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Coexpression of Hard and Epidermal Keratins in the Nail Unit during Human Fetal Skin Development

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Summary. **To investigate the fetal development of the nail epithelium, the expression of hard keratin and epidermal keratin in human fetal skin at various stages of gestation was studied by immunofluorescence microscopy using anti-hair keratin and anti-epidermal keratin 10 monoclonal antibodies. Specimens were obtained from digits and scalps of fetuses at 11, 13, 16 and 20 weeks estimated gestational age. At 11, 13 and 16 weeks, hard keratin was coexpressed with epidermal keratin 10 in the nail epithelial field. At 20 weeks, the epidermal keratin 10 was no longer observed and only hard keratins were expressed in the nail matrix and bed, which were covered by an adult-like mature nail plate. In the hair apparatus and epidermis, such a coexpression of hard keratin and epidermal keratin 10 was not observed in any of the fetal stages examined.**

In conclusion, the coexpression of hard and epidermal keratins is one biological characteristic of the nail epithelium distinguishable from those of the epidermis and hair apparatus during human fetal development of keratinizing epithelia of the skin.

Key words—hard keratin, epidermal keratin, coexpression, nail unit, fetal development.

INTRODUCTION

In the keratinized epithelia of normal human skin, hard keratin is known to be expressed in the nail unit and hair apparatus, but not in the epidermis.[1,6,12,13] Hard keratin has been biochemically and immunologically investigated and proved to be different from epidermal keratin.[2,3,11] Hard keratin is expressed in the keratogenous zone of hair in the adult human hair apparatus,[8,12] and in the upper layers of the nail matrix and bed in the adult human nail unit.[3] In the adult human epidermis, the epidermal keratins 1 and 10 are expressed in the suprabasal layers.[4,16,18] The monoclonal antibodies that specifically recognize these differentiation-related keratins of stratified epithelia have been shown to be very useful in investigating epithelial differentiation.[4]

Although the expression of epidermal keratin during human fetal skin development has been studied by some investigators,[5,10,14] the expression of hard keratin in the fetal skin is not fully understood. In the present study, the expression of hard keratin and epidermal keratin in developing human fetal skin—especially in the nail unit—at various stages of gestation was studied by immunofluorescence microscopy using anti-hair keratin and anti-epidermal keratin 10 monoclonal antibodies, in order to determine the biological characteristics of the fetal nail epithelium.

MATERIALS AND METHODS

Specimens obtained from the digits and scalps of human fetuses at 11, 13, 16 and 20 weeks estimated gestational age were immunohistochemically investigated. The specimens were frozen in liquid nitrogen and stored at −70℃ until use. Some specimens were processed for light microscopy. Four μm-thick frozen sections were made in a cryostat at −30℃ and examined by an indirect immunofluorescence method; the sections were incubated with an anti-keratin monoclonal antibody for 30 min at room temperature, washed in phosphate-buffered saline, and then incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG antibody (Cappel Laboratories, West Chester, PA) for 30 min at room temperature.

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After washing in phosphate-buffered saline, the sections were mounted in glycerin buffer and observed under a Zeiss Standard 18FL fluorescence microscope. As negative controls, phosphate-buffered saline was substituted for the primary antibody.

The anti-keratin monoclonal antibodies used were HKN-2, HKN-4, HKN-5, HKN-6, HKN-7 and RKSE60. HKN-2, HKN-4, HKN-5, HKN-6 and HKN-7 were produced in our laboratory, and their characteristics in the adult human skin have been previously reported.[7,8,17] Briefly,HKN-2 and HKN-4 react to both the epidermis and hair apparatus. The reaction of HKN-5, HKN-6 and HKN-7 is confined to the hair apparatus, and the epidermis is not positively stained by these three antibodies. RKSE60 was purchased from Bioscience Products (Emmenbrücke, Switzerland). RKSE60 recognizes keratin 10 and reacts to the epidermis in the suprabasal layers, but not to the hair apparatus below the isthmus.[15]

RESULTS

Nail unit

At 11 weeks, the nail matrix was beginning to develop. The epithelia in the nail field were stratified and the periderm was being shed (Fig. 1a). HKN-2 (Fig. 1b) and HKN-4 stained the entire strata of the epithelia in the nail field continuously with the neighboring epidermis. HKN-5 (Fig. 1c), HKN-6 and HKN-7 stained the upper layers of the nail field epithelia. RKSE60 similarly stained the upper layers throughout the nail field, although the neighboring epidermis was also positive for this antibody (Fig. 1d).

