portionately, keeping the GFR/TmG ratio unchanged. The reason for the lack of ACTH effect in these 2 children is not apparent. However, in case 7 the lack of response may be related to the short period of ACTH administration, (6 days).

Conclusions. Glycosuria resulting from ACTH administration in premature infants is due to an imbalance of glomerulotubular function demonstrated by a rise in GFR/TmG ratio. The cause of the rise in this ratio varied and was due either to a disproportionate rise in glomerular filtration rate or fall in glucose Tm. The nonglycosuric ACTH-treated infants did not show this rise in the GFR/TmG ratio.

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Received July 29, 1952. P.S.E.B.M., 1952, v81.

A Glass Wool Matrix for Roller Tube Tissue Cultures.* (19935)

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The isolation and identification of the poliomyelitis group of viruses by means of tissue culture has stimulated interest in the application of the roller tube technic to viral and rickettsial agents in general(1-6). The above method involves imbedding fresh tissue in a chicken plasma clot and the cytologic examination of the fibroblast outgrowth at intervals. The use of a clot as a matrix for growing cells has a number of recognized disadvantages among which are listed the following: 1) preparation and preservation of plasma and chick embryo extracts is time-consuming and costly; 2) there is often clouding and liquefaction of the clot; 3) subcultures are tedious and exacting to prepare; 4) staining procedures often necessitate sectioning; 5) the composition of plasma and embryo extract is variable and chemically undefined; 6) plasma may contain contaminating viruses. Some of these objections may be circumvented by means of the glass wool roller tube technic herein described.

Methods. A very thin mat of glass wool† is cut into an approximately 2¼ by ¾ inch piece. This is placed between two metal blocks measuring 1¾ inches long and ¼ inch wide, and the free edges of the fibers are fused with a flame. The glass mat is then washed with ethyl ether and sterilized in the oven at 160°C for 1 hour. Heart or chorioallantoic membrane from a 10-day chick embryo is minced in a petri dish containing 1 part Simms' ox serum ultrafiltrate and 3 parts Hanks' balanced salt solution(5), and a concentration of 100 units of penicillin and 0.1 μg of streptomycin per ml. Three to 6 fragments of thoroughly washed tissue are arranged lengthwise along the inner surface of a pyrex glass tube measuring 16 x 150 mm. The glass wool mat is then placed over the tissue, pressed down gently and the tube cooled for a few seconds in an ice water bath. With a capillary pipette 2-3 drops of sterile, liquid, 2.5% washed agar-agar in saline are placed over the glass mat at the extreme top and bottom. The tube is again immersed in the ice bath in order to solidify the agar. This procedure serves to fix the mat to the sides of the tube and to trap the tissue beneath the glass wool. One

* Aided by a grant from the National Foundation for Infantile Paralysis.

† Pyrex Brand Filtering Fibre; Owen-Corning Fiberglas, Corning, N. Y.,

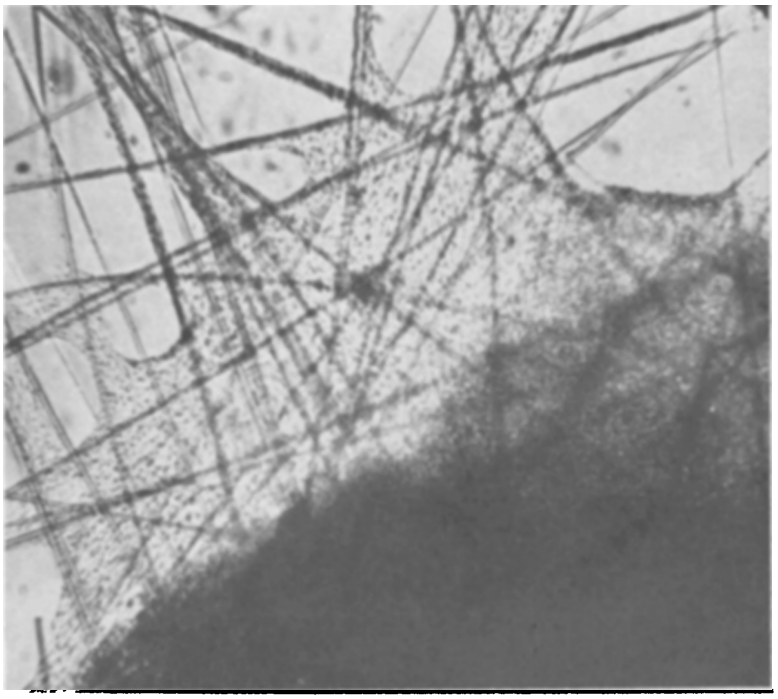

FIG. 1. Fibroblast outgrowth from a fragment of chick embryo heart under a glass wool matrix after 48 hours incubation at 35°C. Note the proliferation along the margins of the fibers and subsequent filling of interstices. × 65.

