W., Douglas, B., and Anderson, K., *J. Exp. Med.*, 1938, v68, 891.

3. Blank, H., Coriell, L. L., and Scott, T. F., McNair, PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 341.

4. Goodpasture, E. W., and Anderson, K., *Am. J. Path.*, 1944, v20, 447.

5. Goodpasture, E. W., and Anderson, K., *Am. J. Path.*, 1942, v18, 563.

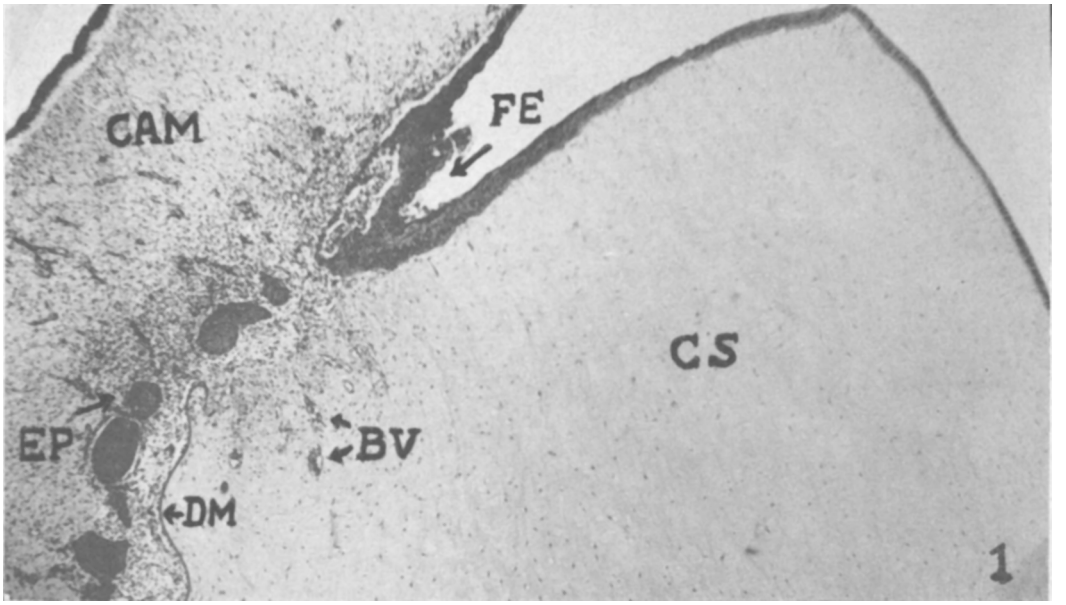

FIG. 1.
Rabbit cornea 72 hours after grafting onto the CAM of the chick embryo. Fusion is apparent in the area where Descemet's membrane ends abruptly. CS—corneal stroma, FE—fusion of epithelium of cornea with epithelium of CAM, DM—Descemet's membrane, EP—epithelial pearls, BV—blood vessels of the chick invading corneal stroma, CAM—chorio-allantoic membrane. (H & E × 50).

blood vessel. The eggs were then sealed with two layers of scotch tape and incubated at 37°C. At various intervals thereafter the cornea with the adherent underlying CAM was removed, fixed, sectioned and stained with hematoxylin and eosin.

The viruses of vaccinia and herpes simplex were used in these tests. The former was obtained from a commercial source as calf lymph and passed once on the CAM; The latter had been adapted by passage through hen's eggs and was used in the form of a 10% suspension of allantoic membrane infected with the HF strain.

Observations and results. In earlier experiments with corneal tissue of the rabbit no effort was made to remove Descemet's membrane. Under those circumstances the graft failed to become attached to the CAM, it increased in thickness, and presented an opaque, yellow gray appearance. It became necrotic in a few days. However, in one experiment the cut surface of the cornea happened to be in apposition with the CAM and in that case fusion occurred in the small area