At 13 weeks, the cells of the nail matrix showed a vacuolated appearance (Fig. 2a). HKN-2 (Fig. 2b) and HKN-4 stained the entire strata of the epithelia in the nail field continuously with the neighboring epidermis. The epithelial region positive for HKN-5, HKN-

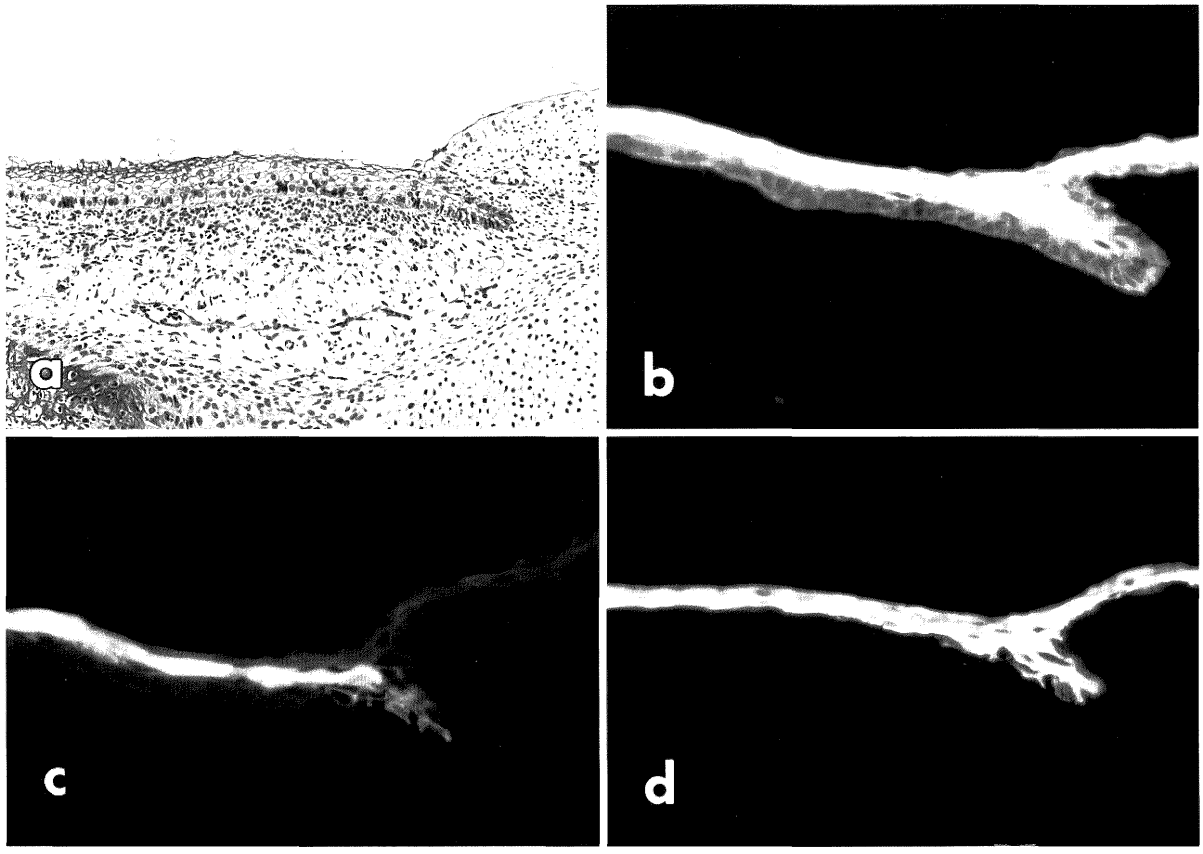

Fig. 1. Hematoxylin-eosin staining (**a**) and fluorescein isothiocyanate-immunofluorescence staining with HKN-2 (**b**), HKN-5 (**c**) and RKSE60 (**d**) at 11 weeks. **a.** The nail matrix beginning to develop. ×120 **b.** The epithelia of the nail field in the entire strata are stained continuously with the neighboring epidermis. ×200 **c.** The epithelia in the upper layers of the nail field are positively stained. ×200 **d.** The epithelia in the upper layers throughout the nail field are stained continuously with the neighboring epidermis. ×200

6 (Fig. 2c) and HKN-7 was still confined to the upper layers of the nail field. RKSE60 still stained the epithelia of the nail field continuously with the neighboring epidermis (Fig. 2d).

