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Embryonal feather growth in the chicken

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ABSTRACT

Prenatal feather growth development in the chicken was studied in 7 body regions in HH stages 27–45, using direct measurements, specific histological and immunohistochemical methods, and scanning electron microscopy. The results from measurements of absolute length values, and, particularly, growth rate development in each HH stage revealed a distinct phase of most intensive growth in HH stage 40–41, which was preceded by feather follicle insertion and accompanied by the occurrence of α-keratins in barbule cells. Specific regional evaluation demonstrated that growth in the feather follicles of abdominal skin generally showed the slowest progression from absolute values and that in the feather filaments of the developing wings the most rapid progression occurred during HH stage 40–41 from growth rate values.

Key words: Feather growth; embryo; chicken.

INTRODUCTION

Feathers represent a unique kind of integumental system beautifully adapted to a great variety of biological needs. Such evolutionary uniqueness is also reflected by feather ontogenesis which has been studied extensively, both histologically and ultrastructurally. It has been demonstrated that this highly differentiated skin derivative develops according to complex epithelial-mesenchymal interactions and homeobox gene expression that obviously control induction, growth, histogenesis, and the course of keratinisation (for literature see e.g. Lucas & Stettenheim, 1972; Dhouailly, 1975; Sengel, 1976; Haake et al. 1984; Sawyer et al. 1985; Noveen et al. 1995; Bellairs & Osmond, 1997).

In contrast to these results, information about the growth in length, i.e. axial growth of the embryonal feather, is still lacking. This is surprising in view of the fact that axial growth of the juvenile, adult, and regenerative feather, respectively, was already a matter of scientific interest at a relatively early stage (Juhn, 1931, 1938; Lucas & Stettenheim, 1972). In order to contribute to this knowledge, the present study was designed to give insight into the development of embryonal feather growth. Some histological and immunohistochemical observations are added for a better understanding of specific growth events found.

MATERIALS AND METHODS

53 White Leghorn chick embryos of different age were obtained from the Clinic for Poultry of the School of Veterinary Medicine, Hannover. The animals were staged according to the criteria of Hamburger & Hamilton (1951), extending from 6 to 20 d of incubation (HH stages 27–28 to 45, hatching at 46). The embryos were narcotised and killed by chloroform vapour, fixed for 48 h in Bouin's solution and 4% formol-calcium (Lillie & Fullmer, 1976), serially dehydrated in graded ethanol (15–100%), and embedded in the glycol methacrylate embedding resin Technovit 7100 (Kulzer) (Gerrits & Smid, 1983). In this way, measurements could also be performed very accurately from histological material as Technovit 7100 minimises shrinkage artefacts (Hanstede & Gerrits, 1983). Such advantages became apparent, in particular, during studies on skin ontogenesis (see e.g. Meyer & Görgen, 1986). A series of embryos was embedded via the intermediary Bioclear (Bio-Optika) in paraffin Histoplast (Serva). Several embryos were

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also directly embedded in Technovit 7100 via dehydration in this medium.

Plastic sections (3–5 μm) were stained with haematoxylin (Delafield's haemalum) and eosin, methylene blue-azure II (Morgenstern, 1969), and periodic acid methenamine silver (PAMS) as modified from Jones (1957). Paraffin sections were used for the immunohistochemical demonstration of α-keratins employing peroxidase-labelled rabbit antikeratin (prekeratin) (Medac, from human stratum corneum, diluted 1:10 with phosphate-buffered saline (PBS), pH 7.2; MW ~ 58000–68000 Da; see Moll et al. 1982). The latter sections were developed in a diaminobenzidine solution according to Yamada & Shimizu (1977).

After careful dehydration in ethanol (see above), several skin specimens were critical-point-dried through CO_2 in a Polaron E 3100 Series I drying apparatus. Finally all specimens were sputtered with gold (100 nm layer) in a Balzers SCD 040 sputter coater and viewed in the Zeiss DSM 940 scanning electron microscope.

