*Pharmaceutical Research, Vol. 7, No. 11, 1990*

*Technical Note*

# Epidermal Differentiation and Permeability in Fetal Pig Skin

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*Received March 14, 1990; accepted May 15, 1990*

KEY WORDS: epidermal differentiation; fetal skin; gestation; stratum corneum; transdermal permeation.

## INTRODUCTION

Stratum corneum, the outermost layer of mammalian epidermis, is generally acknowledged as the principal barrier to penetration of molecules through the skin (1,2). Differentiation of the epidermis to stratum corneum takes place in the third trimester of fetal life with the formation of completely keratinized cells, and by the time full-term gestation is reached the barrier property of the stratum corneum is essentially equivalent to that of adult skin (3). Yet not all babies are born full term, and therefore, many new infants face the first few weeks of their life with an incomplete skin barrier, making them particularly susceptible to excessive dehydration and absorption of potentially toxic substances. On the other hand, incomplete development of other organs poses a myriad of complications for the neonatologist in his attempts to intervene medically, and the incomplete skin barrier may provide a convenient route of administration for drugs intended for systemic therapy. A better understanding of the relationship between skin permeability and the embryogenesis of the stratum corneum is fundamental to assessing the potential for this mode of drug administration.

The extreme sensitivity of the permeability barrier to damage by lipid solvents (4–7), and the selective penetration of most nonpolar materials across the stratum corneum have long suggested that lipids are important determinants of skin penetration. In addition to lipids, several other stratum corneum structural parameters, including thickness (8), number of cell layers (8,9), and geometric organization (10), have been considered as determinants of stratum corneum permeability. Up to the present, differences in thickness and the number of cell layers in stratum corneum alone have been found insufficient to account for differences in percutaneous transport and total lipid concentration may be the critical factor governing skin permeability (10,11). None of these earlier studies have linked permeability phenomena directly with epidermal keratinization. In this context, fetal pig skin has attracted interest because pig skin is often used as an experimental model for the study of physiological and biochemical processes of human skin (12,13). However, information on fetal pig skin is still sparse, although a few reports have been written regarding the histology of swine neonates (14). Furthermore, there have been no transepidermal penetration studies with fetal pig skin undertaken specifically to correlate its permeation properties with epidermal keratinization and embryonic development. Therefore, we examined the morphology of fetal epidermis to correlate, *in vitro*, skin penetration properties with differentiation of the epidermis. We now report the significance of the lipid pathway in the stratum corneum as a function of the stage of epidermal keratinization.

## MATERIALS AND METHODS

Swine fetuses (15) at 55, 75, 84, and 96 days of gestational age and full term (115 days) were obtained from artificially inseminated sows by cesarean delivery. Skin specimens were immediately taken from the dorsal area of the fetuses for light microscopic examination and skin transport studies. Skin was excised with a scissor and the underlying fatty tissue was carefully removed. These skin samples were then frozen by a cascaded freezing technique until further use.[3] For histological observation 1-μm-thick plastic and 6-μm-thick paraffin sections of the skin of all but the 84-day fetuses were prepared by appropriate means.[4] We determined the *in vitro* steady-state transport rates of arecoline (methyl-1,2,5,6-tetrahydro-1-methylnicotinate) through full-thickness skin from aqueous solution.[5]

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[3] The pig fetuses are progeny cross bred of two commercial strains, Landrace/Large White. The pregnant SPF swine for these fetuses were artificially inseminated at the Pig Improvement Company, Franklin, Kentucky. Skin specimens were excised, then treated with gentamycin and glycerin before being cascade-frozen for shipment.

[4] The paraffin section was stained by Gill's hematoxylin and eosin, and the plastic section was stained by 1% toluidine blue for microscopic observations.

[5] All permeation experiments were conducted in diffusion cells possessing an effective diffusional area of 1 $cm^2$ and the downstream receiver volume of 4 ml saline solution was thermostated at 32°C. Steady-state flux values from 0.1 *M* arecoline solutions were determined for all experiments.