At 16 weeks, the horny material of the nail field was not yet so compact as the adult-like mature nail plate (Fig. 3a). HKN-2 (Fig. 3b) and HKN-4 stained the entire layers of the epithelia of the nail field. HKN-5, HKN-6 (Fig. 3c) and HKN-7 stained the upper layers throughout the nail field epithelia. RKSE60 still stained the epithelia of the nail field continuously with the neighboring epidermis (Fig. 3d).

At 20 weeks, an adult-like mature nail plate covered the entire nail bed (Figs. 4a and b). HKN-2 (Fig. 4c) and HKN-4 stained the entire epithelial strata in the nail matrix and bed. The nail matrix and bed were stained with HKN-5, HKN-6 (Fig. 4d) and HKN-7 in the upper layers. HKN-5 further stained a few layers of cells adjacent to the keratinizing nail plate in the proximal nail fold (Fig. 4e). On the other hand, positive staining with RKSE60 was no longer found in the nail matrix and bed, whereas the epithelia lining the ventral surface of the proximal nail fold were stained with RKSE60 continuously with the neighboring epidermis (Fig. 4f).

Hair apparatus

At 13 weeks, hair pegs were observed in the scalp skin. HKN-2, HKN-4 and RKSE60 stained the epidermis and hair pegs. However, the hair pegs as well as the epidermis showed no positive staining with HKN-5, HKN-6 and HKN-7.

At 16 and 20 weeks, adult-like hair apparatuses were observed; they formed the outer root sheath, inner root sheath and hair. HKN-2 and NKN-4 stained the hair apparatuses continuously with the epidermis. Positive stainings with HKN-5, HKN-6 and HKN-7 were confined to the hair apparatuses; HKN-5, HKN-6 and HKN-7 stained the hair and inner root sheath, and HKN-5 further stained the innermost cell layer of the outer root sheath.

