Growth of Herpes Simplex Virus in Adult Human Skin Organ Cultures[1] (34225)

CLAYTON E. WHEELER, JR. AND ROBERT A. BRIGGAMAN
Division of Dermatology, Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

Herpes simplex virus (herpesvirus hominis) multiplies in many different cell types in tissue culture and produces disease in most common laboratory animals, including the chick embryo and its membranes (1). Although much information has been obtained from these host–virus models, they do not represent the natural host–cell system for the virus. The natural host is man in which cells of the skin and mucous membrane are most often infected although cells of the brain, lung, liver, adrenal, and other organs may be involved. It is not practicable to perform extensive experimental studies on human skin *in vivo* although selected experiments of this type can be done. Human skin maintained *in vitro* appears to offer the next best alternative. Skin organ cultures where normal epidermal–dermal relationships are maintained may be superior in many instances to tissue cultures prepared from fibroblasts or epithelial cells which have grown out from explanted skin.

Despite the availability of human skin from biopsies, operations, amputations and autopsies, there have been very few *in vitro* studies of infection with herpes simplex or other viruses, using this organ. Adult human skin grafted upon the chorioallantoic membrane of the chick embryo has been shown histologically to support the growth of herpes simplex, vaccinia, and zoster viruses (2–4). Embryonic human skin in organ cultures has developed typical herpetic cytopathogenesis when infected with herpes simplex (5, 6). To our knowledge viral infection in these models has not been studied beyond these morphologic demonstrations. The main reason for infrequent use of human skin organ cultures in viral studies has apparently been the impracticality of preparation, manipulation, and maintenance of this type of culture.

We are reporting a simple, practical, effective, and reliable way to use adult human skin organ cultures for growth and study of herpes simplex and possibly other dermatropic viruses.

Materials and Methods. Preparation of skin organ cultures. Skin was obtained from adults at operation after the usual preoperative washing with soap, water, and alcohol. Thin, split-thickness sheets were made by cutting the skin at a depth of 0.2 mm with a Castroviejo keratotome. At this level the sheets consisted of epidermis with a thin dermal component which contained epidermal appendages. These sheets were spread out on a board and circular pieces of uniform size were prepared with a 4-mm biopsy punch. Pieces of skin were placed in a screw-capped 16 × 125-mm culture tube which contained 1 ml of growth medium (7). The growth medium consisted of 20% horse serum, 79.9% Hanks' balanced salt solution, 0.1% yeast extract, 100 units of penicillin, 100 μg of streptomycin, and 2 μg of amphotericin B /ml and 0.015% phenol red. Cultures were incubated in a roller drum at 35°. The culture medium was changed daily.

Infected organ cultures. Six to nine pieces of thin, split-thickness skin prepared with a 4-mm circular punch were added to each screw-capped culture tube containing 1 ml of growth medium. To this was added 0.1 ml of herpesvirus (8) suspension containing 10^4 to 10^5 pfu of virus. Cultures were incubated at 35° on a roller drum for 3 hr. The growth

[1] Supported by Research Grants AM 10546 and Al 04733, Dermatology Training Grant AM 05298 and Special Fellowship 5 F03 AM 35608.