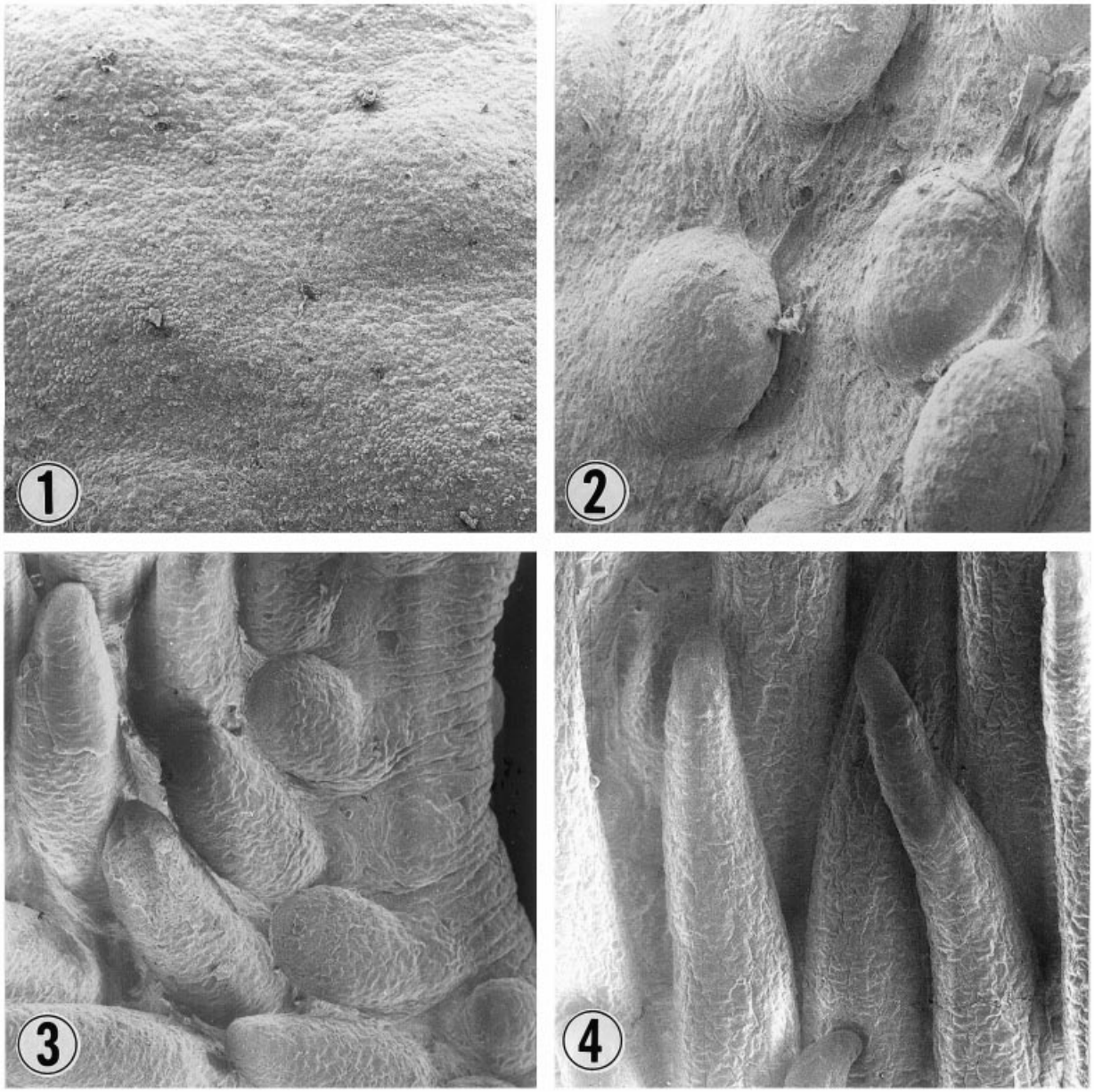
Skin samples from 7 different body regions were studied (for anatomical nomenclature, see Romanoff, 1960; Lucas & Stettenheim, 1972): 1, dorsal neck; 2, dorsal trunk; 3, dorsolateral shank; 4, abdomen; 5, forearm (radius and ulna); 6, hand (metacarpals and phalanges); 7, tail. Measurements (n = 15–45 for each region) of external length development of outgrowing feather buds were directly made from the complete embryo or/and separately from feather buds or filaments freshly excised from the embryos using a Zeiss binocular microscope with a calibrated eyepiece. The results are the arithmetrical means for both methodological approaches and for each body region, but it was not possible to follow the typical sequence of feather patterning (see e.g. Mayerson & Fallon, 1985; Bellairs & Osmond, 1997). Additionally, H.E. stained plastic sections were used for measurements from very early HH stages, and for the control of structural development in the dermis. Specimens prepared for SEM purposes were not included in the measurements because of possible shrinkage artefacts.