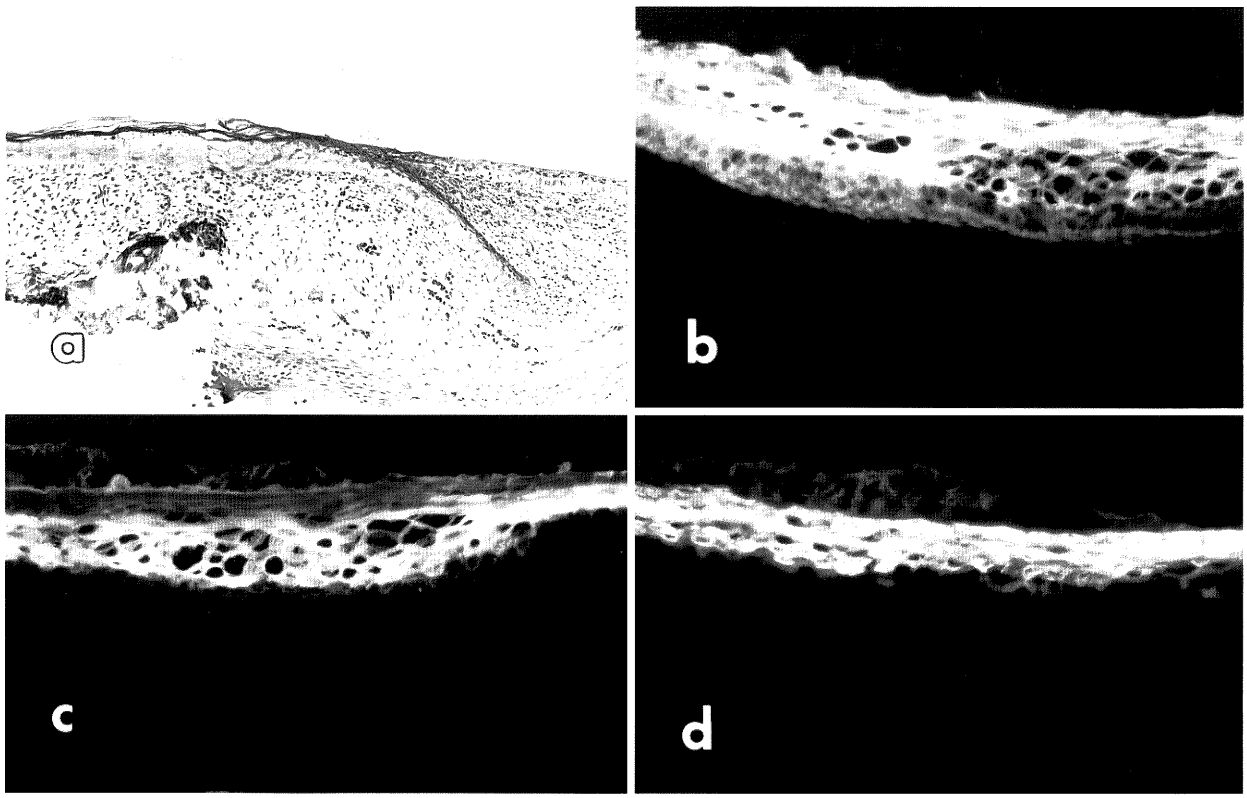

Fig. 2. Hematoxylin-eosin staining (**a**) and fluorescein isothiocyanate-immunofluorescence staining with HKN-2 (**b**), HKN-6 (**c**) and RKSE60 (**d**) at 13 weeks. **a.** The cells of the matrix have a vacuolated appearance. ×90 **b.** The epithelia of the nail field in the entire strata are stained. ×150 **c.** The positively stained epithelial region is still confined to the epithelia in the upper layers of the nail field. ×150 **d.** The epithelia in the upper layers throughout the nail field are still stained. ×150

DISCUSSION

The results of the present study showed the expression of hard keratin in the epithelia of the human nail unit up to 11 weeks, when the nail matrix began to develop. The epithelial area expressing the hard keratin was extended from the nail matrix toward the distal area during the ontogeny of the human nail unit. In this area, the hard keratin was expressed in the upper, more differentiated layers in accordance with immunohistochemical results obtained in the adult human nail unit.[3]

The epidermal keratin 10, which is expressed in the suprabasal layers according to epidermal differentiation in adults, has been revealed to appear coincident with the appearance of the intermediate cell layers in the human fetal epidermis.[5,10,14] The results of the present study showed that the epidermal keratin 10 was also expressed in the upper layers throughout the nail field continuously with the neighboring epidermis, even after the appearance of hard keratin. Furthermore, the epidermal keratin 10 continued to be present in the upper layers of the nail field before the appearance of the adult-like mature nail plate at 20 weeks. This corresponds to findings that the epidermal keratin 10 is not biochemically detected in the normal human adult nail.[6,12] Such a coexpression of hard keratin and epidermal keratin 10 in the same epithelial cells was not found in the hair apparatus or epidermis during fetal development in the present study. Therefore, the coexpression of hard keratin and epidermal keratin 10 seems to be one of the characteristic features of the nail unit during human fetal development. The coexpression of hard and soft keratins may have a relation to the finding that the premature nail plate, which is not so compact as the adult-like mature nail plate, is histochemically different from the neighboring epidermis, although it can not be histologically distinguished

