Growth of Human Embryonic Tissues in Cortisone-treated Laboratory Animals.*† (21178)

HELENE WALLACE TOOLAN

From the Sloan-Kettering Institute for Cancer Research, New York City.

Previous papers have reported the subcutaneous growth and serial transfer of human neoplasms and normal tissues in x-irradiated and/or cortisone-treated laboratory animals (1-5). Human embryonic tissues have been implanted also in cortisone-treated small animal hosts and successfully maintained, as will be noted herein. Although propagation of human embryonic tissues in heterologous hosts is not new since Greene(6) has reported growth of such material in the anterior chamber of the rabbit eye, the following preliminary observations are presented as offering a comparatively simple and easily reproducible procedure for growing human embryonic lung and other embryonic tissues in rats and hamsters.‡

Materials and methods. Six human embryos derived from therapeutic abortions and varying in age from 10-16 weeks furnished the material for this study. All tissues were received within 2 to 6 hours after operation of the maternal donor. Samples of lung and cartilage were obtained in 6 cases; skin in 4, intestine and trachea in 2, and liver in one. As soon as available, the tissues were minced with scalpels, suspended in a buffered Locke-Ringer's solution (ordinary isotonic saline was also satisfactory), and implanted subcutaneously in the flank of weanling rats x-irradiated 1-3 days previously with 150 r total body x-irradiation. The number of rats implanted varied from one to 6, depending on the amount of tissue. Immediately after implantation, the animals were injected subcutaneously in the nape of the neck with 3 mg of cortisone. Three similar doses of this drug were given on alternate days following: *i.e.*, if the implantation and first injection were on a Monday, they received subsequent injections on the next Wednesday, Friday and Monday.§ Two weeks after implantation, the rats were sacrificed and (in the case of the last 3 embryos received) the best growths removed and cut into pieces of approximately 25-40 mg each. These were implanted, according to the method of Lutz *et al.*(8), in the cheek pouches of anesthetized (nembutal) weanling, non-irradiated hamsters which received 3 mg of cortisone subcutaneously at the time of implantation and one mg about every 5th day following. The tissues were allowed either to remain in these hamsters or they were transferred to other hamster hosts similarly treated or to another group of x-irradiated and cortisone-treated rats. In some instances both the first and second generations were placed in rats and in one case embryonic lung was transferred for 7 generations (100 days) in rats alone. However, for reasons which will be given, the optimal procedure was found to be to use the rat as a "screening" host and the hamster thereafter. Details of all the methods and materials mentioned herein have been described previously(3,5).

The implanted tissues could be examined at any time by anesthetizing the hamster hosts, pulling out the pouches and viewing the contents thereof.

Results. All of the human embryonic tistues which were implanted survived and grew in the x-irradiated and cortisone-treated rats

* This investigation has been aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research, the National Cancer Institute of the U. S. Public Health Service, the American Cancer Society, and the Damon Runyon Memorial Fund for Cancer Research.

† The cortisone acetate (Cortone, Merck) used for these experiments has been supplied through the courtesy of Merck and Co., Rahway, N. J.

‡ A recent note has appeared by Handler, and Yerganian(7) on the growth of cartilage and brain from a 40 mm human embryo in cortisone-treated hamsters.

§ If radiation facilities are not available, non-irradiated rats can be used if the cortisone dosage, given in the manner described, is increased from 3 to 6 mg per injection.