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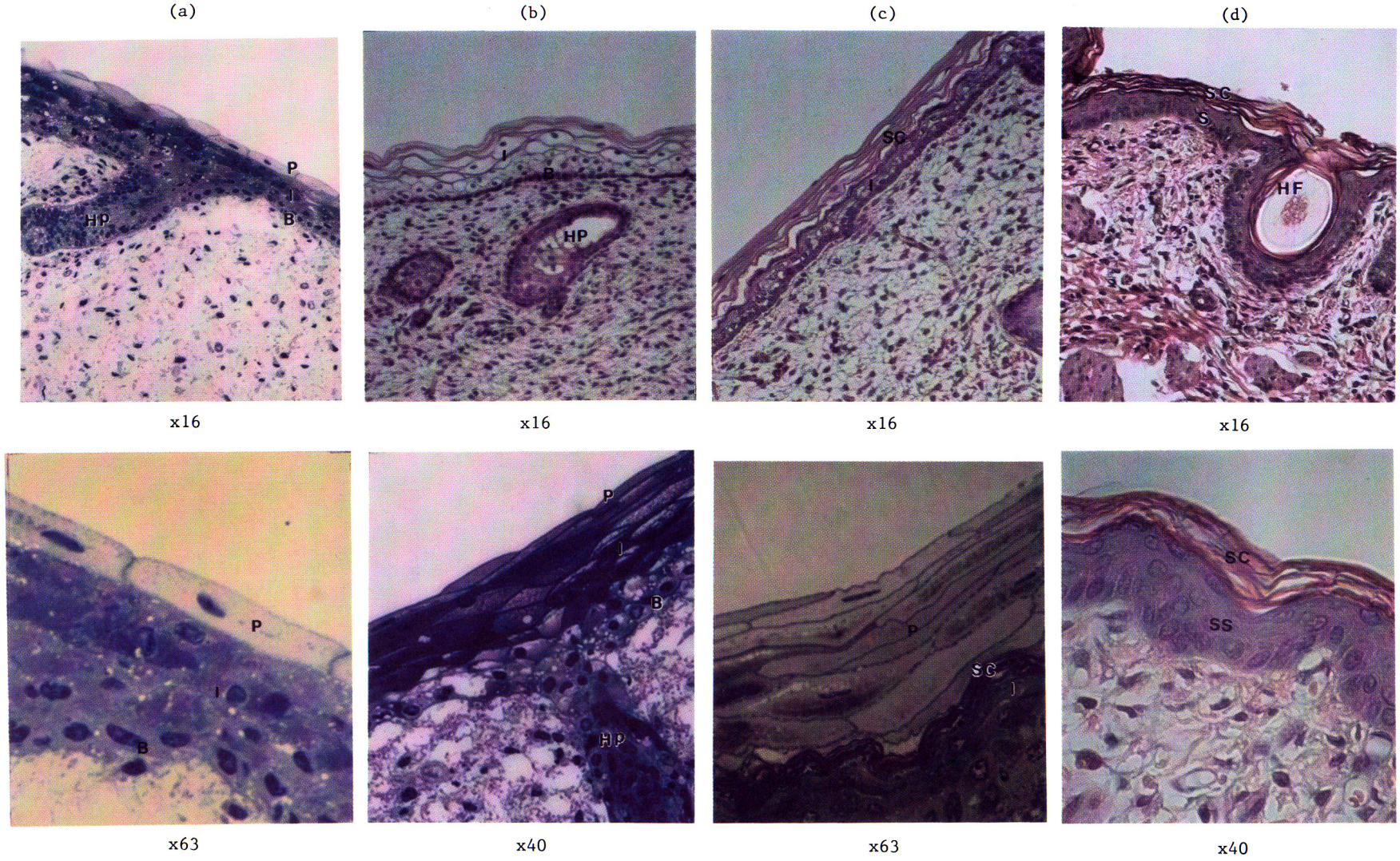


**Fig. 1.** Microscopic cross-sectional views of fetal pig skin at varying stages of gestation. Skin taken at (a) 55, (b) 75, (c) 96, and (d) 115 (full term) days of gestation. See footnote 4 for staining procedures. P, I, B, HP, HF, SC, and SS indicate periderm, intermediate cells, basal layer, hair peg, hair follicle, stratum corneum, and stratum spinosum, respectively. Upper panel: a–d, ×16. Lower panel: a, ×63, b, ×40; c, ×63; d, ×40. Reduced 20% for reproduction.

