importance. It is noteworthy that, among 5 samples which had been used in studies on humans, the four which had given frequent untoward reactions showed a much greater complement-fixing capacity with *S. typhi* antiserums than did the fifth sample which had given no untoward reactions. This apparent correlation was of course not evidence that the difference in the tendencies to give untoward reactions was due to the difference in the capacities of the samples to react with *S. typhi* antibodies. The data are insufficient to permit definite conclusions. Nevertheless, until evidence to the contrary is obtained, the capacity of clinical dextrans to react with *S. typhi* antiserums is one of the factors to take into account in the use of those products. Theoretically, the importance of this particular factor would vary in different geographical and occupational groups, depending upon the frequency of prophylactic typhoid vaccination and upon the incidence of typhoid infection. With some people, the capacity of dextrans (or of accompanying substances) to cross-react with other antibacterial antibodies might be more important.

Summary. Solutions of dextran, both in the unhydrolyzed or native form and in the partially hydrolyzed or clinical form, gave precipitation reactions with antiserums of rabbits immunized with *S. typhi,* including vaccines prepared for human typhoid prophylaxis. Reactions also occurred with antiserums of some other *Salmonella,* particularly *S. oranienburg.* Among 5 samples of clinical dextran that had previously been used in studies on humans, 4 that had given fairly frequent untoward reactions had greater capacities to fix complement with *S. typhi* antiserums than did the one that had given no untoward reactions.

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Proliferation and Vascularization of Adult Human Epithelium in Subcutaneous Tissues of X-Irradiated Heterologous Hosts.* (19133)

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Recently a preliminary report was made on the growth and transplantation of human neoplasms in the subcutaneous tissues of x-irradiated rats and mice(1). The present paper describes the proliferation ad vascularization of normal adult human epithelium in similarly treated rats and hamsters. The findings are of interest in that successful transfer of tissues between heterologous species has been considered as possible only when embryonic or malignant tissues are used(2).

Material and methods. Young female albino rats 30-50 days of age (Carworth Farms) were used as hosts in all 6 experiments and 50-70 g female hamsters (*Mesocricetus aurats*) were employed in 3. These animals were of "weanling" age since it had been learned that human tumors could be supported successfully in such x-irradiated hosts for approximately 15 days, in contrast to the 8-10 days reported for older animals (1). Half of the experimental animals were x-irradiated, both the rats and the hamsters receiving a total of 2 doses of 150 r each on alternate days from two 180 kv tubes (one above, one below the animal) with the last irradiation treatment usually on the day prior to implantation. The other half of the ex-

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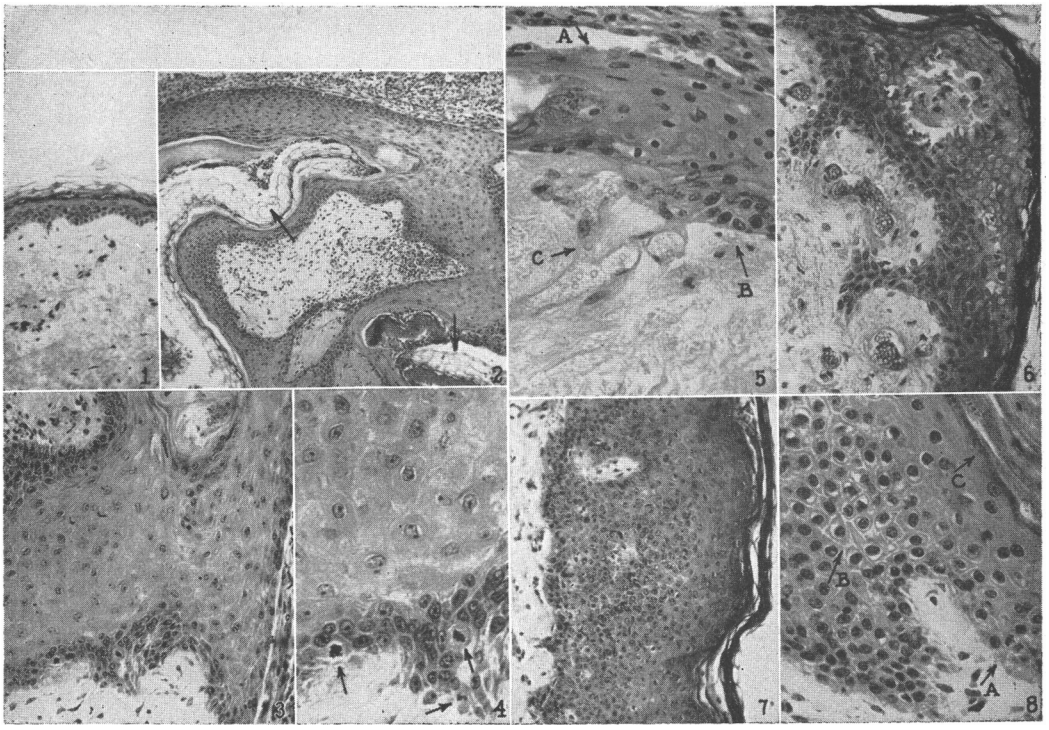

FIG. 1. Original human epithelium, used for one experiment, from chest wall of 63-year-old female. × 80.

