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Inductive Interactions between Human Dermis and Chick Chorionic Epithelium

Abstract. *In a study of specificity in mesenchymal-epithelial interactions, human embryonic dermis has been recombined with chick chorionic epithelium and cultured for 7 days on a host chick chorioallantoic membrane. Dermis from the sole of the foot or palm of the hand induces chick chorionic epithelium to form an epidermis that resembles chick rather than human epidermis. Chick epithelium, though it has the capacity to respond to a human dermal stimulus, is limited to forming chick-type tissue. The human dermis was modified in its turn by culture in combination with chick epithelium.*

That differentiation of embryonic skin and the other specialized derivatives of the integument is based on specific interactions between dermal and epidermal components is well established (*1–3*). Similarly, maintenance of epidermal specificity in adult skin is dependent on the presence of dermis (*4*). Maintenance alone may not, however, require a specific dermis, for Briggaman and Wheeler, using chorioallantoic membrane grafting, reported that adult human epidermis is maintained in recombinations prepared from either human or guinea pig dermis (*5*).

Opportunities to investigate dermal-epidermal interactions were expanded by the elegant demonstration (*2, 6*) of the potentialities of the chick chorionic epithelium (CE) as a responding tissue in combinations with different dermal inducers.

A schematic representation of the methods used in isolating, recombining, and grafting of human and chick tissue on the chick chorioallantoic membrane (CAM) is shown in Fig. 1. Isolated dermis was obtained from either 14- or 15-week-old human or 16-day-old chick embryonic skin. In both cases dermis was easily separated from its epidermis after about an hour in a solution of double-strength calcium- and magnesium-free Tyrode's solution with 0.25 percent disodium ethylenediaminetetraacetate dihydrate (EDTA) at 37°C. Samples of each type of isolated dermis were fixed in Bouin's solution before histological comparison. Samples for experimental recombination were kept in cold Tyrode's solution for about 30 minutes and then wrapped in freshly isolated chick chorionic epithelium obtained from 8-day-old embryos. Isolated epithelium was obtained by rinsing the chorioallantoic membrane in cold Tyrode's solution and then placing it also in the EDTA solution. After 20 minutes at 4°C, the epithelium was easily separated as a continuous sheet (*2*). The recombinants were transplanted onto the chorioallantoic membrane of 11-day-old chick embryos and grown for 7 days.

Two types of control cultures were prepared. Both naked human sole or palm dermis and chick anterior shank dermis were grown for 7 days in Millipore filter chambers on the chorioallantoic membrane. Use of these chambers prevents interaction of the graft with the chorionic epithelium of the host, which ordinarily migrates over naked dermis and undergoes transformation (*7*). Intact skin (dermis and epidermis) from both human and chick were cultured for 7 days directly on the membrane.

Histological comparison of embryonic chick and human skin at explantation showed that the skins are easily distinguishable (Fig. 2, A and B). Cells of the intermediate layers of the epidermis differ; those of the chick have a denser staining cytoplasm. The distinctive subperidermal and peridermal layers characteristic of chick epidermis were not present in the human skin. Swollen cells were commonly seen at the surface of the human epidermis. In both, the basal cell layer was columnar. Human dermis had a denser accumulation of fibers positive to either aniline blue or eosin than chick dermis, and distinctive cells with dark-staining nuclei were present.

