THE DEVELOPMENT OF HAIR FOLLICLES IN THE MARSUPIAL *TRICHOSURUS VULPECULA*

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Summary

The development of hair follicles in the brush-tailed possum *T. vulpecula* has been studied in skin samples from animals ranging in age from embryos to adults. The first follicles appear at about 2 days after birth and develop rapidly, producing minute non-medullated hairs (about 0·5 mm long) during the first hair cycle. After the formation of these hairs, further follicles are initiated and these grow larger medullated hairs (up to about 2·0 cm in length).

Groups of follicles are formed and within each group the following follicles may be distinguished: a single original central primary follicle (two populations are recognized), a pair of original lateral primary follicles, and usually one or two original secondary follicles. All of these follicle types arise directly from the epidermis and open independently on the skin surface. Additional follicles, referred to as derived follicles, develop by branching from the original follicles and they share a common orifice with their parent follicles. The first-formed population of original central primary follicles, however, does not branch.

Special features in the development of the different types of follicles are described and their rates of development are compared. All central and most lateral primary follicles develop sweat glands. The development of the sweat glands of the first-formed population of central primary follicles is retarded until these follicles reach the end of the first period of hair growth.

The manner in which the hair follicles are formed and other features in the development of the coat in *T. vulpecula* are compared with those in other mammals.

I. INTRODUCTION

The first study of the development of hair follicles in a marsupial was made on the brush-tailed possum *Trichosurus vulpecula* more than 30 years ago (Gibbs 1938). The only other detailed study of marsupial hair follicle development is that of Lyne (1957*a*) on the bandicoot *Perameles nasuta*. Mann (1968) referred briefly to the development of hair follicles in the American opossum *Didelphis virginiana*.

Although Gibbs (1938) examined skin samples from *T. vulpecula* ranging in body length from 4 to 25 cm, many aspects of hair follicle development were not described. The ages of the animals sampled by Gibbs ranged from about 12 days to 6 months as estimated from the observations of Lyne and Verhagen (1957) and Lyne (unpublished data).

The present study is part of an investigation of the development of the integument in *T. vulpecula* and was undertaken to provide additional information on the development of the hair follicles. Studies of the melanocytes in the skin and of the epidermis have been completed (Lyne 1970; Lyne, Henrikson, and Hollis 1970).

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II. Material and Methods

Skin samples were taken from the dorsal and lateral aspects of the body of each of the 41 animals referred to previously (Lyne 1970). Two of the animals were embryos (15 and 18 days after mating), three were new-born young (the gestation period is 17–18 days), and 36 were pouch young when first sampled. The young first emerge from the pouch at about 120 days after birth. The number of samples taken before or at birth was 5; birth to 30 days, 34; 31–60 days, 31; 61–90 days, 23; 91–120 days, 17; 121–180 days, 24; 181–365 days, 23; 1–7 years, 13.

For histological studies, samples of skin were fixed in Zenker's fluid, Bouin's fluid, or 10% formalin. Serial sections were cut both perpendicular and parallel to the skin surface. The histological and histochemical techniques used were similar to those described by Lyne and Hollis (1968).

The follicles and hairs were counted at a magnification of $\times 215$ in serial sections cut parallel to the skin surface. Counts were made on two to six fields each of 1 mm^2. These counts were not corrected for skin shrinkage.

The follicle terminology is essentially that used by Lyne (1957*a*) for bandicoots, and the stages of follicle development are based on those described by Hardy and Lyne (1956) and Lyne and Heideman (1959). The various stages are: 0, no follicles; 1, follicle plug; 2, pre-papilla; 3, papilla; 4, hair cone; 5, advanced hair cone; 6, hair tip keratinized; 7, hair tip in epidermis; 8, hair tip emerged; 8+, hair growth; 10*c*, resting phase. Stage 1 is divided into 1*a*, when the length of the plug is less than its diameter, and 1*b*, when the length is equal to or greater than the diameter. Beyond stage 8, four stages (9, 10*a*, 10*b*, and 10*c*) of follicle development were used by Lyne and Heideman (1959). For convenience only two stages are used in the present study. Stage 8+ replaces stages 9, 10*a*, and 10*b*, and stage 10*c* is again used for follicles which have entered the resting or telogen phase.

III. Observations

(*a*) *Development of Original Follicles*

Two distinct populations of original central primary (PCO) follicles are recognized. The two populations are referred to as PCOX and PCOY follicles. PCOX follicles are formed before the PCOY follicles and are similar to the tylotrichs in other species (Mann 1969). Original lateral primary (PLO) follicles and original secondary (SO) follicles are formed in association with the earlier formed PCOX and PCOY follicles.

