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The Development of Ordered Structure in Neonate Rat Epidermis

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The establishment of a columnar pattern of organization in rat backskin and earskin was examined by using frozen sections expanded in alkaline buffer and by labeling with ^{3}H-TdR and autoradiography. An adult columnar pattern of organization was established earlier in backskin than earskin. In both tissues the appearance of cell columns was related to a decreasing rate of cell proliferation and, for ear, to a decreasing rate of lateral growth of the epidermis.

An alignment of flattened suprabasal cells of the epidermis to form columnar units of structure has been demonstrated in man, other primates, rodents and birds [1–5] but the initial establishment of this pattern of organization has not been studied. A preliminary examination of rat earskin showed that epidermal cell columns were not present at birth, but were present 2 weeks later. The histological appearance of rat epidermis during the first weeks after birth was therefore examined and, as a number of observations suggest that the formation of cell columns in adult tissues is related to the rate of basal cell proliferation [6,7], a measure of cell proliferation during this period was obtained by labeling with tritiated thymidine (^{3}HTdR). Some additional data were collected concerning the rate of increase in area of the ear during the period of growth.

MATERIALS AND METHODS

Timed-pregnancy Sprague-Dawley rats were purchased (Flow Laboratories, Dublin, VA) and tissues collected from the offspring on days 1, 2, 3, 5, 7, 10, 15 and 20 after birth. On the morning of each day of tissue collection, 2 animals were removed from the litters, injected subcutaneously in the abdominal region with 1 μCi of tritiated thymidine (Schwartz-Mann methyl-labeled, S.A. 1.9 Ci/ml) per gm body weight and killed 1 hr later.

One ear and a strip of backskin lateral to the midline of each animal were frozen in isopentane cooled in liquid nitrogen. Specimens of ear and backskin for frozen sections were also collected from rats 12 weeks old to examine the structure of fully mature epidermis. Frozen tissues were sectioned in a cryostat at a thickness of 8 μm, picked up on cover slips and fixed in 1% acetic acid in 70% ethanol for 20 min. These sections were then stained for 1 min in 0.01% methylene blue and mounted in half-strength Sorensen-Walbum buffer [8] at pH 12.5 to expand the stratum corneum. Additional frozen sections were fixed in formalin and stained with hematoxylin and eosin for routine histological examination.

The other ear and further strip of backskin were taken from each animal for autoradiography. These tissues were fixed in buffered neutral formalin solution, processed for wax embedding, sectioned at 6 μm and mounted on subbed slides. Using standard methods [9], slides were dipped in Kodak NTB2 emulsion, exposed for 4 weeks at 4°C, developed, fixed and stained with Harris's hematoxylin. A labeling index for the epidermis of each specimen was obtained by counting the labeled nuclei in the interfollicular epidermis of 20 randomly selected microscope fields (diameter 340 μm). Nonserial sections of each specimen were used for counts and cells with 5 or more silver grains overlying the nucleus were counted as labeled. The total number of interfollicular "basal" cells per field of vision was also counted. The "basal" cell region was defined using criteria of cell morphology and the observed level of cell labeling. For the first few days after birth it was more than one layer of cells in thickness.

To provide a measure of the rate of increase in surface area of the ear during the period of growth, the area of the dorsal surface of the ears of 2 animals was measured on days 1, 3, 6, 9, 13, and 27. To compare the rate of interstitial growth of the epidermis with the rate of growth of the ear as a whole, 3 tattoo marks were made on the dorsal surface of the ears of these animals once they had reached a sufficiently large size (day 6). The area within these marks was determined on days 6, 9, 13, and 27. The daily rate of increase in the areas so measured was determined from the percentage increase in area between observations. Because of the variable and extended periods between observations, these data were corrected to a mean daily compound growth rate for the period by using formulae to obtain rates of compound interest.