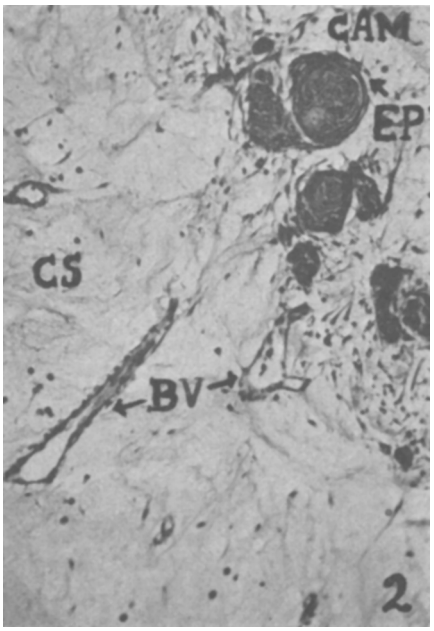

FIG. 2.
Area of invasion of chick blood vessels into rabbit cornea 8 days after grafting. CAM—chorio-allantoic membrane, EP—epithelial pearls, CS—corneal stroma, BV—blood vessels. (H & E × 250.)

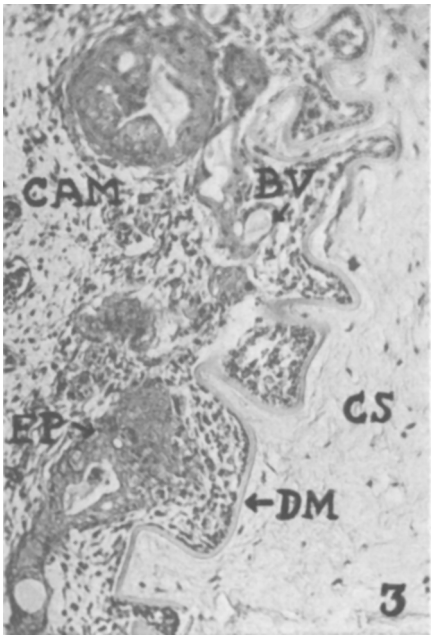

FIG. 3.
Rabbit cornea 9 days after grafting. Descemet's membrane is thrown into folds and forms a firm barrier against invading vessels. Epithelial pearls and newly formed blood vessels appear in the area of apposition. CS—corneal stroma, DM—Descemet's membrane, BV—blood vessels of chick, EP — epithelial pearls, CAM — chorio-allantoic membrane. (H & E × 111.)

of apposition (Fig. 1). This observation led to attempts to remove Descemet's membrane before grafting the cornea onto the CAM. Under these conditions fusion was obtained regularly. The cornea appeared swollen, bluish and opaque and could not be dislodged from the CAM. On histological examination it was found that the epithelium of the CAM disappeared rather rapidly except for remnants in form of epithelial pearls. The corneal stroma showed marked separation of the lamellae with an increase of tissue fluid. This change was apparent within 24 hours. After 48 hours polymorphonuclear cells of the chick were found between the lamellae. Blood vessels were seen to invade the stroma from the mesodermal layer of the CAM where Descemet's membrane had been removed (Fig. 2). Where removal was incomplete the membrane was seen to form a firm barrier against the invading blood vessels and leucocytes (Fig. 3). Vascularization of the stroma was noted after 48 hours of incubation and was observed to extend to Bowman's membrane by the 8th day. The corneal epithelium, as a rule, was well preserved (Fig. 4), however, on occasion the superficial cells were cast off and the basal layers showed vacuolization of the