RESULTS

The first feather germs (anlagen) could be identified morphologically on the body surface at about HH stage 31–32 in the region of the pterylae dorsales, as well as laterally at the still relatively long future tail complex where the rectrices are formed. During embryonal growth, the feather tracts mentioned above increased in area, and additional pterylae emerged in ventral and lateral body regions and in the wing integument. It was not before HH stage 36 that the length of feather buds could be measured exactly. However, soon after the feather germ had become apparent as a slight hillock on the body surface (Fig. 1), it bulged out (Figs 2, 3) and elongated rather rapidly to form the early feather filament that showed clear axial growth. This developing feather system not only presented a clear straight axis, but was also readily measurable because it was entirely enclosed in its sheath (Fig. 4) before this 'pin-feather' was transformed into the neoptile feather with hatching.

Length development in the feather buds of the embryo was regularly observed in the 7 body regions studied and the results are summarised in Figure 5. These findings revealed that absolute length values of the feather filaments more or less continuously increased during HH stages 36–45, i.e. from lengths of 0.5–1 mm to those of 15–25 mm at the time of hatching. When comparing the different body regions, it was evident that feather growth developed most slowly in the abdomen and progressed quite rapidly in the dorsal body parts, the wings showing an intermediate axial growth of feather filaments. In contrast to a continuous growth development observable from absolute values, clear differences with regard to the developmental stage and body region studied could be detected when the ratio of growth was determined as percentage of increase in length compared with the preceding developmental stage. Thus it became obvious that the distinctly highest growth rate occurred during HH stage 40–41. Before this stage, growth ratios increased rather slowly and proportionally, or decreased after HH stage 41, respectively. Compared with the development of absolute values, regional differences also had changed. This means first of all that growth rates were highest in feather filaments of the wings in HH stage 40–41, but not before or after this stage, whereas growth rate development in the other body regions varied more or less irregularly.

It was of particular interest to follow histological differentiation in the chicken integument as related to growth development of the feather follicles. Beginning with HH stage 34, the protruding feather buds established good capillary supply that penetrated into the young feather papilla (Fig. 6). In addition, histological observation made it possible to establish that during HH stages 37–39, the epidermal base of the outgrowing feather buds had been pushed downwards into the dermis now forming the early feather follicle. The depth of this inserted follicle base had reached about 0.30 mm in HH stages 40–41 and remained relatively constant at a level of 0.50–



Figs 1–4. Morphological development of the outgrowing embryonal feather follicle: 1, HH 34, dorsal neck; 2, HH 36, dorsal trunk; 3, HH 39, forearm; 4, HH 40, hand; SEM, all figures $\times 130$.

0.60 mm until hatching. However, the most conspicuous events in this connection could be detected during HH stages 38 and 39 (Fig. 7), just before the enormous increase in growth rate of the elongating feather follicle during HH stage 40–41 occurred. Moreover, it was not before HH stage 38 that our immunohistochemical control reaction demonstrated positive staining for α-keratins in the basal pulpa epithelium and the newly-formed single layer of sheath cells. This reaction remained distinct during HH stage 40–41 in the sheath, the pulpa epithelium, the cortical cells and the barbule cells (Fig. 8), but became less prominent and disappeared in HH stages 42 and 43.

DISCUSSION

The results obtained in this study on growth development in the embryonal feather of the chicken have demonstrated that there are distinct phases of varying growth intensity during feather ontogenesis. This was most evident from the evaluation of growth rates, whereby the increase in feather length occurring between each of the HH stages was ascertained as accurately as possible. Thus HH stage 40–41 stood out as unique stage with the most rapidly growing feather filaments of all body regions. Until now the most active period of growth had been limited to HH

