Appearance of growth-inhibiting activity (G_1 chalone) during ontogenetic development of rat and chick epidermis in vivo and in vitro[1]

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Summary. A comparative study was carried out between the stage of embryonic development of epidermis and its content of growth-inhibitory activity (G_1 chalone). Injections of aqueous extracts from keratinized fetal rat or chick embryo epidermis led to a depression of DNA-synthesis in adult mouse epidermis, whereas extracts from undifferentiated epidermis did not contain such an activity.

Based on a theoretical concept of Weiss and Kavanau[3], it was proposed by Iversen[4] and others that tissue growth is controlled by negative feedback in that the postmitotic cells of the differentiated compartment produce specific factors which inhibit proliferation of immature cells in the same tissue. Those factors have been called chalones[5].

Convincing evidence for the existence of chalones has been provided for epidermis. 2 factors have been described which control either the entry of cells into the phase of DNA synthesis (G_1 chalone)[6,7] or the G_2-M transition (G_2 chalone)[8]. When cell layers of adult mouse epidermis were separated, most of the G_1 chalone activity was found in differentiated, i.e. keratinized layers[9]. Here we show that during skin ontogenesis epidermal G_1 chalone activity appears concomitantly with the onset of keratinization.

A 1st set of experiments was carried out using fetal rats. As shown in figure 1, the epidermis of rat fetus starts to keratinize exactly at day 18 of fetal life. At day 17 the tissue still consists only of non-keratinized cell layers and periderm[10].

To measure growth-inhibiting activity, extracts from epidermal tissue in different stages of development were prepared. The isolation of the epidermis was performed by the acetic acid method with exception of day-17-fetus where only an extract from the whole skin could be made.

The activity was tested by determining the incorporation of ^{3}H-thymidine into DNA of adult mouse back skin epidermis 15 h after i.p. injection of the extracts, i.e. at the time point when the G_1 chalone effect reaches its maximum[11]. As a control, 2 mg of an extract from adult mouse epidermis was injected which led to a 50% inhibition of DNA labelling. Epidermal extracts (2 mg) from 18-21-day-old fetal rats show the same inhibitory effect. In contrast, no inhibition could be achieved with extracts from 17-day-old fetal skin, even with a dose of 8 mg (figure 3).

These results provide evidence for a connection between inhibitor production and epidermal keratinization. They are hampered, however, by the fact that at the critical stage of development (day 17) a preparation of pure epidermis was impossible. Therefore, further experiments were made using tissue cultures of chick embryo epidermis. This system offers the possibility of inducing the differentiation of a fully unkeratinized epithelium into keratinized epidermis under controlled conditions, for example by means of serum or epidermal growth factor[12].

Sheets of undifferentiated chick embryo epidermis were isolated at day 9 of embryonal development by means of collagenase digestion of skin, placed on millipore filters and grown in the presence of 15% fetal calf serum[12,13]. After a cultivation period of 8 days, a thick keratinized layer had developed (figure 2).