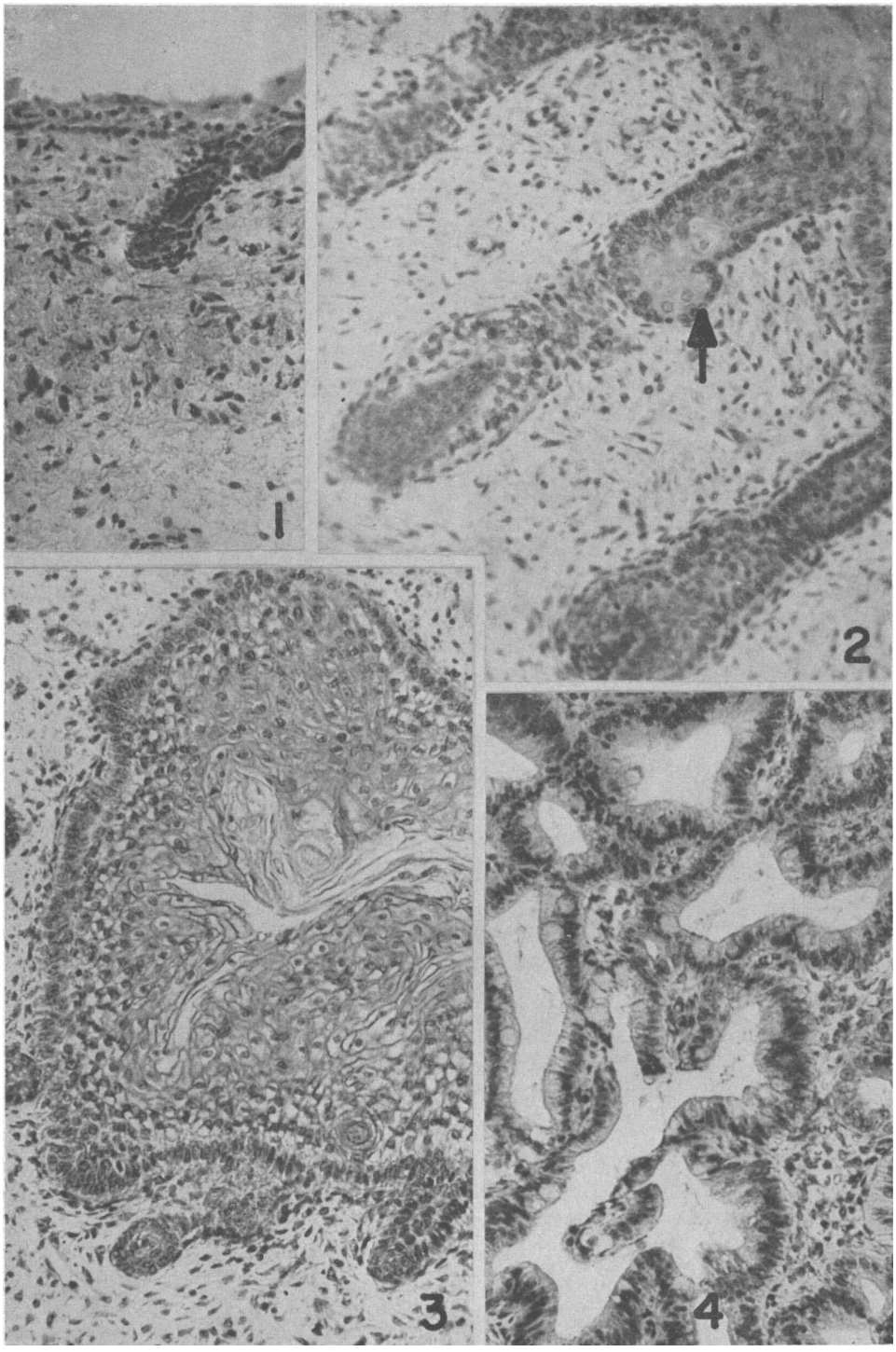

FIG. 1. Original skin obtained from a 16-week-old human embryo. A primitive hair follicle is present. ×160.

FIG. 2. Skin from embryo of Fig. 1 after 2 generations (27 days) in the subcutaneous tissues of x-irradiated rats treated with cortisone (generation 1 = 13 days; generation 2 = 14 days). Hair

follicles are well developed and arrow points to sebaceous gland. ×320.

FIG. 3. Cyst of human embryonic skin in the same animal as Fig. 2. Note multiple layers and differentiation. ×160.