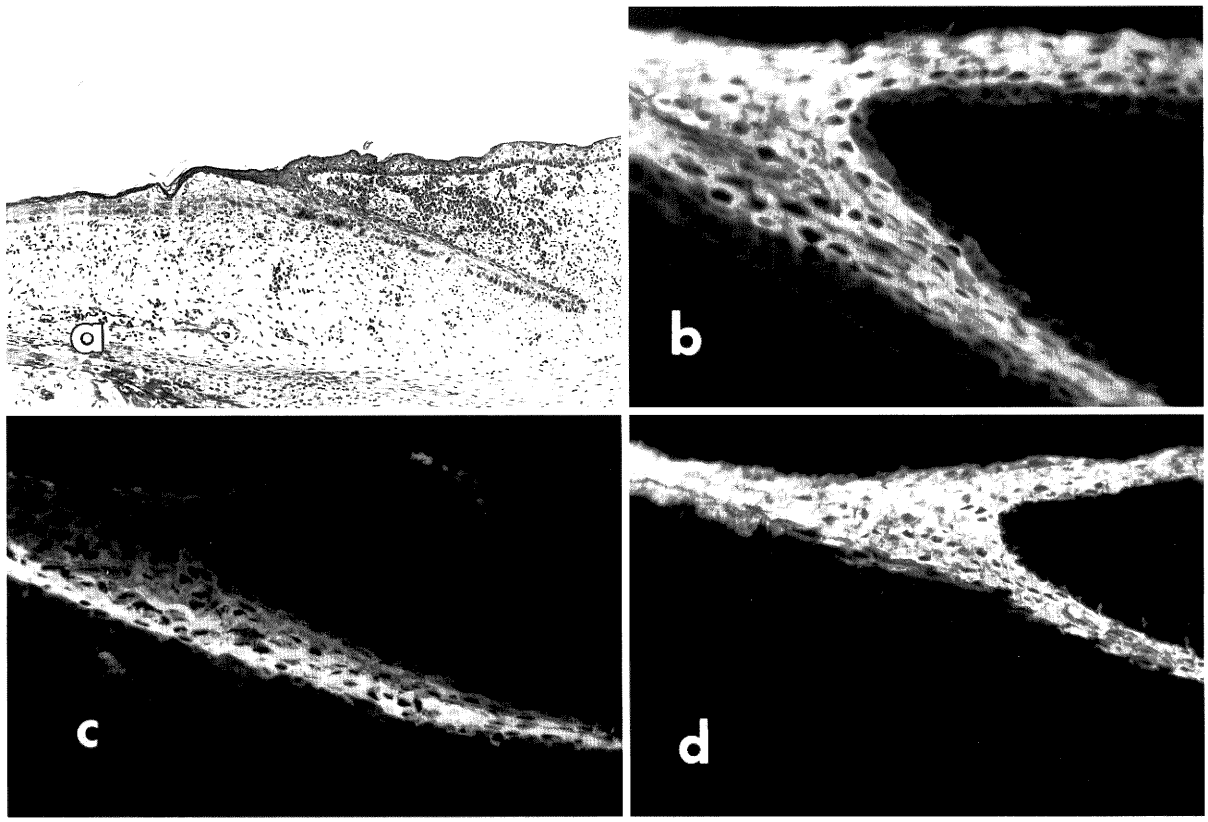

Fig. 3. Hematoxylin-eosin staining (**a**) and fluorescein isothiocyanate-immunofluorescence staining with HKN-2 (**b**), HKN-6 (**c**) and RKSE60 (**d**) at 16 weeks. **a.** The horny material of the nail field is not yet so compact as the adult-like mature nail plate. ×90 **b.** The entire layers of the epithelia of the nail field are stained continuously with the neighboring epidermis. ×170 **c.** The epithelia throughout the nail field are positively stained in the upper layers. ×170 **d.** The epithelia of the nail field are still stained continuously with the neighboring epidermis. ×170