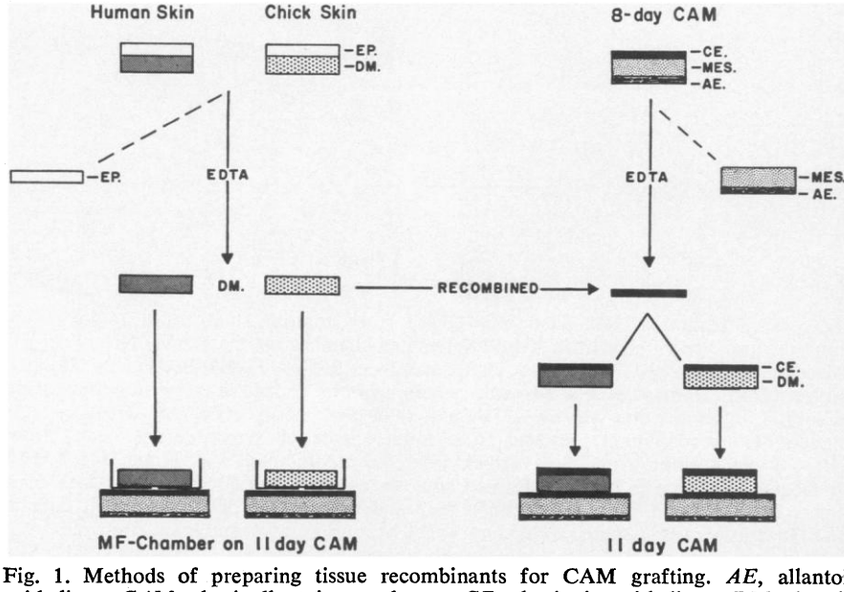

Fig. 1. Methods of preparing tissue recombinants for CAM grafting. *AE*, allantoic epithelium; *CAM*, chorioallantoic membrane; *CE*, chorionic epithelium; *DM*, dermis; *EDTA*, disodium ethylenediaminetetraacetate; *EP*, epidermis; *MES*, mesenchyme; *MF*, Millipore filter.

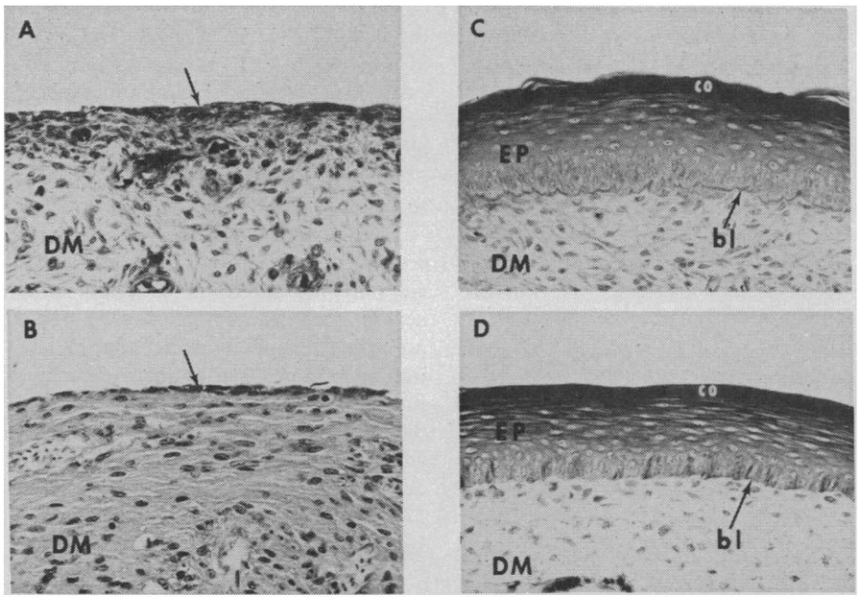

Fig. 2. (A) Section of anterior shank skin from a 16-day chick embryo: *b*, columnar basal layer; *i*, approximately six intermediate cell layers; *sp*, subperiderm; *p*, periderm. (Mallory's triple stain, × 255). (B) Section of sole skin from a 14-week human embryo: *b*, columnar basal layers; *i*, approximately four intermediate cell layers. The blebbing superficial cells (arrow) are commonly seen. (Mallory's triple stain, × 255). (C) Section of anterior shank skin from a 16-day chick embryo after 7 days' growth on the CAM. Note the thick cornified epidermal layer, *co*; *bl*, basement lamella. (Hematoxylin and eosin stain, × 300). (D) Section of sole skin from a 14-week human embryo after 7 days' growth on the CAM; *bl*, basement lamella. (Hematoxylin and eosin stain, × 300).