FIG. 4. Embryonic intestine of a 16-wk human embryo after 2 generations (47 days) in cortisone treated laboratory animals (generation 1 = 14 days in x-irradiated rat; generation 2 = 33 days in hamster's pouch). Goblet cells are prominent. ×160.

with the exception of the one sample of liver. Varying degrees of differentiation occurred in the first and subsequent transfer generations. Skin which was 3-4 layers thick with a few primitive hair follicles in the original embryo (Fig. 1) developed, after 27 days in rats, into well differentiated integument (Fig. 2 and 3) with mature hair follicles and associated sebaceous glands. Intestine, in the 2 samples observed for 30-47 days, did not change much in character (Fig. 4) although there was a tendency for this material to produce cysts which were lined by intestinal cells piled upon one another and sloughing into the central areas. Such formations were obviously a result of the abnormal environmental conditions. Areas of healthy proliferating smooth muscle cells were often seen in these implants.

Cartilage grew very well in all the animals and gradually became calcified (Fig. 6). Of note was the finding that primitive cartilage appeared in association with embryonic trachea or lung bronchioles (Fig. 5,10) long after the initial implantation of these tissues. Apparently it was formed from the embryonic mesenchyme or connective tissue in which mitotic figures were common. Typical bone formation by osteoblasts was observed (Fig. 7).

Of the tissues implanted, embryonic lung apparently underwent the most immediate comparative maturation. The immature typically fetal lung composed of small lobules imbedded in a mass of spongy connective tissue (Fig. 8) changed to a postnatal type of lung tissue after one generation in the animal hosts (Fig. 9-11). As far as could be determined, this picture was produced by a dilatation of all the lobules and bronchioles with fluid from the host animal. After this initial alteration, further changes were less startling although the lung tissues grew slowly but progressively whether maintained in rats for 7 generations (100 days) or, in another case, implanted in hamsters for 43 days after 2 generations in rats (27 days).

Complete descriptions of the different types of host-transfer combinations which were employed after the initial generation in rats, would entail detailed charts for each embryonic tissue specimen implanted: *i.e.*, transfer to the second generation was often in both rats and hamsters and implanted tissues from these, in turn, might supply either rats or hamsters or both in the third. Such manipulation led to the conclusion that the simplest and most effective method for growing embryonic tissues is to make all initial implantations in rats, a procedure which takes less time and skill than implantation into the hamster pouch. From the implanted embryonic material harvested 14 days later, a selection is then made of obviously choice areas. These can be cut up for placement in the hamster pouch wherein they may stay over long periods of time—thus furnishing excellent material for experimental use which can be viewed at any time. Further, the embryonic tissues do not increase in mass in the manner of the rapidly growing transplantable tumors(4,5) so that it is desirable to have a site such as the hamster pouch in which they can remain and develop. Constant transfer every 14 days from rat to rat is traumatic to the tissues and causes a loss which, for slow growing tissues, is of import.

Since this study is as yet in its preliminary stages, it is not known just how long the embryonic transplants can be maintained. Material from the first 3 embryos obtained was discontinued for microscopic examination after 47-100 days in laboratory animals. Lung, cartilage, skin, and intestine from the other 3 embryos are still being carried by cortisone-treated hamsters and, in some instances, x-irradiated rats. The oldest of these is now 8 weeks and is in good condition. It would seem that a long term experimental procedure, employing this material, is feasible.

It should be noted that samples of all the embryonic material received were implanted