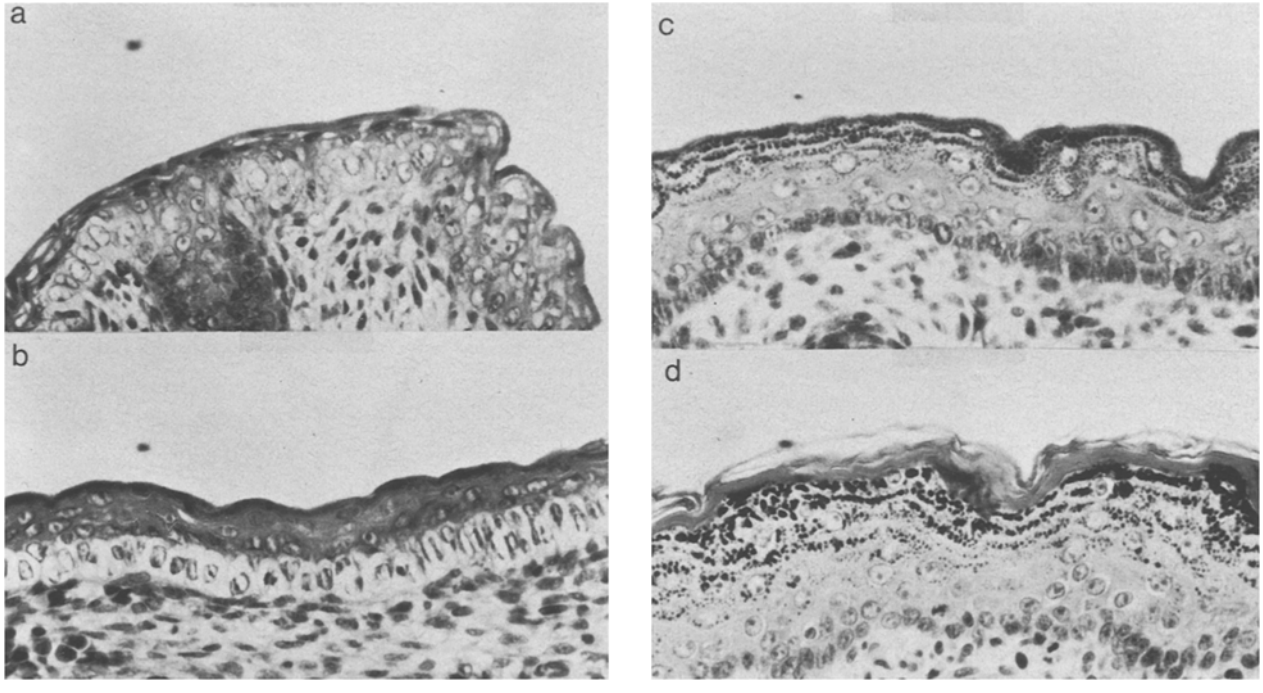

Fig. 1. Cross sections through fetal rat skin at an age of 17 (a), 18 (b), 19 (c), and 21 (d) days. Note the development of a heavily keratinized epithelium. H & E stain, × 300.

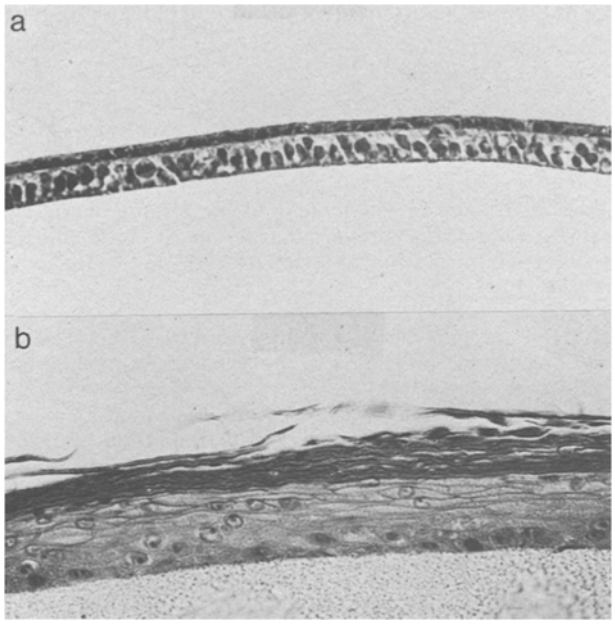

Fig. 2. Cultivation of chick embryo epidermis. Explants of back skin of 9-day-old chick embryos (white leghorn) were dissected. The epidermis was separated from connective tissue by incubation with 0.1% collagenase (Boehringer Mannheim) in Dulbecco's phosphate-buffered saline (PBS) at 37 °C for 25 min. The epidermal sheets thus obtained were placed on millipore filters (Solvinert, pore size 0.25 µm) with the peridermal side facing upwards. The filters were placed on agar platforms and incubated in plastic Petri dishes (Falcon, 3.5 cm diameter) with 1.5 ml of Eagle's minimal essential medium supplement with 15% fetal calf serum at 37 °C in a humidified atmosphere containing 5% CO_2 for a period up to 8 days. The medium was changed every 3 days.