ml of nutrient fluid consisting of chick-embryo extract (1 part), unheated horse serum (1 part), Simms-Hanks 1:3 mixture (8 parts), and antibiotics in the concentration previously indicated is then added. The tubes are stoppered and incubated at the selected temperature in the roller drum. If a combination clot and glass wool matrix is desired the following procedure is employed. One drop of chicken plasma is added first and the tissue fragments are arranged in the plasma. The glass wool mat is pressed down over the tissue and 3 drops of chick embryo extract is dropped on the mat and spread over the surface. When clotting is complete the ends of the mat are fixed to the tube with agar-agar. Nutrient medium is then added and the tubes are stoppered and incubated.

Results. Experience with glass wool preparations has been largely confined to whole chick embryo, chorioallantoic membrane, chick-heart muscle, and human testicular tissue. The experiments have been designed to test the efficacy of the glass wool matrix as compared with the standard clot when an accepted type of nutrient medium is used. Under such conditions the fibroblasts begin to proliferate within 12 hours and grow out along the glass fibers. By 24 hours, a few of the spaces between fibers are filled with a film of cells, and by 48 hours, an irregular zone of growth approximately 1-2 mm in diameter may be observed (Fig. 1). If the nutrient fluid is changed as soon as the pH becomes slightly acid, the outgrowth gradually enlarges until the major portion of the matrix is covered with a thick sheet of fibroblasts.

Transplantation is accomplished by removing the glass wool mat to a petri dish containing balanced salt solution. The zone of outgrowth is cut with scissors into 2 mm squares which are then placed in a second tube. The explant is fixed to the side of the tube with a new glass wool sheet as previously described. Fresh nutrient fluid is added and the tube is returned to the rotator apparatus. If transplantation is successful, the fibroblasts invade the glass wool beyond the original explant within 48-72 hours.

Tissue outgrowths on glass wool mats lend themselves particularly well to staining procedures since portions can be removed, placed on slides and permitted to air dry. Any type of fixation and staining may be utilized depending upon the needs of the investigator. Permanent preparations are sealed under a cover slip and can be examined at leisure under all magnifications of the light microscope. In the thicker portions of the slide the cells are arranged in several layers and tend to be crowded together. At the periphery of the outgrowth the organization of the tissue and the cytology of the individual cell is clearly visible.

Discussion. The present communication is concerned with the substitution of the glass wool matrix for the plasma clot in the roller tube cultivation of chicken, human, and other tissues for reasons previously outlined. From the results obtained it seems likely that tissues which can be grown readily, such as chick embryo, will propagate as well on a glass wool matrix as they do along the surface of a glass tube. For more fastidious types of cells it may be necessary to incorporate the mat into a homologous or heterologous clot. Thus with human fibroblasts from adult testicular tissue, growth on glass wool has not been as luxuriant as with the chicken clot alone. This problem is being investigated.

Strips of perforated cellophane sheets which have been fixed to the side of the tube with agar-agar will also support outgrowth of fibroblasts from chick-embryo fragments. The cells, however, tend to disintegrate and cannot be visualized clearly because of artifacts in the cellophane. Furthermore, the cellophane mats are difficult to handle, slip along the sides of the tube, and fold over. Transplantation, therefore, is cumbersome. A comparison between cellophane and glass helices has already been reported by Shannon, Earle and co-workers(7,8). In these studies the glass proved as satisfactory for growing large numbers of cells as the cellophane. They also employed glass wool as a three dimensional substrate for mass cell culture with some success but discarded this matrix in favor of glass helices(9). A combined cellulose sponge and clot substrate has been described by Leighton(10) for the propagation of mouse mammary adenocarcinoma and chick embryo tissue. The method, however, does not permit observation of individual cells and requires sectioning prior to staining. Other types of matrices such as agar-agar, cotton, and starch particles have not supported fibroblast outgrowth under the experimental conditions imposed(11).

Summary. A simple tissue culture technic is described in which a thin glass wool mat, either alone or in combination with a plasma clot, is employed. The use of glass wool as a matrix for supporting growing tissue fragments circumvents some of the objections to a plasma clot in roller tube cultures.

The technical assistance of Virginia Jentoft is gratefully acknowledged.

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Received September 9, 1952. P.S.E.B.M., 1952, v81.