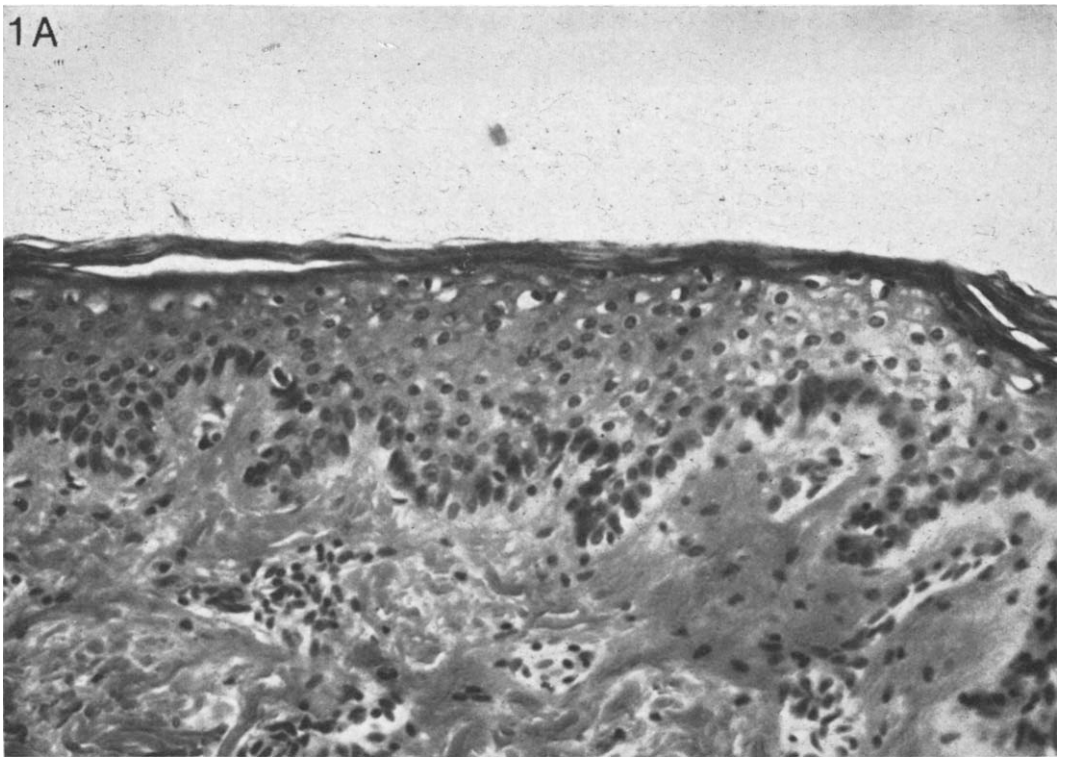
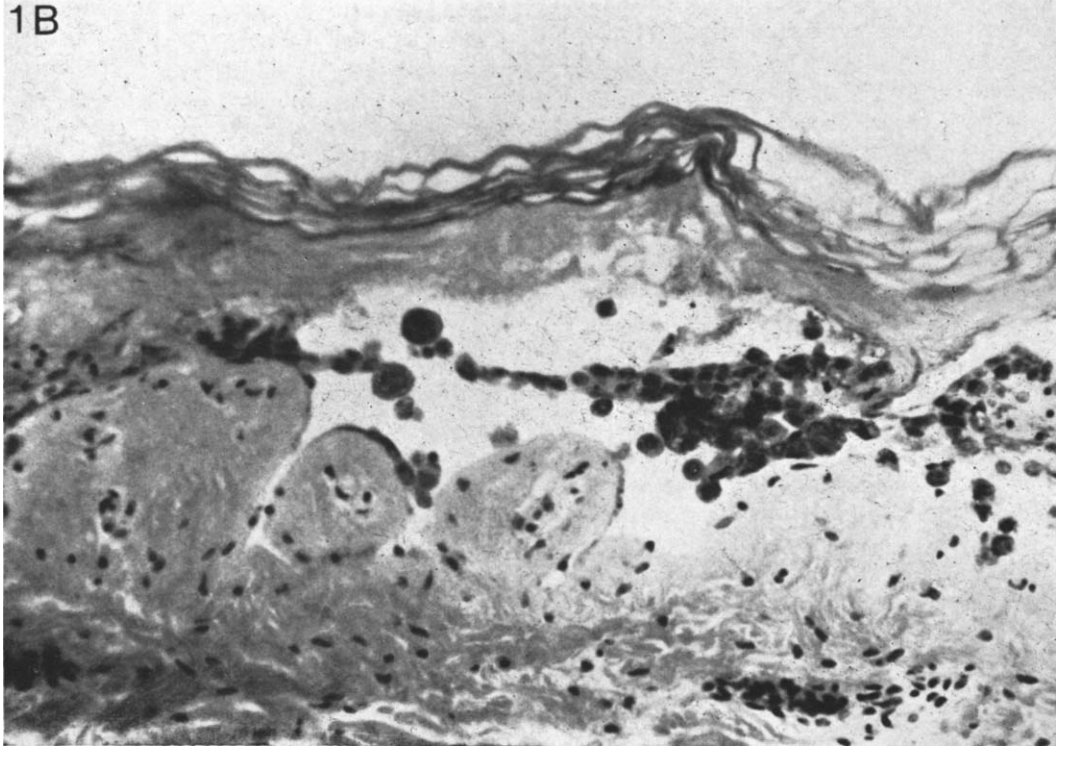