## RESULTS AND DISCUSSION

In the 55-day-old fetus (Fig. 1a), the epidermis varies from 5 to 10 cell layers. It is comprised of an inner or basal layer (stratum germinativum), intermediate cells (stratum intermedium), and nonkeratinized transient outer layers, the periderm. Cords of cells of the developing hair follicles in the hair peg stage extend into the dermis, where they are surrounded by mesenchymal cells, the predecessor of most of the indigenous cells of adult connective tissues, of the developing hair bulb. Holbrook *et al.* (15) report the same observation in the developing human epidermis at 14–15 weeks of gestational age (the beginning of the second trimester). The skin of the 55-day-old fetal pig skin resembles morphologically that of a human fetus during the early second trimester. In the 75-day-old fetus (Fig. 1b), the cells of stratum intermedium appear to be enlarged and those of the periderm somewhat flattened. The periderm is approximately one-third as thick as the epidermis. Hair follicles extend deep into the dermis. At this stage no evidence of keratinization appears in any part of the epidermis. Based on these observations by light microscopy, the morphological characteristics of the 75-day-old fetal pig skin are qualitatively similar to those of the skin of a human fetus at the middle of the second trimester as described by Holbrook *et al.* (15). We infer that both 55- and 75-day-old fetal pig skins are morphologically similar to the stage of skin development found during the second trimester in humans.

In the 96-day-old fetus (Fig. 1c), the morphology of epidermal cells appears to have progressed further toward flattened squames. This stage of fetal skin is more accurately described as a stage of epidermal differentiation. The epidermis is partially keratinized and the stratum corneum is just becoming visible, although its thickness is only about 9 μm, significantly less than that of the full term pig. The beginning of the stratum corneum appears, correspondingly, in the human at the end of the second trimester. At first the stratum corneum consists of only a few cell layers, but it increases in thickness during the third trimester, and at full term, it is approximately as thick as the adult stratum corneum (3). It is also clear that fragments of degenerated periderm cells are all that remain above the keratinized cells of newly formed stratum corneum. In full-term pig skin (Fig. 1d), however, no remnants of the periderm remain in the entire epidermis. The granular cells also appear to be formed in the stratum intermedium of 96-day-old fetus. In the human fetus, the granular layer is established at the sixth month, and the intermediate cells become known as spinous cells; thus, each layer of the epidermis assumes the adult nomenclature as the tissue assumes the adult characteristics (3). These microscopic studies demonstrate the previously unreported findings that, in swine fetus, the periderm and stratum intermedium appear in the epidermis and then disappear when epidermal keratinization is completed. These findings with 96-day-old fetal pig skin are known also to occur in the third trimester of human fetal life. Collectively, our observations of fetal pig skin development are morphologically comparable with developing human skin on a time scale roughly proportional with respect to the gestation periods for each species.

Gestational age has been shown to be a determinant of skin permeability, *in vitro*. The steady-state transport of arecoline through fetal pig skin decreases significantly with increasing gestational age (Table I). Given the structural evidence for the relationship between gestational age and stratum corneum development, it is clear that the major barrier to transport and, consequently, absorption into the systemic circulation is the stratum corneum. Numerous reports during the last 50 years have documented poisoning in infants after the use of topical products (16). There is *in vivo* evidence that the skin of premature infants is more permeable to phenylephrine than is the skin of mature full-term infants (17). The results presented here indicate that the fetal pig not only develops its skin barrier property in the same way as the human, but does so at the same relative point in its gestational age.

Table I. Permeability of Arecoline in Fetal Pig Skin Derived from Fetuses of Varying Gestational Ages[a]

| Gestational age (days) | Solution pH | Permeability (cm/sec) × $10^8$ |
|---|---|---|
| 55 | 4.2 | 1715 (1) |
| | 9.0 | 1829 ± 115 (2) |
| 75 | 4.0 | 1409 ± 340 (3) |
| | 9.0 | 1430 ± 145 (3) |
| 84 | 4.0 | 668 ± 21 (2) |
| | 9.0 | 1868 ± 152 (2) |
| 96 | 4.0 | 43 ± 27 (5) |
| | 9.0 | 655 ± 212 (3) |
| Full term, 115 | 4.0 | 2 ± 1 (3) |
| | 9.0 | 40 ± 5 (6) |

[a] Permeability data are given as means and standard deviations, with number of replicates shown in parentheses.

