TABLE I.
C3H Hybrids (Mixed Sexes) Sacrificed 48 Hours After Initial Therapy.

Treatment	Dose schedule	No. animals	Organ weights, g per 100 g body wt: Mean spleen wt ± S.E.	Mean thymus wt ± S.E.
None	—	15	1.348 ± 0.0802	0.153 ± 0.0194
95% ethyl alcohol	1.5 ml/kg daily for 2 days	7	1.050 ± 0.1259	0.051 ± 0.0101*
4% formaldehyde	5 ml/kg 2 times daily for 2 days	9	0.605 ± 0.742*	0.050 ± 0.0056*
40% glucose	15 ml/kg daily for 2 days	10	1.055 ± 0.1187	0.112 ± 0.0152
48-hour fast	—	9	1.138 ± 0.1031	0.107 ± 0.0147*

* Upon statistical analysis weights are significantly smaller than untreated controls.

temporarily inhibit growth or produce temporary regression of the malignant lymphoid tumor 6C3HED. A similar effect is produced by a 48-hour fast. Fasting begun 30 hours after the initial stimulus gives an additional "lympholytic" effect.

It has been shown that stimuli which are known to produce an alarm reaction with resulting atrophy of the normal thymus and normal spleen produce a similar type of reaction in tissue composed of malignant lymphocytes.

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Human Skin Grafted Upon the Chorioallantois of the Chick Embryo for Virus Cultivation.*

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(Introduced by Werner Henle.)

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Goodpasture, Anderson, and Douglas have described a technic for grafting human skin onto the chorioallantoic membrane of embryonated eggs.[1,2] They demonstrated that viruses such as herpes simplex and vaccinia, which multiply readily in the normal embryonated egg, would also grow in the grafted skin with the appearance of characteristic inclusion bodies in the epithelial cells. In addition, by using this technic they recorded an experiment in which they were able to grow herpes zoster on a single occasion.[3] They also indicated the value of such heterologous skin grafting in immunologic studies, by demonstrating that skin taken from chickens immune to fowl pox would lose its immunity after being grafted onto the chorioallantois.[4] It is assumed that the immune bodies are diluted out of the skin as it grows in the egg.

The obvious usefulness of vascularized, morphologically distinct human skin, actively growing in a sterile host that does not produce antibodies of its own, prompted the

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† Fellow in the Medical Sciences, National Research Council, during part of this study.

‡ Senior Fellow in Virus Research, National Research Council.

1 Goodpasture, E. W., Douglas, B., and Anderson, K., *J. Exp. Med.*, 1938, **68**, 891.

2 Goodpasture, E. W., and Anderson, K., *Am. J. Path.*, 1942, **18**, 563.

3 Goodpasture, E. W., and Anderson, K., *Am. J. Path.*, 1944, **20**, 447.

4 Goodpasture, E. W., and Anderson, K., *Arch. Path.*, 1940, **30**, 212.

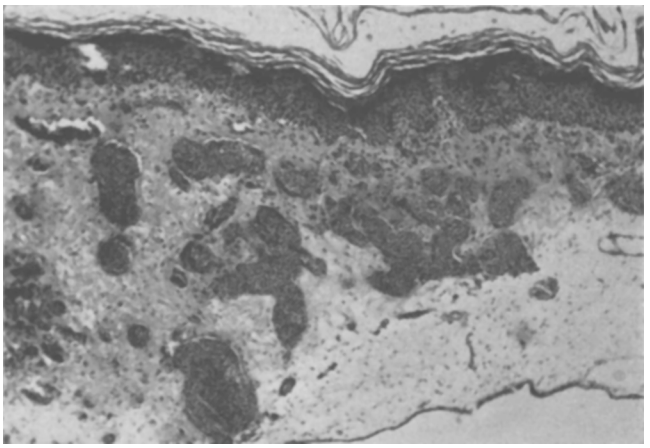

FIG. 1.
Human skin, 7 days after grafting onto chorioallantois. Large blood vessels contain nucleated erythrocytes, and are prominent in the denser human corium as well as the chick membrane. (H & E × 40).