To provide an estimate of the length of the DNA synthesis phase in neonate epidermis, a litter of 6 rats (18 hr old) was injected subcutaneously with 0.05 ml ^{3}H-TdR per rat (1 μCi/gm body wt.) and 2 hr later with 0.05 ml ^{14}C-TdR (0.1 μCi/gm body wt.). Specimens of backskin were taken 1 hr later and processed for autoradiography using a thick layer of Kodak NTB emulsion. Labeled nuclei were counted, discriminating by track length [9] between those labeled with ^{3}H-TdR alone and those labeled with ^{14}C-TdR with or without concommitent labeling with ^{3}H-TdR.

RESULTS

The hematoxylin and eosin sections of neonate rat backskin showed that the epidermis was thicker than that of the adult consisting at this stage of 6–8 layers of nucleated cells, a thick and densely staining stratum granulosum and a well-formed stratum corneum. Developing hair follicles were regularly spaced and had penetrated 200–300 μm into the underlying dermis. During the first 10 days after birth the epidermis became progressively thinner. Emergence of developing hair shafts was first seen on day 5. The appearance of earskin at birth was similar to backskin except that hair formation was at an earlier stage with the developing follicles only just beginning to invade the dermis. Epidermal thinning occurred more slowly and the eruption of hair did not occur until after day 7.

The appearance of alkali-expanded sections of ear and backskin at various times after birth is shown in Fig 1 and 2 and that of the adult rat in Fig 3. At birth, the stratum corneum of the epidermis of both earskin and backskin consisted of 10–12 layers of flattened keratinized cells. A continuous superficial layer of cells showed an intense patchy staining with methylene blue and a discontinuous layer of cells with this appearance remained at the surface until about day 3 (Fig 1*a, b*). An increasingly ordered pattern of cell alignment within the stratum corneum was observed with time after birth. On day 1, neither region showed a clearly detectable alignment of stratum corneum cells but an ordered structure comparable to adult skin, with alignment of 2–3 layers of nucleated cells beneath a columnar pattern throughout the thickness of the stratum corneum, was established by day 7 in backskin and by day 10 in earskin.

In backskin, some alignment of cells in the stratum corneum was clearly apparent by day 3. Although at this time columns were not present through the full thickness of the sectioned stratum corneum, runs of 4–8 aligned cells were seen passing through the stratum corneum at acute angles (Fig 1*b*). By day 5 the stratum Malphighii was still somewhat thicker than that of the adult (Fig 3**b**) but columns of cells passing through the entire thickness of the stratum corneum were seen. By day 7

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Abbreviations:

^{3}HTdR: tritiated thymidine

backskin showed a regular vertical alignment of cells both in the stratum Malphighii and the stratum corneum (Fig 1*d*).

In earskin, no appearance of an ordered alignment of epidermal cells was seen before day 5, when some evidence of alignment of the upper stratum Malpighii cells could be detected (Fig 2*b*). By day 7, a nonrandom alignment of the cells in the stratum corneum was clearly apparent but the cell columns so formed were less regular than those of the adult. By day 10 the ear epidermis was still somewhat thicker than that of the adult (Fig 3*a*) but a clear pattern of alignment of the 3–4 uppermost

