

IN VITRO GROWTH OF MOUSE HAIR ROOT

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Abstract. Hair roots from mouse dorsal skin continued to grow in culture medium for 10 h or more after isolation. Under observation by time-lapse cinematography, the cultured hair roots appeared to grow downward. We observed both the downward growth of the cultured hair roots as well as the transfer of melanosomes from melanocytes to cortical cells. The hair roots were also capable of incorporating the labelled amino acid and thymidine.

Key words: Hair roots; Culture medium; Cortical cells; Melanocytes; Time-lapse cinematography

Although it has long been known that isolated hair follicles can survive for some time in culture medium, biodynamic observations have never been made on such hair follicular cells (1, 2, 3, 4). In the present investigation, hair roots were isolated from mouse skin and cultured in Rose chambers (5). Under time-lapse cinematography, the cultured hair roots appeared to grow downward and it was also seen that melanosome transfer from melanocytes to cortical cells takes place in the hair root. During this

time, ^3H -thymidine and ^{14}C -leucine were added to the culture medium and became incorporated into the hair follicular cells. Hair roots cultured in this way can serve as a quite useful model for studying the various pharmacological and hormonal effects on hair growth.

MATERIALS AND METHODS

Dorsal skin from 5-10 day old C57BL/6 mouse was sterilized by wiping with 70% alcohol and skin specimens, measuring 1×1 cm were excised with scissors. The specimens obtained were rinsed thoroughly with Tyrode's solution and the dermal connective tissue as well as fat tissue were separated carefully with fine forceps so as not to damage hair roots. The specimens were then torn into small pieces with a pair of fine needles under a binocular microscope in such a way as to contain a few hair roots in each. Each specimen was then sandwiched between two cellophane dialysis membranes and hair shafts were firmly attached to the chamber walls, placed in the centre of the coverslip of the Rose chamber. The medium was then introduced into the chamber via a hypodermic needle

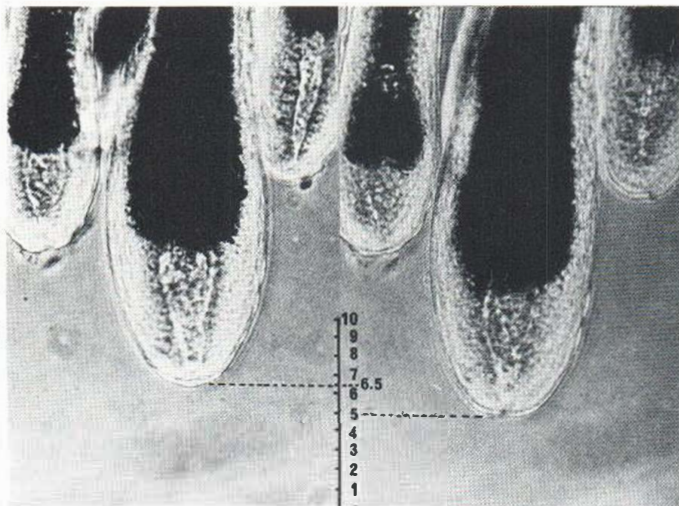


Fig. 1. One division on this scale shows 10 μm . Hair follicles obtained from 7-day-old C57BL/6 mouse were cultured in Ham F12 medium. Phase contrast lenses were used. Left: at 0 time, right: cultured for 6 h.

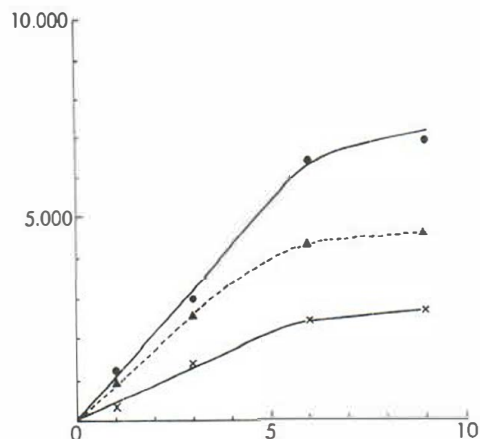


Fig. 2. Abscissa: cpm/hair; ordinate: time (h). Time course of incorporation of ^3H -thymidine and ^{14}C -leucine into the cultures hair follicles. Δ --- Δ : ^3H -thymidine, $\bullet$ — $\bullet$: ^{14}C -leucine, $\times$ — $\times$: ^{14}C -leucine+actinomycin-D ($5 \mu\text{g}/\text{ml}$).

through the rubber ring. The culture medium used was Ham's F12 medium (Nissui Seiyaka Co. Ltd.) supplemented with 20% fetal bovine serum (Flow Laboratories, Rockville, Md., USA). The hair roots thus incubated were observed under the microscope with phase contrast and/or transmission lens. The cellular interaction between cortical cells and melanocytes was also observed, with time-lapse cinematography.