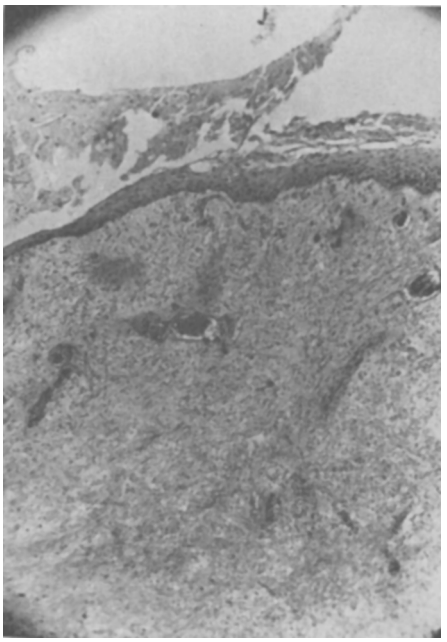

FIG. 2.
Human skin, after grafting onto 3 successive eggs for a total of 21 days. The epidermis remains distinctive. The dense collagen bundles of the corium have been separated by inflammatory cells of the chick. (H & E × 40).

study herein reported. This was to try to develop a relatively simple procedure whereby bacteria-free, growing skin was available which could be kept growing by serial transfer from egg to egg.

Technic. The human skin used in most of this study was obtained at circumcision of white and Negro children.§ The prepuce was cleaned preoperatively with soap and water and alcohol. Other antiseptics were avoided. The skin was used routinely within 2 hours of operation, but occasionally was stored in the ice box at 4°C for as long as 5 days before grafting. The prepuce was further cleaned of clotted blood and debris in the laboratory by bathing with the following solution: Physiological saline solution, phosphate buffered to pH 7.2, to which had been added gelatin to make 0.5% solution, and penicillin and streptomycin to make a final concentration of 500 units and 100 μg per ml, respectively. Previous studies in this laboratory[5] have indicated the antibacterial effectiveness and innocuous character of this solution. The skin was stretched, with sterile pins, on a sterile, solid cork board, epithelial surface down. With forceps and scalpel, the connective tissue was meticulously removed,

§ Obtained through the courtesy of Dr. C. E. Koop and the Surgical Staff of the Children's Hospital of Philadelphia.

[5] Coriell, L. L., Blank, H., and Scott, T. F. McNair, to be published.

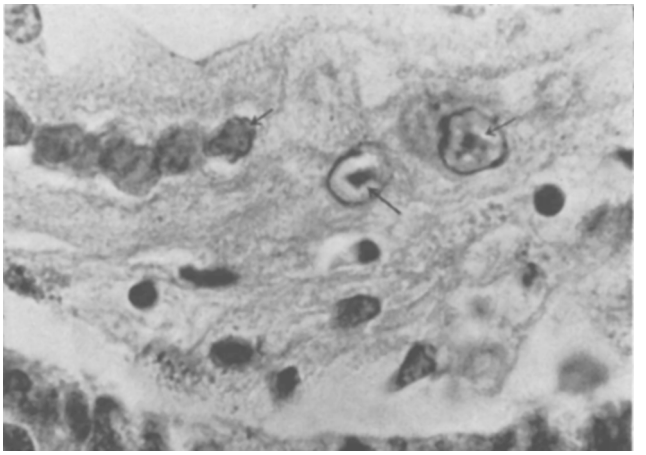

FIG. 3.
Epithelium of grafted human skin infected with herpes simplex 48 hours before fixation. This skin has been growing for 27 days (in 4 eggs) before inoculation with the virus. Intranuclear inclusion bodies in various stages of development are present. (H & E × 700).

leaving the epidermis and attached corium as thin as possible. Throughout this procedure, the antibiotic gelatin saline solution was used generously to prevent the tissue from drying. The thin sheet of skin was then cut into squares of the desired size. Ten-day-old embryonated hens' eggs were prepared by the "false air sac technic,"[6] and a window was made in the side of the egg overlying the false air sac by removing pieces of shell with the aid of fine forceps. A sterile, flat instrument, such as a forceps handle, was used to place the skin on the chorioallantoic membrane of the eggs, with the raw surface in even contact with the membrane. After grafting the skin, the shell defect was sealed with 2 layers of Scotch tape, and the eggs were incubated on their sides at 96°F.