FIG. 2. Material from Fig. 1 after 10 days in the subcutaneous tissues of an x-irradiated hamster (arrows point to cysts formed by the epithelium). × 43.

FIG. 3. Magnification of Fig. 2. × 100.

FIG. 4. Detail showing mitotic activity in basal layer (arrows). × 200.

FIG. 5. Four day section of human breast epithelium in x-irradiated rat. Note death of original prickle cells (arrow A). A few basal cells (arrow B) are still alive and associated with a new capillary (arrow C). × 200.

FIG. 6. Six day section of human breast epithelium in an x-irradiated rat. New capillaries are abundant. The basal layer is prominent and in good condition. Sloughing of the original prickle cell layer is still occurring. × 100.

FIG. 7. 10 day section of human breast epithelium (same experiment as Fig. 5 and 6) in x-irradiated rat. Note great increase in thickness of epithelium. × 80.

FIG. 8. 12 day section of human epithelium from chest wall in an x-irradiated rat. The various cell layers are well defined: the basal layer with a mitotic figure (arrow A); the layer of prickle cells with prominent intracellular bridges (arrow B); and the *stratum granulosum* bordering a cyst formed by the epithelium (arrow C). × 200.

perimental animals were normal untreated controls. Human epithelium was obtained in 5 of the 6 experiments from freshly removed surgical material, 2 from the chest wall and 3 from the breast. The sixth specimen was abdominal skin from an autopsy performed 2 hours after the patient's death. Donors were adult females varying from 43 to 72 years of age. Five of these had a breast or rectal adenocarcinoma at a site other than the source of epithelium; a sixth had a benign breast lesion. As soon as the skin was obtained, it was stretched and as much underlying dermis as possible removed by gentle scraping. The epidermis, handled with aseptic technic, was then cut into strips and placed in a petri dish containing sterile Ringer's solution (buffered to a pH of approximately 7.3 with 3 mg of glucose and 300 units of penicillin added per cc) where it was minced with scalpels until the suspension would pass through a No. 18 needle. 0.5 cc of such a concentrated suspension was injected subcutaneously into each flank of the experimental animals. The hamsters received implantations in their cheek pouches(3) as well. In each experi-

ment no less than 18 rats and 12 hamsters (if used) were implanted, so that one normal and 2 irradiated rats and 1 normal and 1 irradiated hamster could be killed at approximately 3-4, 7, 10, 14, 18 and 25 days after the implantation for histological material. There was some variation in the different experiments as to days of killing.

Findings. Though the results of the implantations were most uniform and successful in rats, the overall microscopic findings were the same regardless of the species of experimental animal used. In the case of the hamsters, there was no difference in any one animal, between material implanted in the subcutaneous tissues of the flank and that inserted in the cheek pouch. A general description of the histological sections will therefore be given.

Three days after the minced epithelium had been implanted it was found surviving in comparable condition in both normal and x-irradiated animals. The epithelial fragments with their small amount of attached dermis lay unvascularized and unincorporated in the host tissues. They were often seen forming small cysts—apparently at this time by a process of stretching of the existing cells rather than by mitotic activity since few cells were dividing. The original prickle cells usually were sloughed off or pyknotic and were rarely in good condition. The basal cells, however, appeared to be in fair to excellent shape with an occasional mitotic cell even in the normal un-irradiated animals.

On approximately the fourth day after implantation, host capillaries penetrated the collagen and elastic fibers of the surviving human dermis in the x-irradiated animals (Fig. 5). With one exception (a normal rat killed 4 days after implantation which showed several young host capillaries) the material in the control animals was never vascularized. Though a few epithelial pieces might be found with surviving cells and even with an occasional mitotic figure in the normal hosts as late as the 7th day, by far the majority of the fragments at this time and in these animals were dead. None survived after this period. By contrast, the human epithelium in the x-irradiated hosts was well vascularized (Fig. 6) and actively proliferating on the 6th or 7th day. Mitotic figures were common and appeared to be confined to the basal cells which is of interest in view of Thuringer's(4) opinion that in normal human epidermis the lower one third of the prickle cell layer contains more mitotic figures than the basal layer. By the 10th to the 14th day the fragments of human epithelium had considerably increased their original diameter (Fig. 1), often as much as tenfold (Fig. 2-4, 7 and 8). It should be noted that here and there among the large cysts "nests" of epithelium were often seen, made up of small groups of cells which had become separated during the implantation.