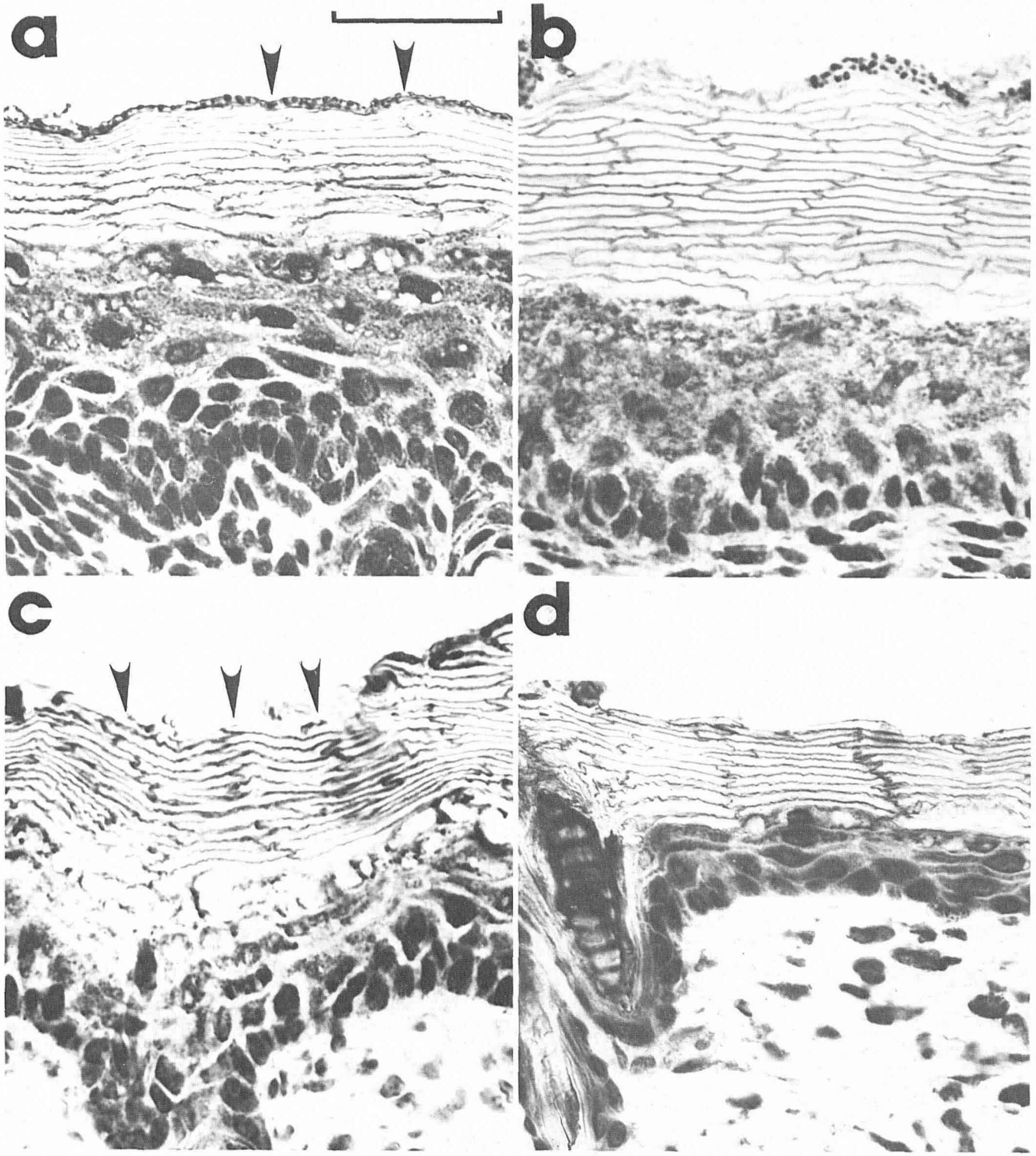

FIG 1. Frozen sections of backskin stained with methylene blue and treated with alkaline buffer; (*a*) First day after birth: the epidermis is well formed with a thick stratum Malpighii and with some 11 layers of cells in the stratum corneum. A continuous layer of periderm remains at the surface. Perhaps some trace of alignment of stratum corneum cells into columns may be observed (*arrows*). (*b*) Day 3: the stratum corneum shows evidence of irregular alignment of cells. A discontinuous layer of periderm remains at the surface and the number of layers of cells in the stratum corneum has increased. (*c*) Day 5: alignment of cells into columns running through the full thickness of the sectioned stratum corneum is seen (*arrows*). (*d*) Day 7: the stratum Malpighii has reduced in thickness and alignment of the superficial nucleated cells beneath regular cell column in the stratum corneum is seen. (Scale = 40 μm).

cell layers of the stratum Malpighii and of cells throughout the full thickness of the stratum corneum was seen (Fig 2*d*). There was still a tendency for the columns in the stratum corneum to be less vertically aligned than in the adult. The appearance of the epidermis on day 15 was essentially similar to that of day 10. No marked change in size of stratum corneum cells of either ear or backskin was observed during the period of observation. For both tissues, measurements of the maximum width of cut profiles of the flattened stratum corneum cells suggested a maximum diameter of about 40 μm at all stages.