The grafted skin was inoculated with virus-containing material 24 hours later, under the assumption that humoral antibodies from the donor of the skin would be largely removed after this interval on the egg.[2] The grafted skin was inoculated through a window cut in the Scotch tape with sterile scissors, after preliminary cleansing with 70% alcohol. Material to be inoculated was placed directly on the skin, which was then pricked with a vaccinating needle or with a 27-gauge hypodermic needle of the syringe used to deposit the inoculum onto the skin. More Scotch tape was used to seal the window again, and the eggs incubated for an additional period, varying from 1 to 7 days as the particular experiment required.

At the appropriate time, the skin was removed with attached chorioallantoic membrane. Material for histological study was placed on a small piece of absorbent paper with the membrane next to the paper, and the whole fixed in 5% acetic acid Zenker's fixative. Hematoxylin- and eosin-stained paraffin sections were prepared for routine study.

For passage to another egg, the skin was removed aseptically and placed in a sterile petri dish. With the aid of iris scissors and forceps, all of the attached chorioallantois was trimmed away, as was any additional connective tissue which had grown in the corium during the initial period on the egg. The skin was then transferred to the chorioallantois of another 10-day egg, prepared in the usual fashion. Such serial passages were usually made at 7-day intervals.

[6] American Public Health Association, Diagnostic Procedures for Virus and Rickettsial Diseases, p. 243, 1948.

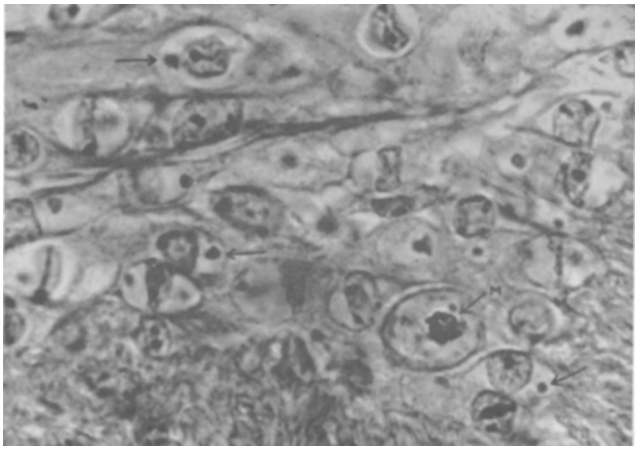

FIG. 4.
Epithelium of grafted human skin infected with vaccinia 72 hours before fixation. Numerous basophilic cytoplasmic inclusion bodies (Guarnieri) are present. A mitotic figure (M) can also be seen. (H & E × 450).

Results. The observations of Goodpasture and his group that human skin would grow quite readily on the chick chorioallantois were confirmed. Of 245 grafts only 13 were unsuccessful. Bacterial contamination was rare in our experience, although fungi such as *aspergillus* or *candida* occasionally grew out. A successful "take" was accomplished by active proliferation of the epidermis and the establishment of chick circulation in the human corium, as indicated by nucleated chick erythrocytes in the blood vessels of the corium (Fig. 1). This epidermal proliferation produced an increasingly thick, horny layer, which was desquamated after 2 or 3 weeks of continuous passage. If the epithelium failed to grow, the histologic picture was quite distinct. The epithelial cells assumed a light pink color with a small, pyknotic, dense nucleus, and there was none of the usual evidence of multiplication such as mitotic figures or increase in thickness. It was further demonstrated that the human skin could be passed serially at weekly intervals from egg to egg, and retain distinctive epithelium (Fig. 2). Of 124 regrafts, 7 failed to take.