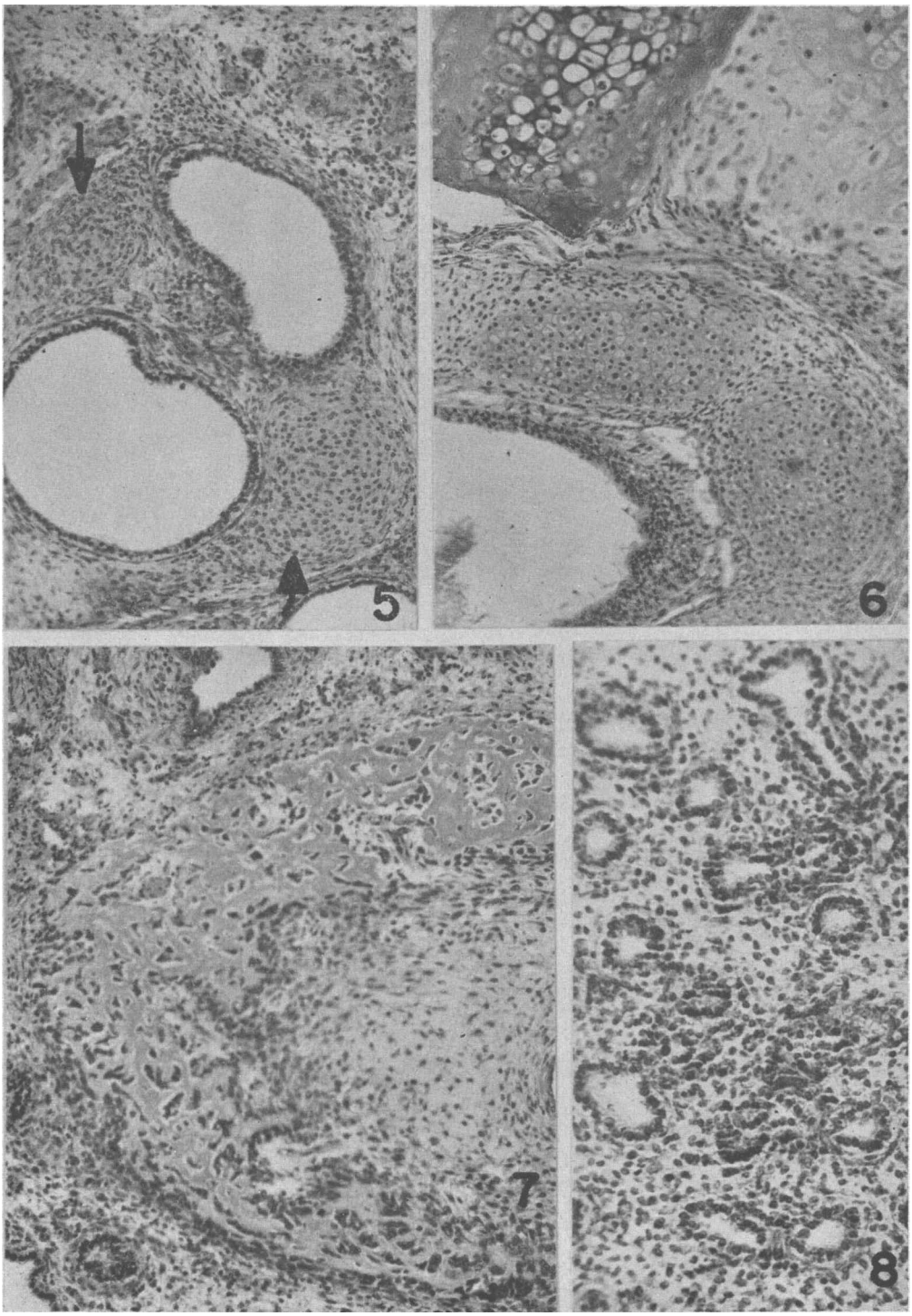

FIG. 5-7. Implants of minced human embryonic chest cage (trachea plus small amount of lung, rib and humerus from a 12-wk embryo) after 2 generations (28 days) in x-irradiated rats. Fig. 5 shows primitive cartilage (arrows) associated with embryonic bronchioles. In Fig. 6 trachea with its surrounding young cartilage is at bottom of picture and pieces of cartilage in various stages (probably derived from humerus) are above. Fig. 7 shows bone formation by osteoblasts. All ×90.

FIG. 8. Original embryonic lung from 16-wk human embryo. Note small tubules lined by columnar epithelium and imbedded in a connective tissue matrix. × 160.