The labeling indices obtained for back and earskin at various times after birth are shown in Fig 4*b*. The indices for both tissues were relatively high on day 1, fell progressively until day 10 and then appeared to remain relatively steady. Throughout the period of observation, the indices for ear epidermis were approximately twice as high as corresponding indices for the epidermis of the back. The number of basal cells per unit length fell with time after birth until about day 10 (Fig 4*a*)

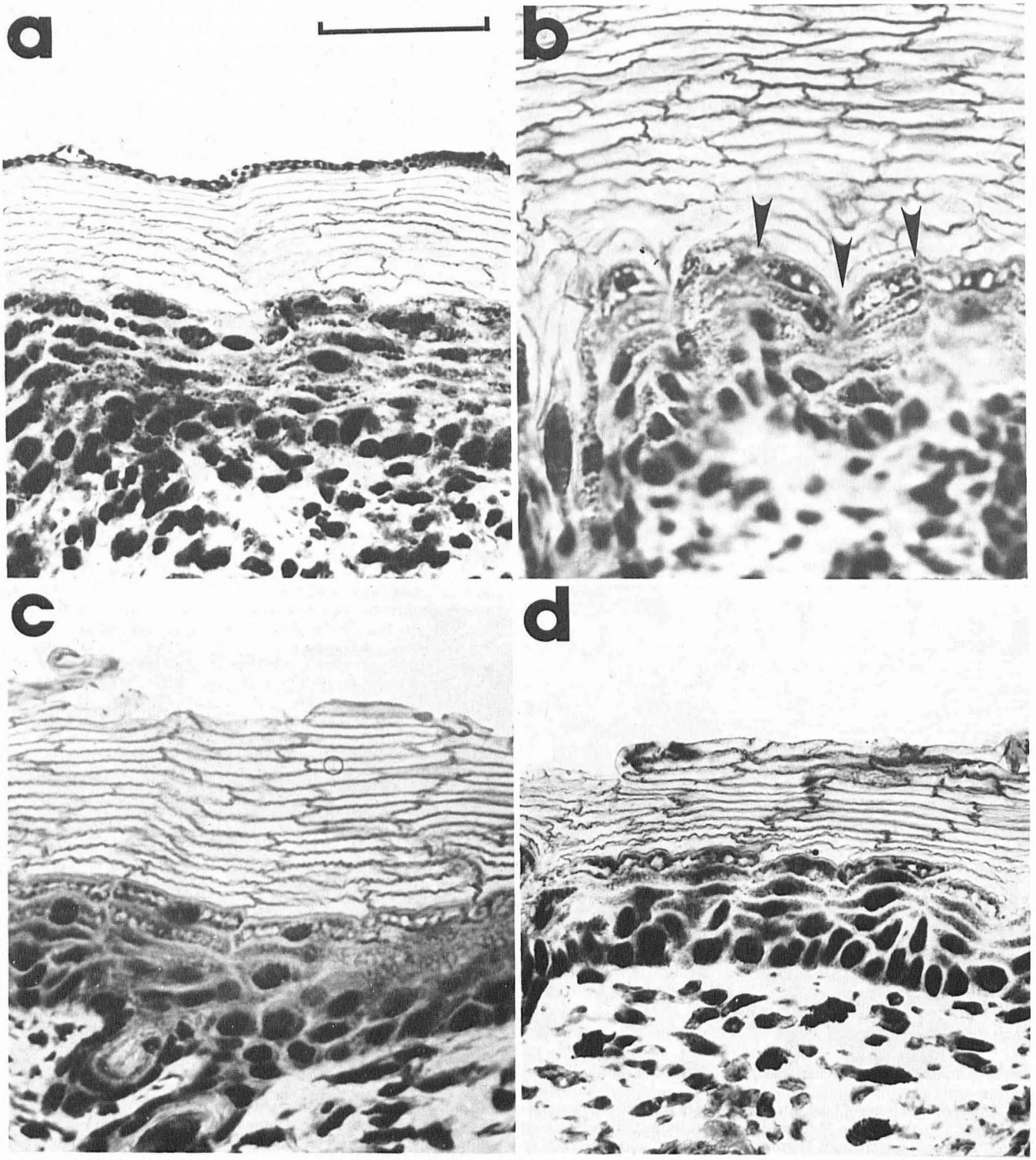