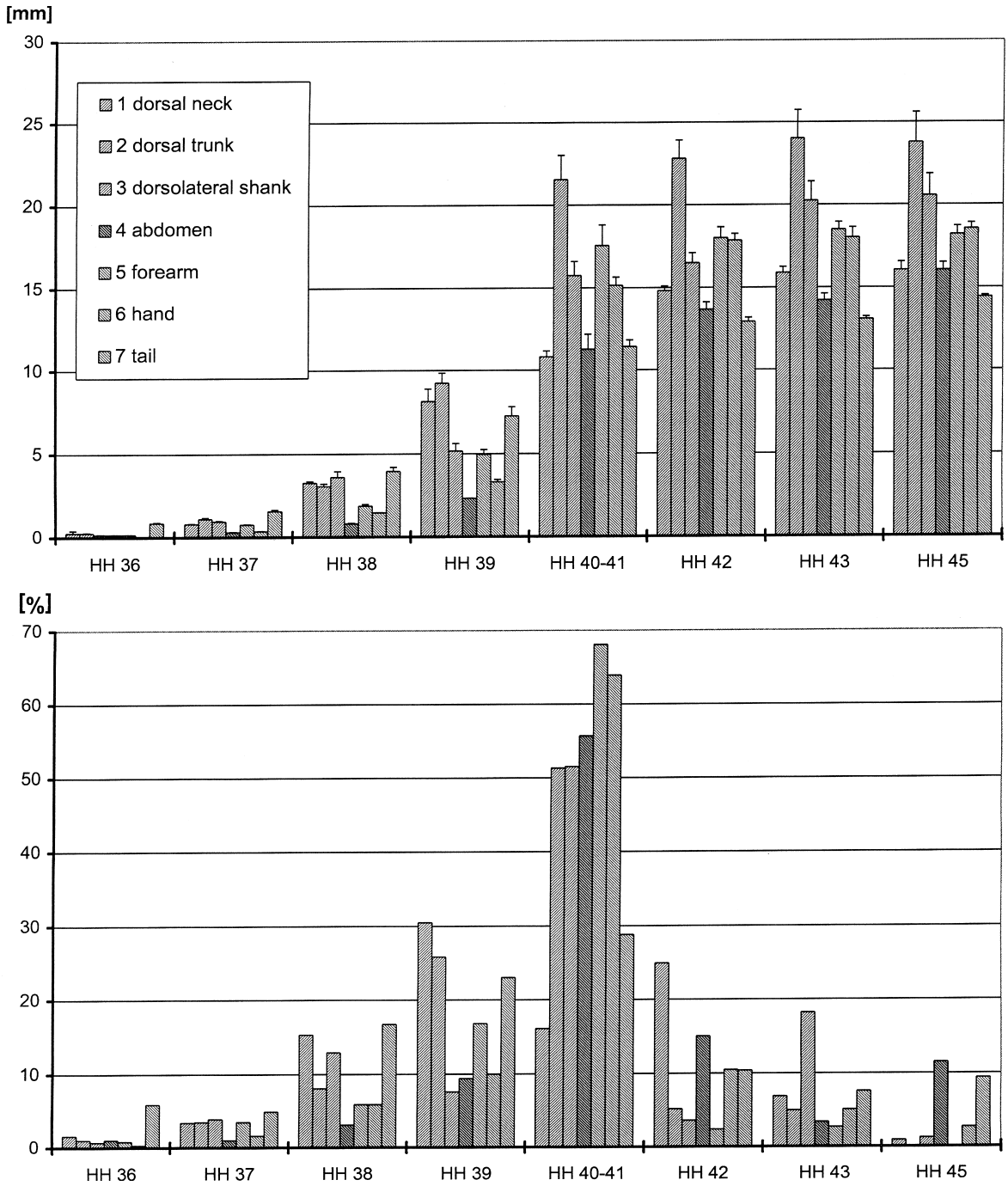
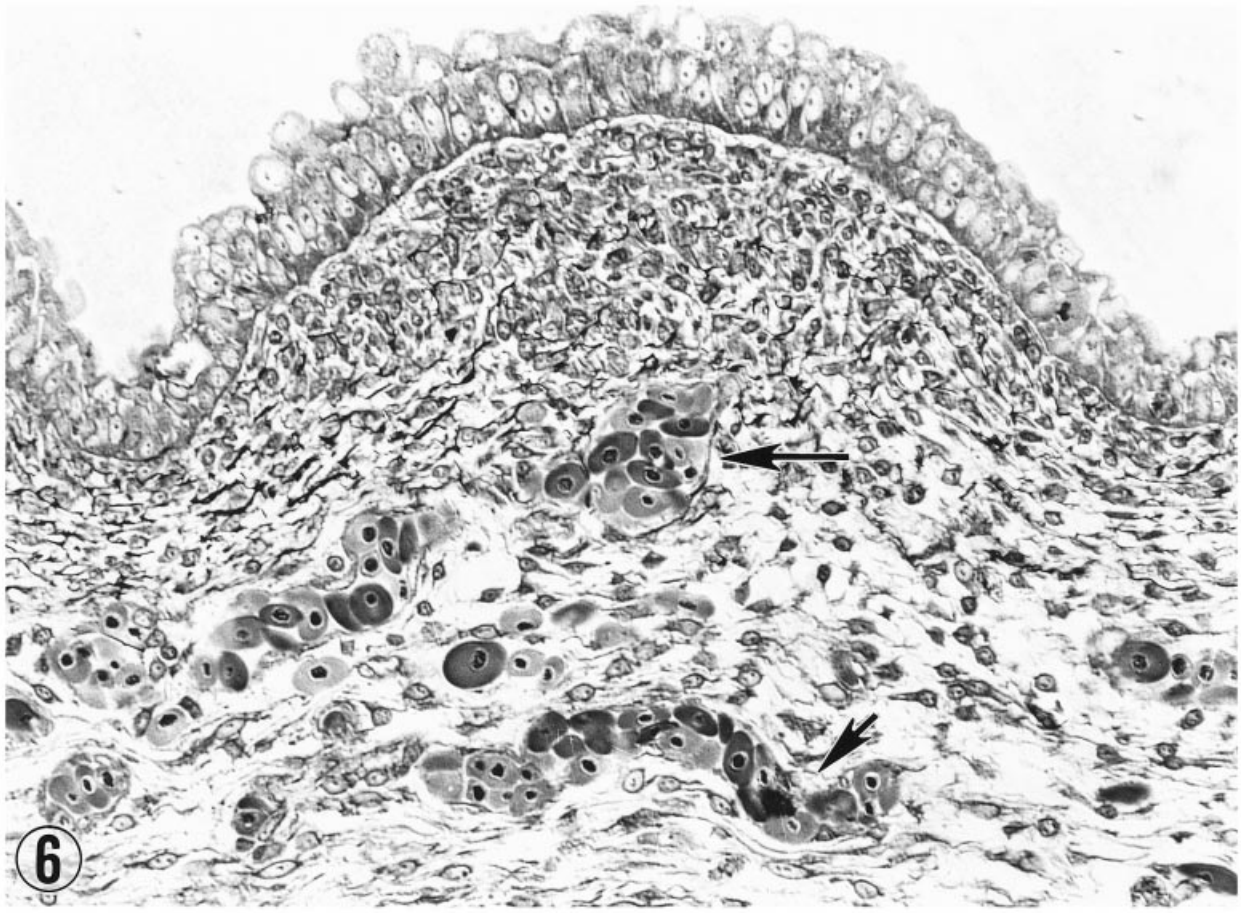