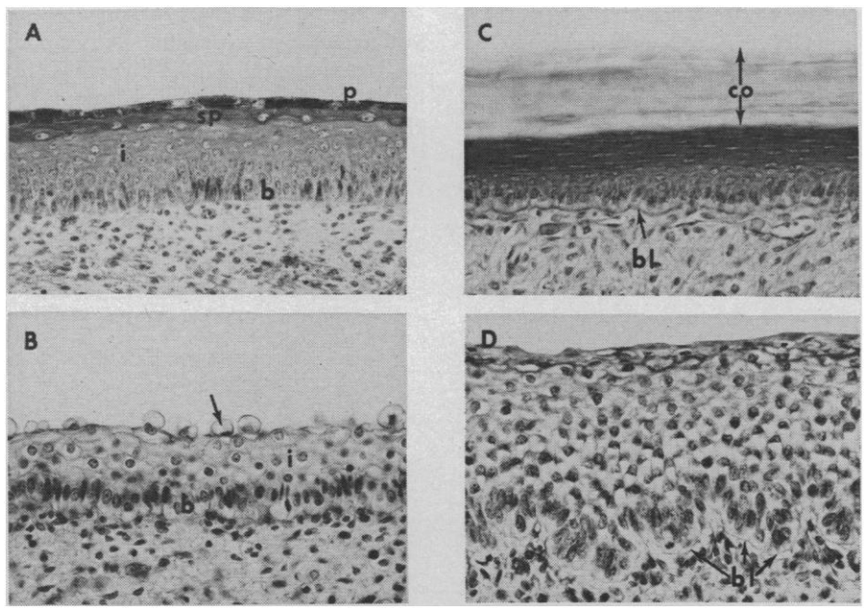

Fig. 3. (A) Section of isolated dermis (*DM*) from anterior shank of a 16-day chick embryo after 7 days' growth in a Millipore filter chamber on the CAM. No epidermal differentiation (arrow). (Mallory's triple stain, × 255). (B) Section of isolated dermis (*DM*) from sole of a 14-week human embryo under the same conditions. No epidermal differentiation (arrow). (Hematoxylin and eosin stain, × 255). (C) Section of a recombinant prepared from isolated anterior shank dermis (*DM*) from a 16-day chick embryo and 8-day chick CE. The recombinant was grown for 7 days on the CAM. The CE has transformed into an epidermis (*EP*) typical of that of a 19-day chick embryo. Note cornification (*co*) and the position of the basement lamella (*bl*). (Mallory's triple stain, × 255). (D) Section of a recombinant prepared from isolated sole dermis (*DM*) from a 14-week human embryo and 8-day chick CE. The recombinant was grown for 7 days on the CAM. The CE has transformed into an epidermis (*EP*) which closely resembles that of the 19-day chick embryo (compare with C). Basal cells (*b*), basement lamella (*bl*), and the extent of cornification (*co*) are identical. (Mallory's triple stain, × 255).

In no case did any epidermal differentiation occur at the outer dermal surface when naked dermis of either human (10 cases) or chick (12 cases) was grown in the filter chambers (Fig. 3, A and B). The chick dermis had accumulations of dermal fibers (positive to either aniline blue or eosin) at its surface, while the human dermis had broad bands of dermal fibers throughout the graft. Chick red blood cells were seen within the blood vessels of the human dermis (*8*), and the number of cells with darkly staining nuclei decreased.

The series of intact skin grafts (Fig. 2, C and D) retained their species characteristics. The chick epidermis (10 cases) lost its peridermal layers and underwent heavy cornification typical of that seen in the hatched chick. Although the human epidermis (10 cases) added some intermediate cell layers, it did not cornify. The dermal tissues of both also retained their individual morphology.

Recombinants prepared from isolated chick anterior shank dermis and chick chorionic epithelium (22 cases) developed an epidermis (Fig. 3C) typical of normal 19-day embryonic scales. Figure 3D indicates that the epidermal differentiation obtained from recombinations of isolated human sole or palm dermis and chick chorionic epithelium are indistinguishable from the homotypic series. Thus this recombinant (31 cases) also developed a thick, keratinized epidermis. The epidermis consisted of columnar basal cells, several layers of squamous-shaped intermediate cells, and a thick cornified surface layer. Figure 3D also shows that alterations occurred in the human dermis. The broad bands of dermal fibers and the cells with darkly staining nuclei seen in Fig. 3B were no longer evident.

This study shows that intact human embryonic skin grown on the chorioallantoic membrane retains its species characteristics. In this respect the results support those obtained with adult human skin (*5*, *8*). While isolated dermis grown in filter chambers on the chorioallantoic membrane survived, modifications in the kinds of cells and in other structures occurred.

A number of investigators have produced chimeric structures by recombining tissues from different species (*9*) or disaggregated cells—for example, chick and mouse (*10*). Furthermore, dermis from one species maintains fully differentiated epidermis from another (*5*). Many examples of xenoplastic inductive

interactions are available, the first being the recombinants between anurans and urodeles (*11*) but examples in which tissues from widely different species were employed are rare. There are virtually no previous studies in which an unspecialized responding epithelium has been used: perhaps the closest to the present combination is that of Coulombre and Coulombre (*12*), who reported that replacement of the lens of a 5-day chick embryo with dermis from mouse flank led to feather formation in the relatively unspecialized host-chick cornea.

This investigation demonstrates that human embryonic skin dermis specifically directs the transformation of unspecialized chick chorionic epithelium into a typical keratinized epidermis, histologically indistinguishable from chick skin. That the differentiated epidermis may exert an influence on human dermis in its turn is suggested by the additional modifications observed in the human dermis after recombination culture. This study clearly distinguishes the roles of both the inducing dermis and the transforming epithelium in a tissue interaction.