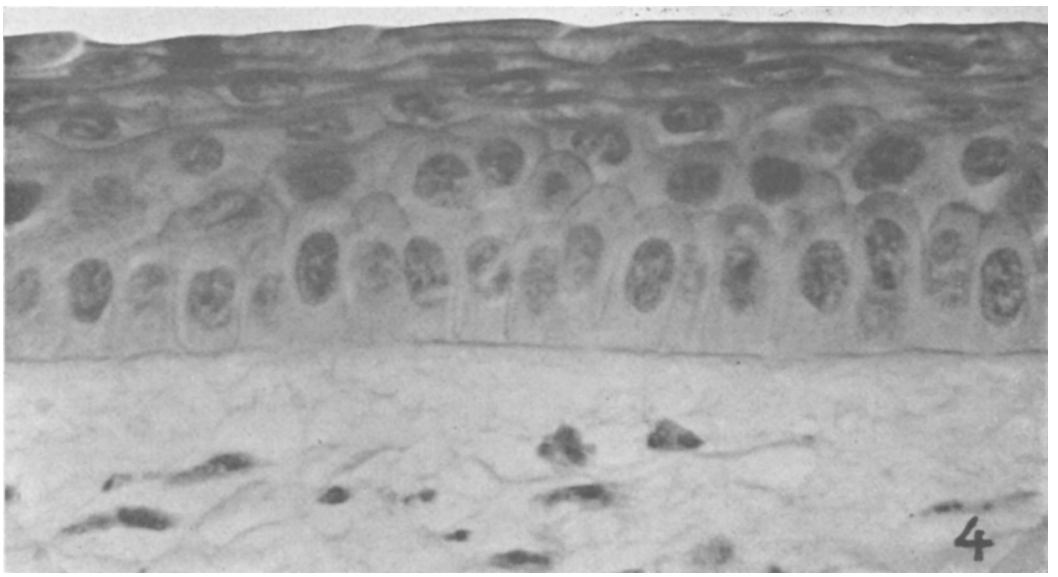

FIG. 4.
Rabbit cornea 8 days after grafting onto CAM showing well preserved epithelium. (H & E × 1,000.)

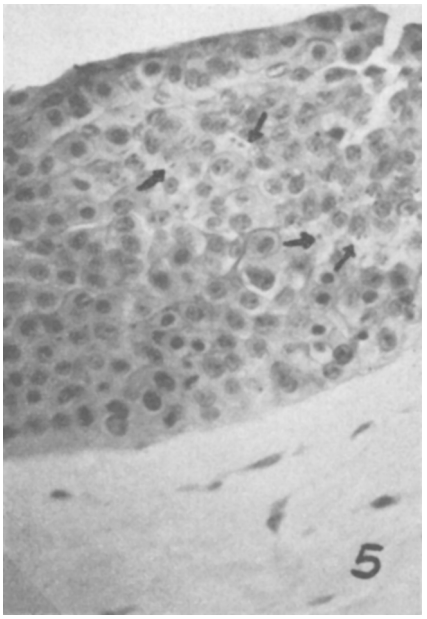

FIG. 5.
Proliferated epithelium of corneal graft 72 hours after infection with vaccinia virus by the needle prick method. Numerous cytoplasmic inclusion bodies marked by arrows. (H & E × 290.)

nuclei. Proliferation was indicated by not infrequent mitotic figures. Viability of the epithelium was further proven by the appearance of characteristic cytoplasmic inclusions following infection with vaccinia virus (Figs. 5,6) and, in cases of infection with herpes simplex, of intranuclear inclusions (Fig. 7).

Since the experimental period was necessarily limited to 8-10 days, attempts were made to preserve viable corneae for longer periods by re-grafting onto a second chick embryo. Successful takes were obtained but the corneal epithelium was shed in most grafts. In only one instance of infection with vaccinia virus on second transplant was the epithelium well enough preserved to permit the demonstration of inclusion bodies.

Experiments with human corneal tissue were limited to only three cases, two stillborn infants and one premature fetus. All 3 were partially macerated. One cornea of one of these showed satisfactory fusion. These studies will be continued.

Summary and conclusions. 1. Human and

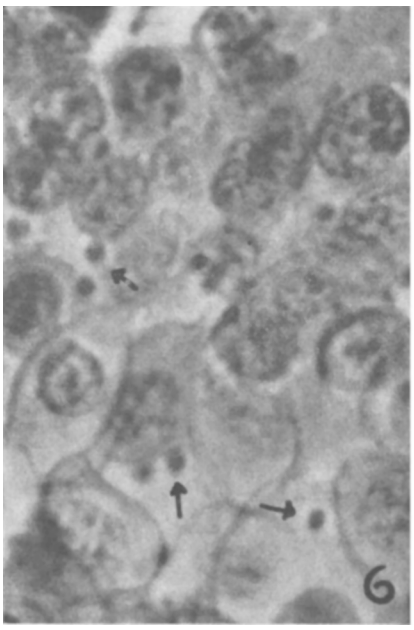

FIG. 6.
Enlarged view of corneal epithelium shown in Fig. 5. Numerous cytoplasmic inclusion bodies. (H & E × 1360.)