FIG 2. Frozen sections of earskin (*a*) First day after birth: no evidence of cell alignment is detectable. (*b*) Day 5: early evidence of alignment of granular cells is seen (*arrows*). (*c*) Day 7: a pattern of columnar organization is clearly apparent. (*d*) Day 10: this tissue is approaching an adult pattern of organization but is still somewhat thicker and the cell columns in the stratum corneum are less regular. (Scale = 40 μm).

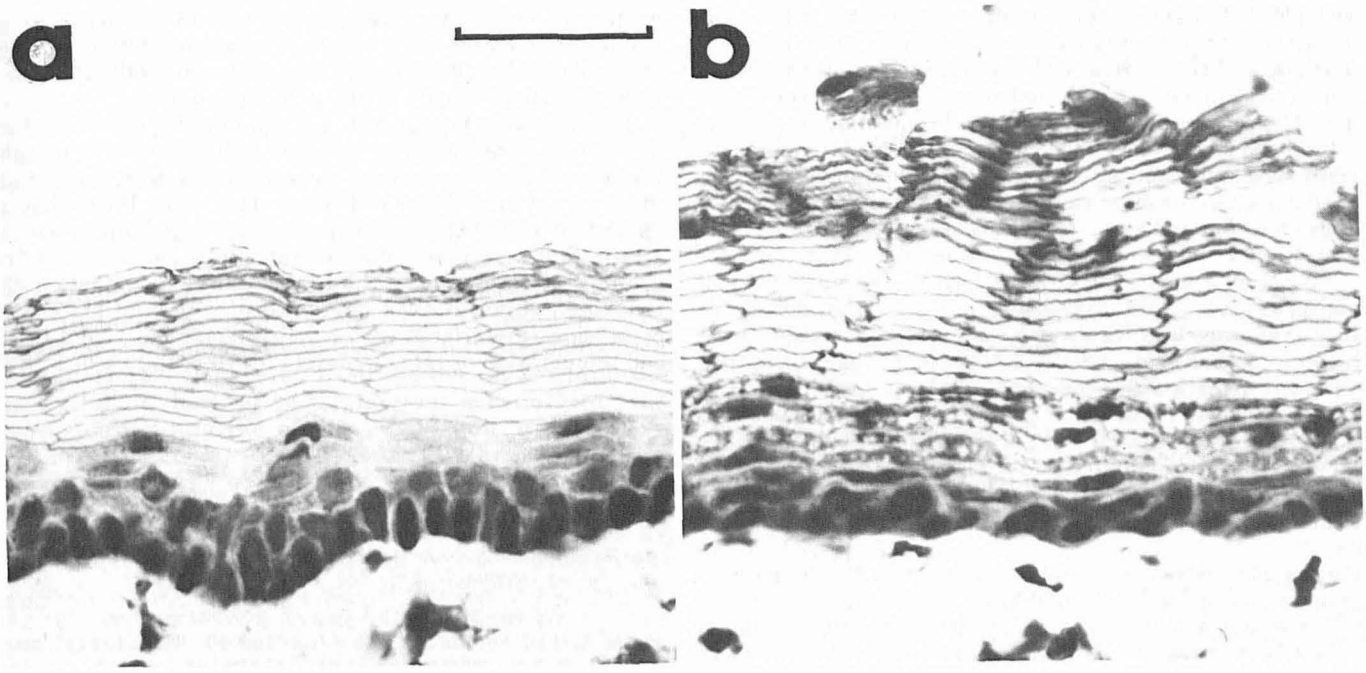

FIG 3. Frozen sections of adult rat; (*a*) earskin and (*b*) backskin. A regular vertical alignment of cells of the stratum corneum and of suprabasal nucleated cells is seen. (Scale = 40 μm).