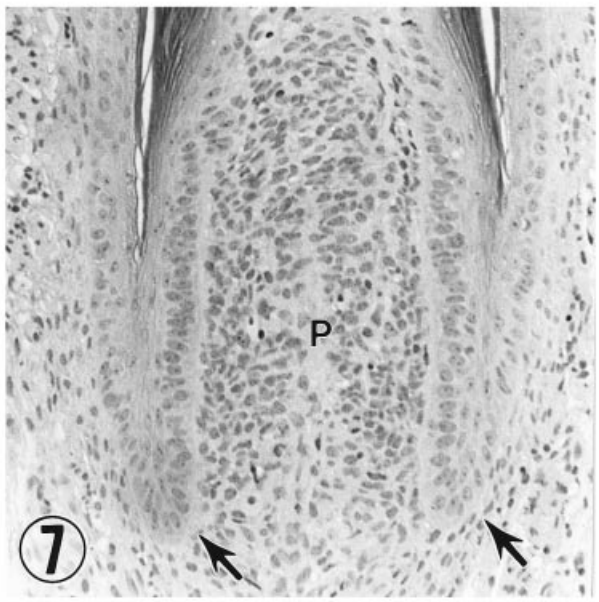
Fig. 5. Development of longitudinal growth of the embryonal feather follicle as found in different body regions. Above: absolute length values in mm; below: % growth ratio; 1, dorsal neck; 2, dorsal trunk; 3, dorsolateral shank; 4, abdomen; 5, forearm; 6, hand; 7, tail.