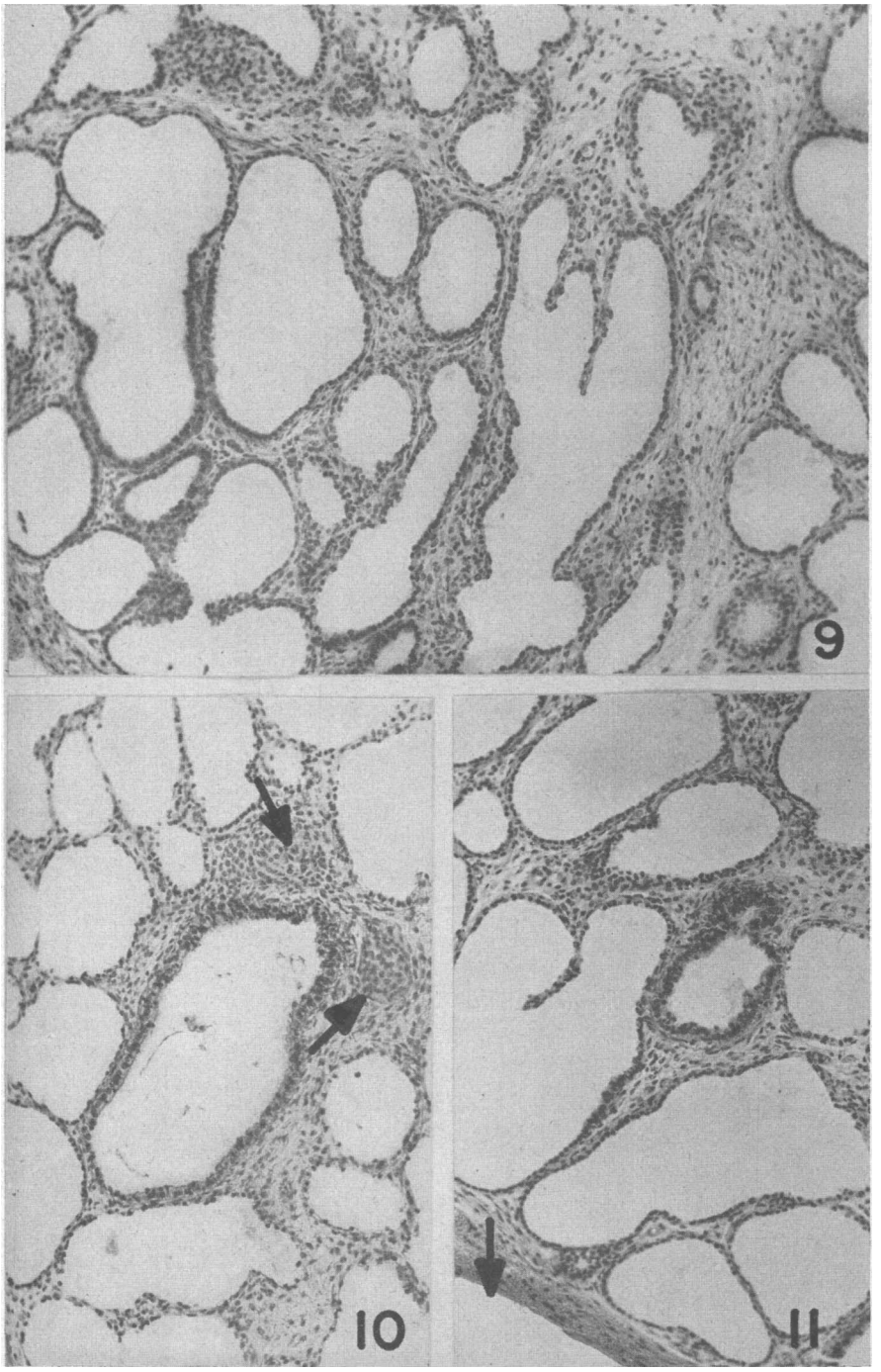

FIG. 9-11. Same lung as Fig. 8 after 47 days in cortisone-treated animals (G1 = 14 days in an x-irradiated rat; G2 = 33 days in hamsters). Each of these tissues was removed from a different hamster. Fig. 9 shows post natal appearance of dilated lobules. Fig. 10 again demonstrates primitive cartilage associated with a bronchiole and apparently derived from surrounding mesenchyme. Fig. 11 illustrates how little host reaction toward human tissue occurs (arrow points to edge of implant in hamster pouch). All ×90.