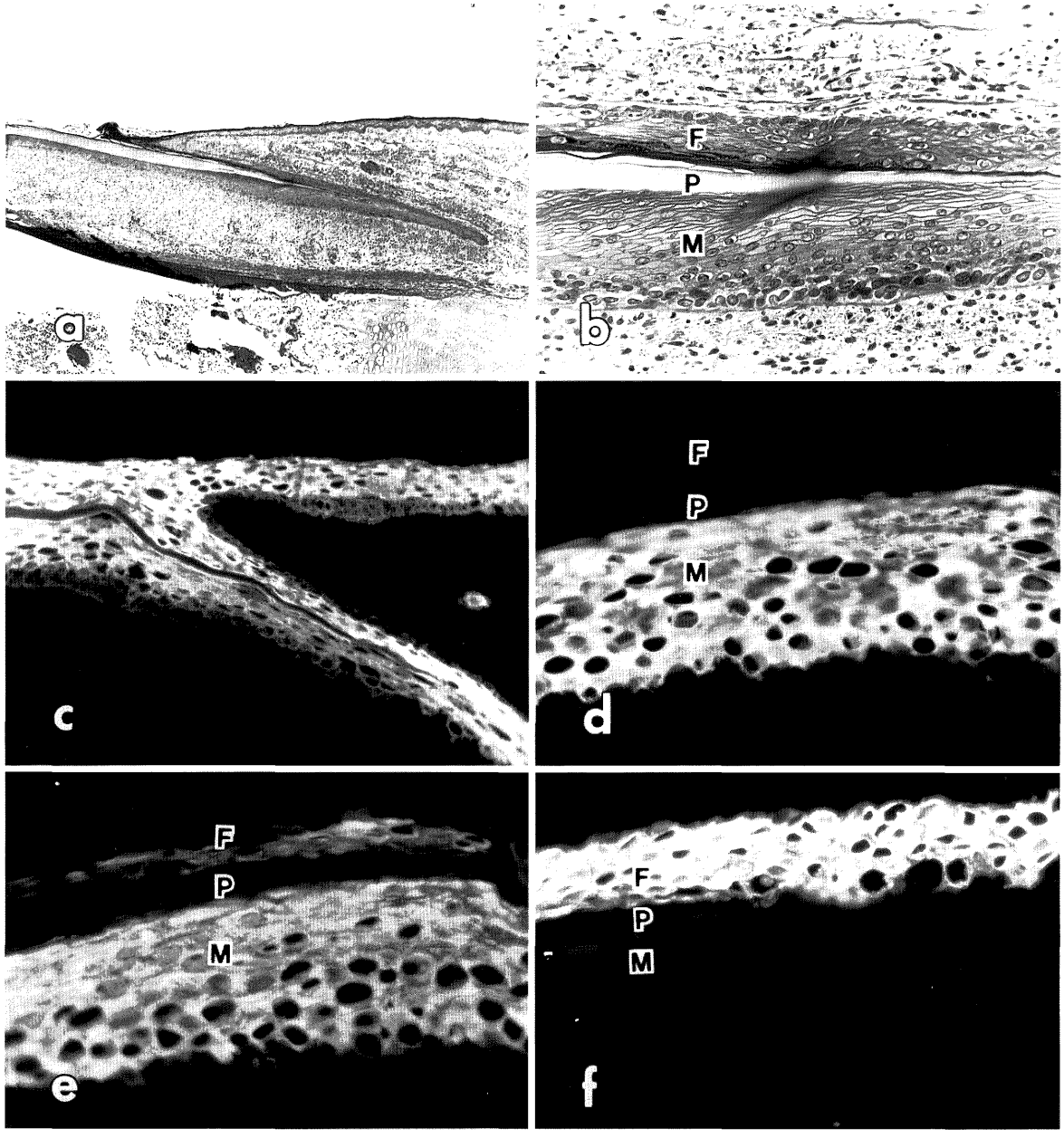

Fig. 4. Hematoxylin-eosin staining (**a** and **b**) and fluorescein isothiocyanate-immunofluorescence staining with HKN-2 (**c**), HKN-6 (**d**), HKN-5 (**e**) and RKSE60 (**f**) at 20 weeks. **a.** The adult-like mature nail plate covers the entire nail bed. ×40 **b.** The keratinizing nail plate *(P)* is found between the nail matrix *(M)* and the proximal nail fold *(F)*. ×230 **c.** The entire epithelial strata in the nail matrix and bed are stained continuously with the neighboring epidermis. ×70 **d.** The nail matrix *(M)* is positively stained in the upper layers, whereas the nail plate *(P)* and the proximal nail fold *(F)* are negatively stained. ×300 **e.** A few layers of cells adjacent to the keratinizing nail plate *(P)* in the proximal nail fold *(F)* are positively stained in addition to the nail matrix *(M)*. ×300 **f.** The positive staining is no longer found in the nail matrix *(M)*, whereas the epithelia lining the ventral surface of the proximal nail fold *(F)* are stained continuously with the neighboring epidermis. ×300

from the neighboring epidermis.[19]

The HKN-5-positive staining of a few layers of cells adjacent to the keratinizing nail plate in the proximal nail fold is very interesting. This became apparent after the appearance of the adult-like nail plate at 20 weeks. The staining pattern of the proximal nail fold with HKN-5 seems to be identical to that of the innermost cell layer of the outer root sheath of hair, which shows a specific staining with HKN-5 and a unique ultrastructural cell differentiation.[8] The reaction of HKN-5 may have a relation to the fact that the proximal nail fold plays a similar role with the outer root sheath; that is, these structures support the respective formation of the nail plate and hair shaft[9] to grow in the proper direction.

In conclusion, the results of the present study show that the nail unit can be distinguished from the epidermis by the expression of hard keratin by 11 weeks gestational age. The expression of the epidermal keratin 10, however, remains in the nail field until the appearance of the adult-like mature nail plate up to 20 weeks. It seems to result from an intermediate character of the nail unit between the epidermis and hair apparatus. Furthermore, the proximal nail fold may be comparable with the outer root sheath of hair in their similar positive staining for HKN-5.

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