To determine the hair root viability, $5 \mu\text{Ci}$ of ^3H -thymidine ($10.5 \text{ Ci}/\text{mM}$, Daiichi Pure Chemicals Co., Ltd.) and $5 \mu\text{Ci}$ of ^{14}C -leucine ($280 \text{ mCi}/\text{mM}$, Daiichi) were added separately to 2 ml of the medium in which isolated hair roots were cultured. After various periods of cultivation, hair roots were taken out and rinsed thoroughly with Tyrode's solution, after which individual roots were sepa-

rated and five to six roots were dissolved in 0.2 ml formic acid and subjected to radioactivity determination. The radioactivity was counted with a Beckman liquid scintillation counter.

RESULTS AND DISCUSSION

We observed that under these conditions the hair root continued to grow for a period of about 10 h. Fig. 1 shows two photographs taken at the beginning of the culture (left) and after 6 h of incubation (right). The growth was measured as the distance from the original level of the hair root tip which was marked at 0 time on the surface of the incubation chamber. The hair root elongated downward by about 0.03 mm during a period of 10 h, thus far observed. Since the hair shafts were firmly fixed by the chamber walls, the elongation of the hair root can only be considered as a growth of the hair. Such growth was observed with some hair roots, though not all. The elongation of the hair roots observed is not due to degeneration nor to hydration swelling, since the following biochemical experiments show that these hair follicular cells are quite active in protein and DNA biosynthesis. Time courses of incorporation of ^3H -thymidine and ^{14}C -leucine into hair follicular cells are shown in Fig. 2. As a control study, $5 \mu\text{g}$ of actinomycin-D together with ^{14}C -leucine was added to the culture medium. The subsequent procedures were exactly the same as in the experimental series. The rapid incorporation of radioactive substances continued up to 6 or 7 h, but actinomycin-D inhibited protein biosynthesis.

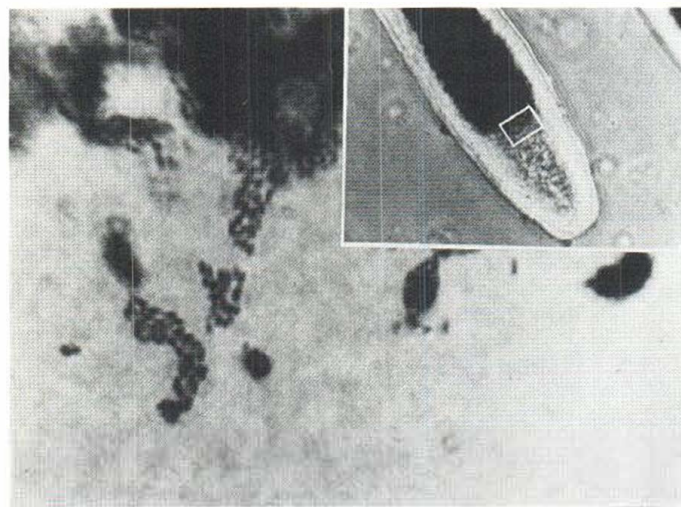


Fig. 3. Light microscopic photographs of cultured hair follicle and melanocytes with dendrites containing numerous melanosomes. $\times 1000$, Inset $\times 70$. As the photograph was taken under transmitted light, the cortical cells surrounding the melanocyte are not visible.

Under the time-lapse cinematography, the transfer of melanosomes from melanocytes to cortical cells was observed clearly in the hair root. As shown in Fig. 3, the dendritic processes of melanocytes loaded with numerous melanosomes are extended among the cortical cells (the cortical cells are not visible under the transmission lens used in this particular photograph) and melanosomes are transferred *en masse* from the tip of a dendrite to the cortical cells (6). When cytochalasin B was added to the culture medium, the dendrites retracted rapidly and melanocytes rounded up and became smaller in size. The melanosome clusters which had transferred to the cortical cells remained there.

These observations provide further evidence to show that the follicular cells, including melanocytes and cortical cells, are both metabolically and biologically active: thus these surviving hair follicles can be utilized as a model of hair growth in various studies.

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