in roller tube tissue culture‖ at the same time they initially were implanted in rats. After the first generation of growth in these animals (and often after 2 or 3 generations of transfer), some of the harvested embryonic material was again placed in tissue culture. Without exception, much better *in vitro* growth was obtained from the rat grown tissue than the original material. The possible explanation for this finding will be discussed elsewhere(5).

Summary. Human embryonic tissues particularly lung, skin, cartilage, and intestine have been propagated in x-irradiated rats or nonirradiated hamsters both treated with cortisone. The method of growing these tissues is simple and so uniformly reproducible that it offers a good source of human embryonic material for experimental use and study.

‖ All tissue cultures were done in the laboratory of Dr. Alice Moore of The Sloan-Kettering Institute.

1. Toolan, H. W., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 572.
2. ———, *ibid.*, 1951, v78, 540.
3. ———, *Canc. Res.*, 1953, v13, 389.
4. ———, *Proc. Am. Assn. Canc. Res.*, 1954, v1, 49.
5. ———, *Canc. Res.*, 1954, (to be published).
6. Greene H. S. N., *ibid.*, 1943, v3, 809.
7. Handler, H. H., and Yerganian, G., *Proc. Assn. Canc. Res.*, 1954, v1, 18.
8. Lutz, B. R., Fulton, G. P., Patt, D. I., Handler, A. H., and Stevens, D. F., *Canc. Res.*, 1951, v11, 64.

Received June 23, 1954. P.S.E.B.M., 1954, v86.

Preparation, Characterization, and Antigenicity of a Saline Soluble Fraction From Shigella Types. (21179)

JOSEPH G. TULLY AND JOSEPH T. TAMURA.

From the Department of Microbiology, College of Medicine, University of Cincinnati, and the Cincinnati General Hospital, Cincinnati, Ohio.

Several investigators have extracted and characterized the somatic antigen of *Shigella sonnei* and the *Sh. paradysenteriae* (Flexner) group(1,2). The complete antigen appears to be a complex of polysaccharide, phospholipid, and protein. The polysaccharide of the antigenic complex determines specificity and it can elicit antibodies in man and the mouse, but not in the rabbit. The protein fraction appears to be the most toxic component of the complex. Numerous attempts have been made to reduce the toxicity of the complete antigen(3) but decreased antigenicity usually accompanies reduced toxicity.

Vaccines made of whole cells have been used in prophylactic immunization against bacillary dysentery. Most preparatory methods(4,5), and most extraction methods for somatic antigens, include the washing of cells with saline solution, an act that may cause a significant loss of antigen from the cells. The successful extraction of Vi antigen from *Escherichia coli* with isotonic saline has recently been reported (6,7). A similar method was used here for the extraction of shigellae. This report concerns the preparation, preliminary analyses, and some immunogenic properties of saline soluble antigens from 3 *Shigella* types.

Materials and methods. The cultures used in this study were *Sh. sonnei,* strain (Ch); *Sh. paradysenteriae, Flexner III,* strain (Be); and *Sh. paradysenteriae, Flexner II,* strains (Km) and (PR).* The organisms were grown in a chemically defined medium (Table I). The (Km) strain of Flexner II required 17 g of dibasic phosphate and 5 g of glucose per liter of this medium. Cultures were incubated with shaking for 24 hours at 37°C in liter quantities. The cultures were centrifuged and the packed cells resuspended in cold acetone and dried on filter paper. One liter of the chemically defined medium yielded approximately 2 g of dry cells.

* Obtained through the courtesy of Dr. M. L. Cooper, The Children's Hospital Research Foundation, Cincinnati, O.