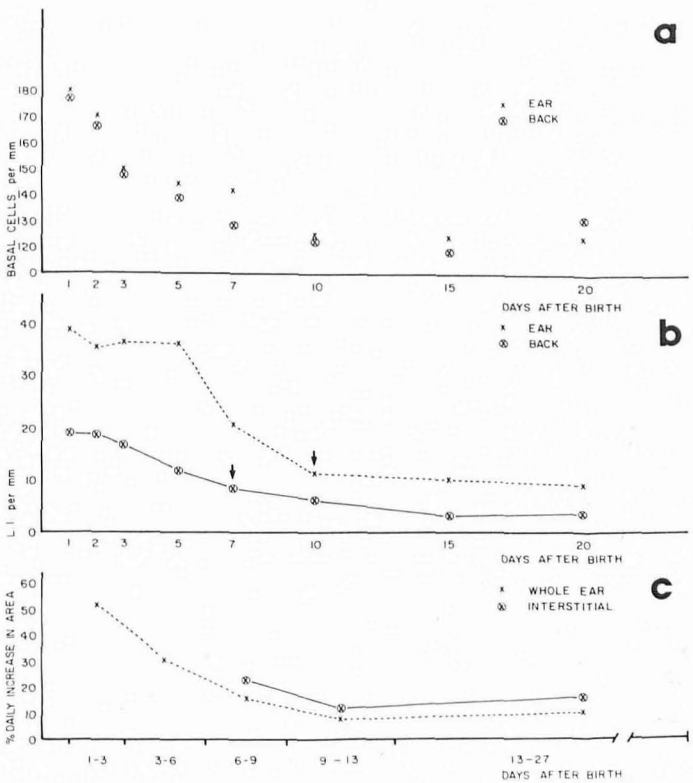

FIG 4. (*a*) The number of basal cells per mm of basement membrane of back and earskin at various times after birth. (*b*) The number of labeled cells per mm of basement membrane of back and earskin at various times after birth. *Arrows* mark the time at which epidermal structure approximated that of the adult. (*c*) The calculated rate of mean daily increase in area of the epidermis of the ear during various periods after birth.

Calculations made for the daily rate of increase of the area of the whole ear and for the area within tattoo marks on the dorsum of the ear are shown in Fig 4*c*. During the first few days after birth, the daily rate of increase in the area of the ear was very high, some 50% per day and this rate fell to about 10% per day after 2 weeks. The rate of increase of the area between tattoo marks was comparable to that of the ear as a whole.

Counts of labeled nuclei in the 6 rats of the double labeling experiment indicated that 26.8 ± 3.5 (SD) of the labeled nuclei were labeled with thymidine alone. Duration of the S phase, was estimated to be 5.4 hr [10].

DISCUSSION

The general histological appearance of the backskin during the first week after birth, and the stages of epidermal thinning and hair follicle formation, correspond to previous descriptions of this tissue [11–13]. Expressed as a percentage of total basal cells, the levels of the labeling indices recorded and their pattern of decrease with time after birth were comparable to the data reported by Stern, Dayton, and Duecy [11].

The densely-staining superficial cells observed in methylene blue stained sections of both earskin and backskin correspond in appearance to periderm. Bonneville [14]) reported that periderm becomes detached from the surface of the epidermis of fetal rat skin during the 2–3 days before birth, but Stern, Dayton, and Duecy [11] reported persistence of the periderm for several days following birth. The persistence of periderm in the present study indicated that desquamation did not start until some days after birth. During the 2-day period between the day 1 and the day 3 observations, the stratum corneum accumulated an additional 6 layers of cells, increasing from 11 to about 17 cell layers in thickness.