FIG. 1. Microscopic appearance of adult human skin organ cultures at 6 days: (A) uninfected skin control (×512); (B) herpes-infected skin showing ballooning, acantholysis and multinucleated giant cell formation (×512).



medium was then changed five times (four washes) in 1-ml aliquots which were saved for viral titration. This was done to remove most of the unabsorbed viral inoculum. Thereafter, cultures were incubated at 35° in a roller drum and culture medium was changed each day and saved for viral titration. At the end of 7–8 days, cultures were ground and the suspension titrated for virus. Viral titrations were performed by a recently described plaque count method (7). Specimens were removed from identically treated controls, uninfected and infected, for histologic study at daily intervals.

Results. Histology of uninfected skin organ cultures. Skin pieces were as well preserved in this simple culture system as in more elaborate systems previously described (9, 10). Epidermal cells of the basal and lower prickle layers appeared to remain viable throughout the 10-days cultivation. The granular cell layer was either lost or diminished during the first several days in culture and was replaced by a parakeratotic layer under the preexisting stratum corneum. From the fourth day on, cells of the upper prickle cell layer developed pyknotic nuclei and vacuolated cytoplasm. A thin layer of epidermal cells grew around the cut surface of the dermis (epiboly) in many of the split-thickness specimens.

Histology of infected skin organ cultures. Infected pieces of skin showed typical herpetic cytopathogenesis (Fig. 1) by the third or fourth day (11). These changes consisted of multinucleated giant cells, basophilic intranuclear inclusions, separation of cells from each other (acantholysis) and ballooning degeneration. Epithelial cells of the epidermis and its appendages were affected but no definite viral changes were observed in dermal tissue. Except for lack of an inflammatory reaction, the specimens resembled biopsies from clinical herpetic lesions (11). The detection of histologic changes on days 3 and 4 correlated well with the appearance of virus in the culture medium between the third and fourth day. This incubation period was also reminiscent of the incubation period of human herpetic infections (1). Based on sequential histologic specimens, small foci of infection appeared first in the epidermis at the cut edge of the specimen. Later, foci were observed in the center of some pieces in the vicinity of skin appendages. These foci subsequently enlarged until the entire epidermis was involved. This sequence suggested that intact stratum corneum protected underlying viable epidermal cells from infection.

Viral growth in skin organ cultures. Representative growth curves of HF herpesvirus, type 2, in skin organ culture are shown in Fig. 2. The initial descending limb of the curve represents unabsorbed virus which is removed with the five changes of medium. New virus is first detected in the culture medium between days 3 and 4. The amount of virus in the medium increases rapidly and then levels off at days 5 or 6. Final samples of skin contain 10^6 to 10^7 pfu of virus/ml of ground suspension. Since skin in the suspension makes up about 0.1 of the total volume, the viral titer in skin itself is about 1 log higher.

Discussion. This organ culture system provides a simple method for study of herpes simplex infections in adult human skin. Organ cultures can be made in relatively large numbers because of ease of preparation and the relatively small amount of skin required. Because of the reproducibility of cytopathogenesis and viral reproduction, this system should be useful for studying herpes simplex virus–host relationships. It may also be useful for studying vaccinia, zoster–varicella, molluscum contagiosum, warts, and other dermatropic viruses.