ROGER H. SAWYER
URSULA K. ABBOTT
Department of Avian Sciences, University of California, Davis 95616
JOHN D. TRELFORD
Department of Obstetrics and Gynecology, University of California Medical School, Davis

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Crystalline Cholera Toxin and Toxoid

Abstract. *The exo-enterotoxin of* Vibrio cholerae *has been obtained in crystalline form. A solution of the crystalline protein was equal in potency to the parent pure toxin in both choleragenicity and skin reactivity. Crystals of the natural toxoid, choleragenoid, resemble those of the toxin in appearance. A solution of crystalline choleragenoid was equivalent to the parent preparation in the flocculation test.*

We have reported the isolation and purification of the cholera exo-enterotoxin, which was designated choleragen, and indicated that the pure protein was both choleragenic and skin reactive (*1*, *2*). Other investigators using cruder preparations have reported some dissociation of skin reactivity and choleragenicity and suggested that the two activities may not be present on the same molecule (*3*). We have also reported the purification and some of the properties of a natural toxoid—designated choleragenoid, also found in fermenter-grown cultures of the highly toxigenic strain of *Vibrio cholerae*, 569B Inaba (*1*, *2*)—and presented some details of how the toxoid is formed from the toxin (*4*, *5*). We now report the application of an additional criterion of purity, the solubility test, and the crystallization of these two proteins. Redissolved crystalline cholera toxin was found to be equally as potent as the parent purified toxin in choleragenicity, skin reactivity and flocculation (Lf) tests.

Choleragen and choleragenoid, purified by either of the methods described (*2*, *5*), were each subjected to an additional chromatographic passage on Bio-Gel P-150 (Bio-Rad Laboratories) to remove residual traces of the other protein and traces of aggregated protein which may have been formed. As an additional test of purity, the phase rule solubility test was applied. Increasing increments of choleragen solution (25 mg/ml) in tris (hydroxymethyl) aminomethane (tris) buffer (*1*) were added to test tubes, and tris buffer was added to a total volume of 1 ml. To each tube 1 ml of 4*M* $(NH_4)_2SO_4$ was added and the tubes were incubated overnight at 22° to 23°C with gentle shaking. The tubes were centrifuged, and the optical density of the supernatant was determined at 280 nm. The results, Fig. 1A, were consistent with those expected from a pure protein. The solubility curve showed a sharp change in slope at the inflection point (at approximately 1.7 mg of protein added), and there was no further increase in absorbance with added increments of protein.

Choleragen was crystallized by adjusting the *p*H of a solution containing approximately 20 mg/ml to the isoelectric point, *p*H 6.6 (*2*), and adding 4*M* $(NH_4)_2SO_4$ (adjusted to the same *p*H) until slight opalescence was observed at room temperature. Sufficient water to clarify the solution was added, and the mixture was placed at 4°C. On subsequent days, a white precipitate was observed in the bottom of the conical test tube which, on swirling, exhibited a refractile sheen characteristic of dispersed crystals. Examination by dark-field microscopy revealed the crystalline nature of the sediment (Fig. 1B). A variety of crystalline forms was observed. Some were needle-like structures and some shield-like forms, but,

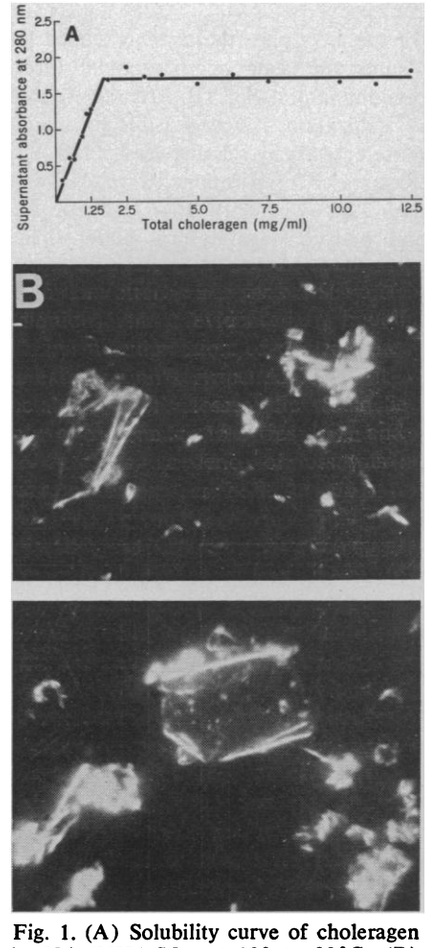

Fig. 1. (A) Solubility curve of choleragen in 2*M* $(NH_4)_2SO_4$ at 22° to 23°C. (B) Two views of crystals of choleragen, dark-field microscopy; original magnification approximately 600 ×.