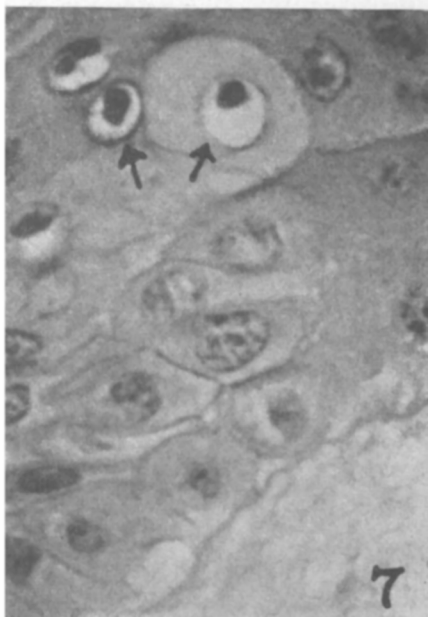

FIG. 7.
Rabbit corneal graft 72 hours after infection with herpes simplex virus showing intranuclear inclusion bodies. (H & E × 1,000.)

rabbit cornea may be grafted onto the chorio-allantoic membrane of the chick embryo and kept viable.

2. When the graft is successful, it presents a bluish appearance and is firmly adherent to the membrane. Histologically, such grafts are seen to be invaded by chicken blood vessels into the normally avascular stroma, and show preservation and proliferation of the corneal epithelium.

3. The viruses of vaccinia and herpes simplex were found to give rise to inclusion bodies within 24-72 hours in the epithelial cells of the corneal graft.

4. Serial passages of corneal grafts were complicated by the frequent loss of the epithelium and in only one case were inclusion bodies found after the inoculation during the second grafting.

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Transformation Type Specificity of *H. influenzae*.* (17721)

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Avery, MacLeod and McCarty(1) isolated from type III *D. pneumoniae* a desoxyribonucleic acid which in a suitable environment endowed R cells, derived from type II *D. pneumoniae,* with characteristics of S cells of type III. The transformed cells became encapsulated, elaborated the specific carbohydrate of type III and exhibited colony traits of this type. McCarty and Avery later reported an improved method for isolating specific pneumococcus desoxyribonucleic acids which they applied to types II, III and VI(2). Boivin and others(3) and Weil and Binder(4) reported the very irregular occurrence of transformations of *E. coli* and *S. dysenteriae* in the presence of crude desoxyribonucleic acid containing extracts made from the specific type into which the R variant was changed. This paper reports 1) the isolation from type b *H. influenzae* of a desoxyribonucleic acid which transforms an R strain Rbl, derived from type b *H. influenzae,* to type b cells during an 18 to 24 hour period of growth and 2) the isolation from type c *H. influenzae* of an unpurified transforming principle which endows the same R strain, Rbl, with properties of type c *H. influenzae.*

Material and Methods. Isolation of transforming principles. The desoxyribonucleic acid of type b *H. influenzae* was isolated and purified by the method which McCarty and Avery(2) applied to pneumococcus. Type b *H. influenzae* grown for 6 to 7 hours on Levinthal agar plates was harvested by washing each plate with 4 cc of a solution containing 0.1 M sodium chloride and 0.1 M sodium citrate. After 2 washings in this fluid the yield from 300 plates was reduced by centrifugation to a volume of 40 to 50 cc and frozen in carbon dioxide ice. After thawing at 4°C the volume was made up to 500 cc with the citrate saline solution and 5 cc of 10% sodium desoxycholate was then added; marked dissolution of the organisms occurs within 30 minutes at room temperature, the creamy appearing suspension clearing almost completely. Three extractions with chloroform and amyl alcohol were carried out to remove protein. The addition

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1. Avery, O. T., MacLeod, C. M., and McCarty, M., *J. Exp. Med.,* 1944, v79, 137.

2. McCarty, M., and Avery, O. T., *J. Exp. Med.,* 1946, v83, 89.

3. Boivin, A., Delaunay, A., Vendrely, R., and Lehoult, Y., *Experientia,* 1945, v1, 334; Boivin, A., Delaunay, A., Vendrely, R., and Lehoult, Y., *Experientia,* 1946, v2, 139; Boivin, A., and Vendrely, R., *Helvet. chim, acta,* 1946, v5, 1938.

4. Weil, A. J., and Binder, M., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 349.