In devising the procedure for the organ culture model, we considered the level and thickness of skin to use and the number of pieces of skin to place in each culture tube. In appropriate experiments, viral production per piece of skin was highest when pieces obtained from split-thickness sheets cut at 0.2 mm were used. Approximately 20% less virus was produced in pieces cut at 0.4-mm thickness which contained relatively more dermis. Pieces cut from dermis after shaving away and discarding epidermis produced 80% less virus; these dermal pieces still contained epidermal appendages. These results of viral growth are consistent with histologic

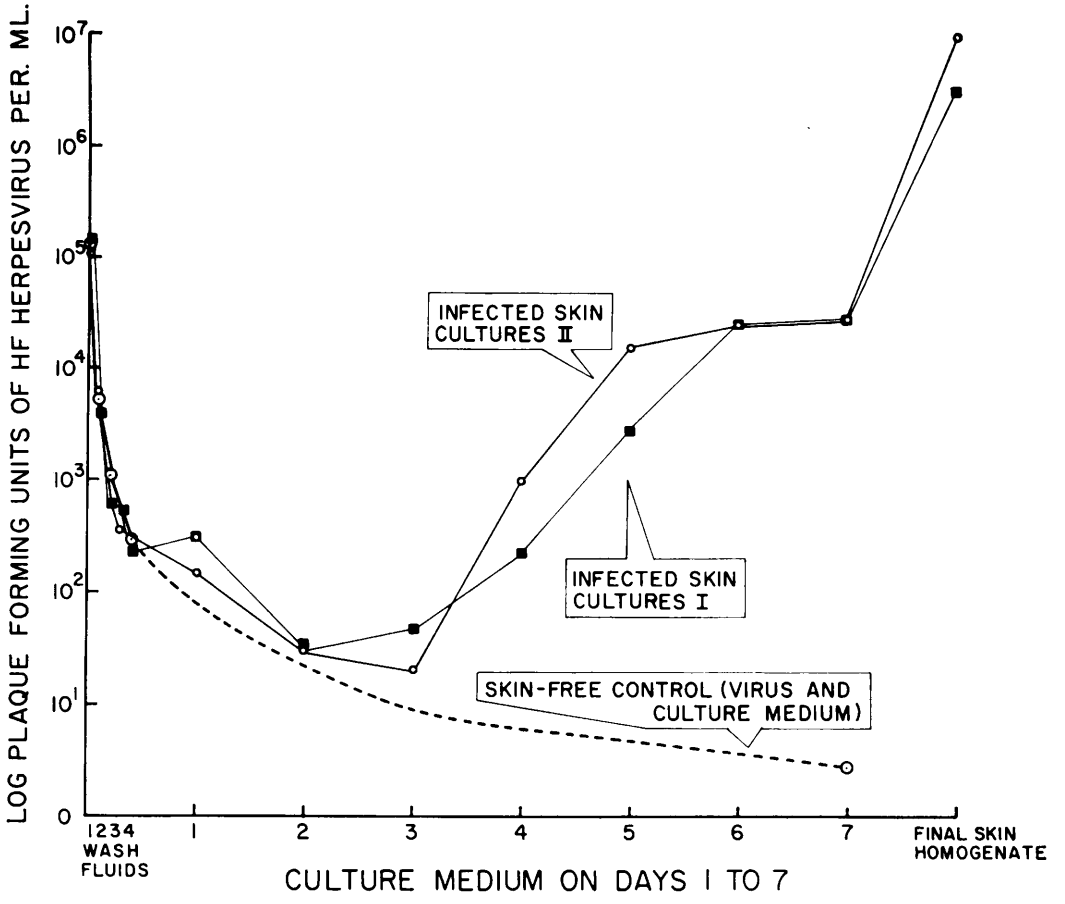


FIG. 2. Viral production in adult human skin organ cultures infected with HF herpes simplex virus.

findings where viral cytopathogenesis was recognized only in the epidermis or its appendageal extensions into the dermis. There appeared to be little difference in viral production per piece of skin whether three, six, or nine pieces were present in a culture tube.

Summary. Infection of adult human skin organ cultures with HF herpes simplex virus (type 2) resulted in appearance of new virus in the culture medium in 3 or 4 days. This was associated with epidermal cell cytopathogenesis which resembled *in vivo* herpes simplex infections. Cultures consisted of 4-mm punches of split-thickness skin incubated in 1 ml of medium in 16 × 125-mm screw-capped roller tubes at 35°. Cultures were easy to prepare in numbers, manipulate, and maintain; and viral reproduction and cytopathogenesis were reproducible.

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Received June 9, 1969. P.S.E.B.M., 1969, Vol. 132.