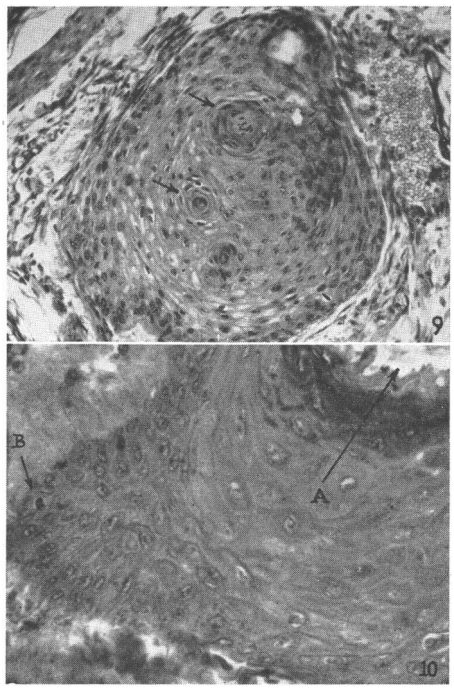

FIG. 9. Typical small "nest" of human epithelial cells in an x-irradiated rat 8 days after implantation. Attempts to form cysts by such a small group of cells produces the "perls" seen here (arrows). × 100.

FIG. 10. Human epithelium originally from the chest wall (Fig. 1) after 3 generations (31 days) in x-irradiated rats. Cysts are still being formed (arrow "A"). Arrow "B" points to a mitotic figure in basal cell layer. × 300.

3. Lutz, B. R., Fulton, G. P., Patt, D. I., Handler, S. H., and Stevens, D. F., *Canc. Res.*, 1951, v11, 64.

4. Thuringer, J. M., *J. Invest. Dermat.*, 1939, v2, 313.

These cells, in attempting to form the usual cysts, often produced what appeared to be typical "perls" instead (Fig. 9).

At approximately 14-16 days, the implanted tissues in the irradiated animals apparently died quite suddenly. In all the 18 and 25 day sections the human epithelium was dead. The cell nuclei were entirely pyknotic, the basal layer separate and shrunken away from the underlying and remaining dermis, and the whole invaded by host cells, particularly polymorphonuclear leukocytes. Lymphocytes, though present, were not conspicuous. Many of the "new" capillaries contained an amorphous basophilic material, not identified. Considerable pigment was found among the basal cells of these late sections. (A small amount had been seen in the 12 and 14 day tissues).

Transplantation. In 4 experiments the material found at the implantation sites in x-irradiated rats killed 10 days after injection, was pooled, minced and re-implanted in other x-irradiated rats. Viable epithelial tissue was found in this second group of irradiated animals 11 days after the implantation in three of the 4 transfer attempts. A second transfer was made of the 3 surviving tissues to a 3rd group of irradiated rats. Two of the three transfers had vascularized epithelial cysts with cells in mitosis 10 days later. Fig. 10 shows such cells 31 days after the original material was obtained from a human patient and more than 2 weeks after the implanted material had ceased to survive in the original group of x-irradiated animals. No transplants beyond the third generation were attempted.

Discussion. Recently considerable interest has been aroused in the intraocular heterologous transplantation of human tissue, since Greene and his coworkers(2) have maintained that such transplantation is a diagnostic test for malignancy. It is the premise of these workers that only embryonic tissues and tumors which have attained a high degree of malignancy can be grown with success heterologously. In view of this opinion, it is noteworthy that the experiments herein described, show that normal human epithelium as well as tumors can become vascularized and can increase as much as tenfold in the subcutaneous tissues of x-irradiated heterologous hosts, and that, by transfer into new groups of these animals, both the normal and malignant human tissues can be maintained in "healthy" and proliferating condition.

The success or failure of the human tissue to grow does not appear to depend upon its autonomy but rather to be a function of the heterologous host resistance after alteration by previous x-irradiation. It should be noted that other normal or benign human tissues, such as colloid goiter, can be grown and transferred, in flourishing condition, in the subcutaneous tissues of x-irradiated heterologous hosts as will be reported in a later paper.

Summary. In 6 experiments, minced fragments of normal adult human epithelium implanted subcutaneously in previously x-irradiated heterologous hosts (rats and hamsters) became vascularized, proliferated and increased for approximately 15 days. After this period they died suddenly. In un-irradiated control animals the epithelial material survived in poor condition for 3-7 days but did not become vascularized. When transferred every 10 days to new groups of x-irradiated animals, human epithelium remained viable and in active mitosis for the duration of the experiment (3 generations or 31 days).

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