An alignment of cells in the stratum corneum developed gradually and it was often difficult to decide subjectively when evidence of a nonrandom pattern of organization was first established. Additionally there was frequently some variability in the appearance within a given specimen. Nevertheless it was clear that an ordered structure was developed earlier in backskin than in earskin. Occasionally possible traces of alignment of stratum corneum cells were seen in backskin even at birth (Fig 1*a*).

A number of observations suggest that a high rate of cell proliferation is incompatible with column formation. For example, cell columns are absent from epidermal regions with a normally high rate of cell proliferation [2,4,15] and are lost from epidermal regions that normally show column formation when the rate of cell proliferation is experimentally or pathologically

raised [15–17]. Explanations of this relationship have been given in terms of competition between simultaneously flattening cells [6] or insufficient time to achieve a minimum surface area [7] or to develop cell surface changes necessary for cell recognition after leaving the basal layer [2]. It is perhaps of interest that backskin, which had a lower rate of cell proliferation than earskin, developed cell columns earlier and that in both tissues, a regular pattern of organization was seen only when rates of cell proliferation had fallen to relatively low levels.

At birth, the rudimentary pinna of the ear is a protruberance approximately 3 x 1.5 mm with a relatively flat interfollicular epidermis. It was therefore puzzling to observe that during the first and second days after birth, the rate of increase in the surface of the ear averaged about 50% per day and from then until day 6 was about 30% per day. The later comparability of the rates of growth of the ear as a whole and of the area between tattoo marks (Fig 4) and the distribution of labeled cells which were scattered fairly uniformly throughout the basal layer of the epidermis, dermis, and central cartilaginous plate suggest that growth is not restricted to growth foci but occurs interstitially.

Estimates of rates of cell proliferation during this period also support a concept of interstitial growth. Using Abercrombie's [18] correction, a measured basal nuclear diameter of 8 μm, a Ts of 5.4 hr and the other data presented in Fig 4 as the basis for calculation, approximately 15,000 cells would be produced beneath 1 mm^2 of epidermal surface in the period from birth to day 3. With a measured width of stratum corneum cells of approximately 40 μm, approximately 24,000 nucleated and stratum corneum cells are present in 1 mm^2 of epidermis on day 1. With the increase in stratum corneum cells being offset by a decrease in basal cells, 1 mm^2 of epidermis on day 3 contains approximately 26,000 cells. Thus, it appears that the rate of cell production during this period (15,000) is greatly in excess of the rate necessary to maintain the epidermis (2,000) unless a rapid rate of interstitial growth is also occurring.

How the epidermis, which is quite well formed and has many layers of stratum corneum cells at birth, is capable of expanding at a rate comparable with the rate of growth of the ear as a whole is uncertain. If the cell layers of the neonate stratum corneum remain firmly adherent to each other during the doubling in surface area which occurs between birth and day 3, a great increase in the area of the more superficial cells would be expected as a result of stretching. However, an increase in length of cut cell profiles within the superficial stratum was not observed in the 3 or 5 day specimens. Possibly the cells within the neonate stratum corneum have the ability to slide laterally over each other during growth and thus allow for lateral spreading of stratum corneum as a whole. Such plasticity of the intercellular cementing mechanism has not been reported. In either case, rapid lateral expansion of the stratum corneum would not seem to be compatible with maintenance of the columnar structure and it may be of significance that alignment of regular cell columns were not observed until the rate of expansion had dropped to about 10% per day.

The mechanisms by which cell columns are established are, at present, uncertain. The observed establishment of morphologically identifiable cell columns in the stratum corneum indicates that it is not until some days after birth that rat epidermal cells express the potential of epidermal cells for establishing an ordered tissue architecture. The absence of cell columns at earlier stages, however, could indicate either that this potential has not yet developed or is present but that its expression is interfered with by such factors as a high rate of cell proliferation or rate of lateral growth of the tissue.

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