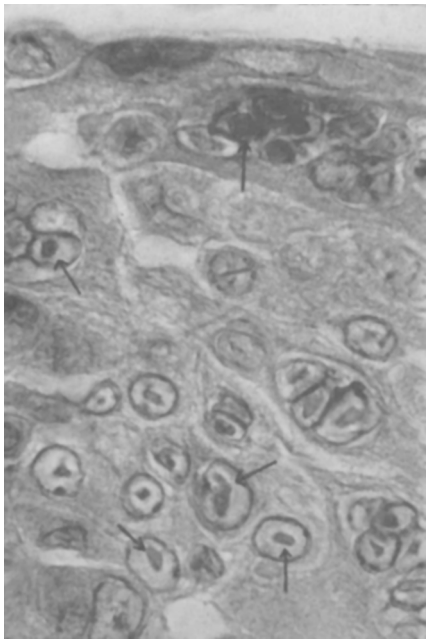

FIG. 5.
Epithelium of grafted human skin infected with herpes zoster 6 days before fixation. Almost every nucleus shows marginated chromatin and contains a characteristic inclusion body. (H & E × 450).

By inoculating skin which had thus been kept alive for 4 weeks (27 days) with herpes simplex virus, and incubating for an additional 48 hours, it was possible to demonstrate the formation of characteristic intranuclear inclusion bodies in the epithelial cells of the skin graft. This was considered addi-

tional proof of the viability of the growing epidermis (Fig. 3). If the skin is inoculated with vaccinia virus, characteristic cytoplasmic basophilic inclusion bodies are formed in the epithelial cells (Fig. 4).

In addition, we were able to grow the virus of herpes zoster on one occasion (Fig. 5). This was done by inoculating skin which had been growing on the chorioallantois for 24 hours. The inoculum was untreated vesicle fluid taken from a patient with herpes zoster ophthalmicus of 48 hours' duration. The skin was removed after 6 days' additional incubation. The microscopic appearance of the infected area, including the presence of a large number of characteristic intranuclear inclusion bodies, confirms Goodpasture's observation in his single successful cultivation of the zoster virus.[3]

Summary and Conclusions. (1) Human skin may readily be grafted onto the chorioallantoic membrane of embryonated eggs. (2) The prepuce removed by usual surgical circumcision is a satisfactory source of adequate amounts of skin. (3) Penicillin and streptomycin obviate most bacterial contamination. (4) Grafted human skin may be passed serially from egg to egg at weekly intervals and remain viable. (5) Herpes simplex and vaccinia grow readily on grafted human skin, with the formation of characteristic inclusion bodies. (6) A successful inoculation with herpes zoster confirms the previous single successful cultivation of this virus outside of the human body.

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Atrio-Ventricular Anastomosis: An Additional Valve.

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In vascular surgery during the last few years communications have been established experimentally and therapeutically between blood vessels. Little has been done in surgery of the heart involving the formation of anastomosis between the cavities of this organ. Schepelmann[1] and Dimitrieff[2] were the first to create artificial communications between the atria or the ventricles. Neither of these workers tried to produce a new opening that would join an atrium to a ventricle.

If such an anastomosis could be created with valvular properties it would be of value in pathological changes of the valves. In stenosis of the mitral valve, for example, the blood which has been dammed back in the atrium could stream through the new pathway into the left ventricle. In insufficiency, on the other hand, the anastomosis could serve as a safety valve guarding against the rising pressure in the atrium. These points, however, remain to be settled experimentally.

Dimitrieff[2], Cutler[3] and Souttar[4] have shown that various operations on the auricular appendage are well tolerated. The appendage also represents the shortest anatomical pathway for producing a connection between atrium and ventricle. Furthermore, by using it for the atrio-ventricular anastomosis, valvular requirements can be fulfilled. Because of its funnel shaped structure, it is most suitable for transformation into a valve and for implantation into the ventricle. In an anastomosis using the appendage, the blood will flow from a broad base to a narrow apex, whereas back flow through this structure would be difficult. The experiments to be described

1 Schepelmann, E., *Deutsche Z. f. Chir.*, 1912, **120**, 562.

2 Dimitrieff, I. P., *Zentralbl. f. Chir.*, 1926, **53**, 715.

3 Cutler, E. C., and Beck, C. S., *Arch. Surg.*, 1929, **18**, 403.

4 Souttar, H. W., *Brit. Med. J.*, 1925, **2**, 603.