The average permeability to arecoline increases dramatically as a function of pH of the donor solution in both full-term and 96-day-old fetal skin. The $pK_a$ of arecoline is 7.9. Hence, at pH 4 the molecule exists almost exclusively in a protonated state, while at pH 9 the neutral free-base form predominates. This phenomenon is characteristic of lipid membranes which selectively exclude charged species. On the other hand, no such selectivity is demonstrated by the 55- and 75-day specimens, demonstrating the absence of a significant barrier property. The 84-day skin exhibits a moderate degree of selectivity but no reduction in permeability to

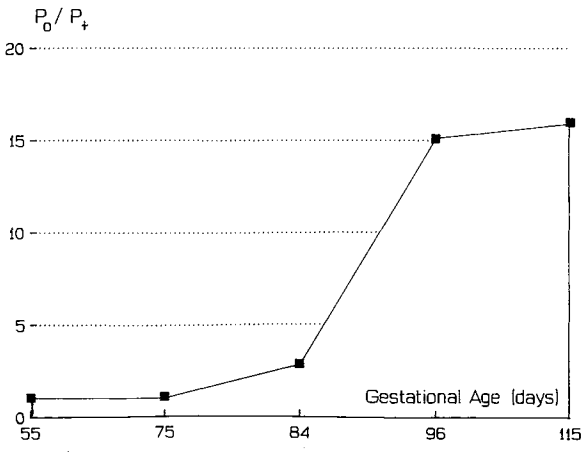


Fig. 2. Relationship between selective permeability to arecoline in the protonated state vs free base and the gestational age of fetal pig skin. $P_0/P_+$ is the ratio of permeability at pH 9 and pH 4.

the neutral molecule, indicating only an incomplete barrier function. A feel for the relative selectivity of the skin can be had by examining the ratio of permeabilities at the two pH values (Fig. 2). The relationship between this ratio and gestational age faithfully mirrors the morphological evidence we have presented and clearly demonstrates the remarkable transition in skin structure and function which takes place in a very small window of time during the third trimester of gestation.

In conclusion, these results demonstrate the importance of the middle to end of the third trimester of pregnancy in the fetal pig to the development of an adequate skin barrier function in preparation for birth. There is much circumstantial evidence that the human fetus undergoes a similar sharp transition at the same relative gestational age and points out the importance of recognizing this in premature infants born in this period of gestation. Moreover, these data indicate that the skin may be a viable route of administration for life-preserving drugs intended for systemic treatment during the critical first weeks of life for the premature infant.

## REFERENCES


1. R. J. Scheuplein and J. H. Blank. *Physiol. Rev.* 51:702–747 (1971).
2. B. Idson. *J. Pharm. Sci.* 64:901–924 (1975).
3. K. A. Holbrook. In *Biochemistry and Physiology of the Skin,* L. A. Goldsmith (ed.), Oxford University Press, 1983, p. 64.
4. G. S. Berenson and G. E. Burch. *Am. J. Trop. Med.* 31:842–853 (1951).
5. I. H. Blank. *J. Invest. Dermatol.* 21:259–271 (1953).
6. J. W. H. Mali. *J. Invest. Dermatol.* 27:451–469 (1956).
7. T. M. Sweeney and D. T. Downing. *J. Invest. Dermatol.* 55:135–140 (1970).
8. K. A. Holbrook and G. F. Odland. *J. Invest. Dermatol.* 62:415–422 (1974).
9. J. G. Smith, Jr., R. W. Fischer, and H. Blank. *J. Invest. Dermatol.* 36:337–343 (1971).
10. D. N. Menton and A. Z. Eisen. *J. Ultrastruct Res.* 35:247–264 (1971).
11. P. M. Elias, E. Cooper, A. Korc, and B. E. Broun. *J. Invest. Dermatol.* 76:297–301 (1981).
12. D. L. Bissett and J. F. McBride. *J. Soc. Cosmet. Chem.* 34:317–326 (1983).
13. A. M. Kligman. *J. Invest. Dermatol.* 62:267–287 (1974).
14. E. H. Fowler and M. Calhoun. *Am. J. Vet. Res.* 25:156–164 (1964).
15. K. A. Holbrook and G. F. Odland. *J. Invest. Dermatol.* 65:16–38 (1975).
16. D. R. Wilson and H. Maibach. In *Neonatal Skin,* H. Maibach and E. K. Boisits, (ed.), Marcel Dekker, New York, 1982, p. 101.
17. R. L. Nachman and N. B. Esterley. *J. Pediat.* 79:628–632 (1971).