stages 37/38 and/or 39 (Koning & Hamilton, 1954; Romanoff, 1960; Kischer, 1963; Ede et al. 1971), taking into consideration, however, that systematic examinations of growth development in the embryonal feather had not been performed previously.

Growth rate analysis also revealed another important feature of regional growth development. This means that the most rapid growth occurred during HH stages 40–41 in the feather filaments of the developing wings. Such a conclusion is reasonable because of the strong feather types produced here, but could not be verified from the course of absolute feather growth.

With regard to results of the histological methods employed, several aspects need to be emphasised more clearly. First, before feather growth progressed intensively, a dense capillary supply had been established, already beginning during HH stage 34. This observation is corroborated by a locally limited tenascin C (AD2 repeat) modulated fibronectin (type





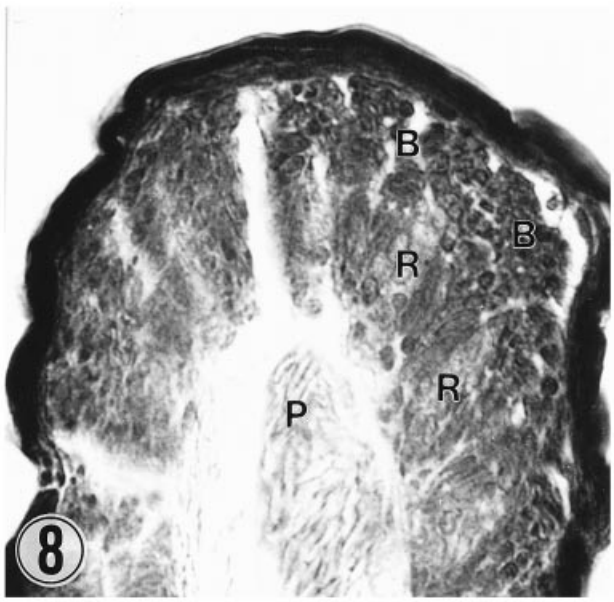


Fig. 6. Sagittal section of the early protruding embryonal feather follicle, HH 34, with centrally ingrowing primary capillary supply (long arrow), derived from early dermal blood vessels (short arrow); a multilayered epidermis and early connective tissue fibres are visible. Technovit embedding, PAMS staining, $\times 375$.

Fig. 7. Longitudinal section of the freshly inserted embryonal feather follicle with its young papilla (P) (epithelial roll marked with arrows), HH 42. Technovit embedding, H.E. staining, $\times 245$.

Fig. 8. Immunohistochemical demonstration of α-keratin in the growing embryonal feather follicle, HH 40–41, distinctly positive reactions in the covering sheath and the barbule cells (B), negative reactions in the rami cells (R) and the papilla (P). Paraffin section, PO staining, $\times 600$.

III) production at the base of feather buds during HH stages 35–36 (Derr et al. 1997). Secondly, the most rapid increase in growth rates during HH stage 40–41 was definitely preceded by the insertion of the feather filament into the protective and supportive dermis during HH stages 38 and 39. The latter process seems to be an indispensable prerequisite for further histological differentiation of the different feather producing parts of the embryonal follicle. This view applies also to the information obtained from our immunohistochemical approach, i.e. feather growth development is accompanied by α-keratin production in the barbule cells. The phenomenon was more or less restricted, however, to HH stage 40–41, and had not been demonstrated in these cells previously, although extensive -S-S- bond formation has been noted (Bell & Thathachari, 1963). Nevertheless, earlier studies had focused on the first appearance of β-keratins during embryonal feather development. This keratin type was not detectable in the barb ridge cells before HH stage 39, and as feather differentiation and β-keratinisation progressed, the α-keratins became less prominent relative to the β-keratins (Haake et al. 1984).

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