The figure shows cross sections through an unkeratinized explant at the beginning of the experiment (a) and through a fully keratinized explant after 8 days (b). H & E stain, × 400.

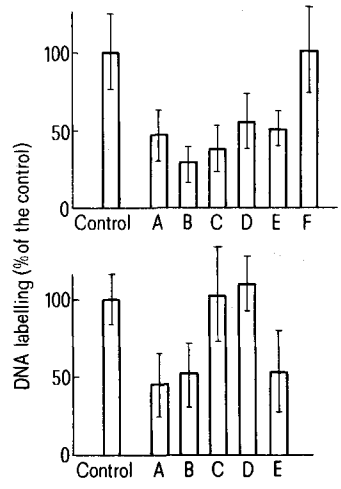

Fig. 3. Effect of skin and epidermal extracts on DNA labelling in dorsal mouse epidermis in vivo. Epidermis from adult mice (after chemical depilation, Bertsch et al.[15]), 3-day-old chicken or fetal rats was isolated by incubating the back skin in 1% acetic acid at 4 °C for 18 h. Chick embryo skin and epidermis were prepared as described for figure 2. Extracts were obtained by homogenizing the tissue in water and stirring at 4 °C for 30 min. The suspension was centrifuged at 105,000 × g and the supernatant was lyophilized and used for the experiments. The lyophilized extracts were dissolved in 0.3 ml 0.9% NaCl solution and i.p. injected into female mice (strain NMRI, age 7–8 weeks). Controls received 0.9% NaCl solution. 14 h later the DNA was labelled by i.p. injection of 30 µCi of ^{3}H-methylthymidine (NEN, Bokston, USA). After an additional h, the animals were killed and the epidermal DNA was isolated and checked for radioactivity as previously described[17]. Upper diagram: *A*, 2 mg mouse epidermis extract, *B–E*, extracts from fetal rat epidermis (2 mg each, *B*, 21 days, *C*, 20 days, *D*, 19 days, *E*, 18 days), *F*, 8 mg whole skin extract from 17-day-old rat fetus.
Lower diagram: *A*, 2 mg mouse epidermis extract, *B*, 2 mg chick epidermis extract, *C*, 6 mg whole skin extract from 9-day-old chick embryos, *D*, 2 mg epidermis extract from 9-day-old chick embryos, *E*, 2 mg extract from 9-day-old chick embryo epidermis after cultivation for 8 days.

Ordinate: specific radioactivity of epidermal DNA in percent of the control (49±12 cpm/µg DNA, N = 40). N = 12, ±SD.

Again extracts were prepared from undifferentiated as well as from keratinized cultures and tested for G_1 chalone activity as described above. As shown in figure 3, extracts from epidermis keratinized in vitro inhibited epidermal DNA labelling in vivo to the same extent as extracts from adult chick or mouse epidermis. In contrast, extracts from undifferentiated tissue did not show such an effect. To exclude that the inhibitory factor was destroyed by the collagenase treatment 6 mg of an extract obtained from untreated whole skin of 9-day-old embryos were injected; again no inhibitory effect could be observed.

The resistance of the activity to proteolytic digestion, as well as its species non-specificity, are consistent with results obtained with purified epidermal G_1 chalone[14].

With the reservation that inhibitory activities rather than defined chemical entities have been demonstrated, these results lend support to the concept of tissue-own growth control by negative feedback. It has to be emphasized, however, that the production of chalone-like inhibitors during functional expression, i.e. keratinization, does not necessarily go along with an increased response of the tissue to these factors, since as indicated by experiments with newborn mice, the G_1 chalone responsiveness of epidermis is developed in a separate ontogenetic step[15].

Regarding our data, recent experiments are comprehensible showing that a non-keratinizing epidermal carcinoma failed to produce DNA synthesis-inhibiting factors, whereas a keratinizing squamous cell carcinoma contains such an inhibitory activity[16].

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