(i) *PCOX Follicles*

Figure 1 shows the relation between age and the stage of development reached by the most-advanced PCOX follicles. Follicle primoridia of the PCOX follicles are first seen at about 2 days after birth. These follicles are easily recognized in samples treated for alkaline phosphatase as the dermal cells beneath the epidermal plug contain this enzyme. PCOX follicles mature rapidly since stage 8 is reached at 14 days after birth. The presence of stage 10*c* follicles at 15 days after birth shows that the active phase of hair growth is very short. The hairs grown by the PCOX follicles during the first hair cycle are about 0·5 mm long but they project only about 0·2 mm above the skin surface. Occasionally, hair growth ceases (stage 10*c*) before the hair emerges (stage 8). The hairs produced by these follicles during the first hair cycle are non-medullated. No attempt has been made to study the cyclic activity of these or the other follicle types. However, the beginning of the second hair cycle is apparent in some PCOX follicles at 85 days after birth, and they produce medullated hairs similar to those produced by the PCOY follicles during the first cycle.

The development of a hair canal is first apparent in the epidermis at about stage 4–5, when a cord of cells appears above the PCOX follicle (Fig. 2). This cord of cells, which lies at an oblique angle to the skin surface, may be partially keratinized before the hair reaches this level. The tip of the hair, with the hair cone still attached, penetrates the epidermis along the centre of the cord of cells. Because of the late development of the sebaceous glands (see below), sebaceous cells do not take part in the formation of a hair canal in the neck region of the follicle.

Sweat glands are formed in association with all PCOX follicles. The gland rudiment first appears at stage 2 and develops slowly. It is only a small outgrowth of epithelial cells when the tip of the hair has reached the epidermis and it is still at about the same stage of development at the end of the first hair cycle (Fig. 3). After the cessation of hair growth in these follicles, sweat gland development continues (Figs. 4 and 5), and reaches the base of the resting follicle at about 40 days after birth.

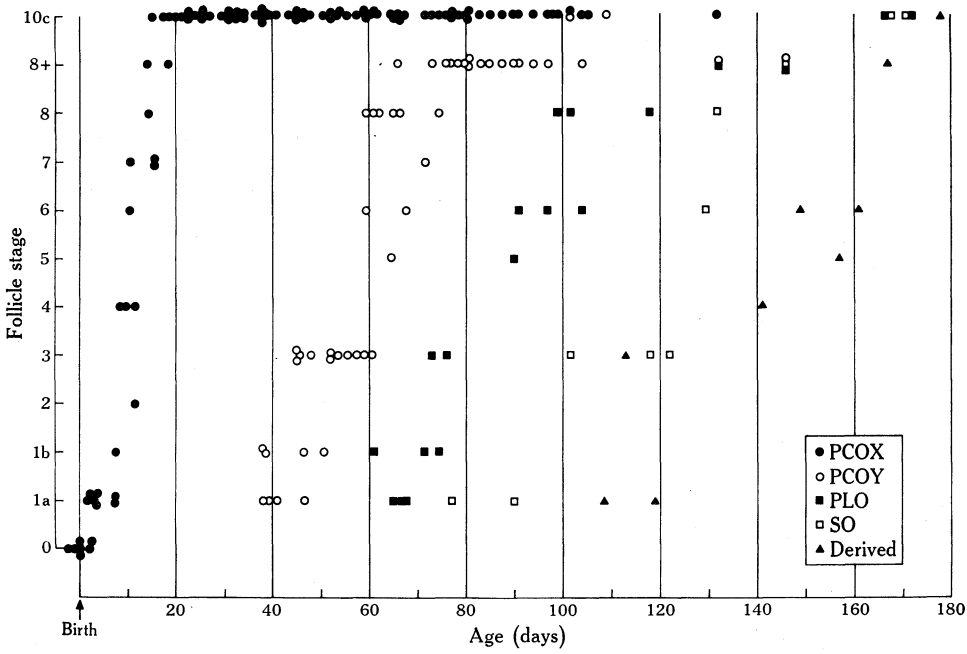


Fig. 1.—Relation between age (birth to 180 days) and the stage of development of the most advanced of the different types of follicles during the first hair cycle in 116 samples from 40 animals. In the later samples, some follicle stages are not included as they could not be identified with certainty.

Sebaceous glands are also formed in association with all PCOX follicles but usually they are not apparent until after the hair emerges. The first sebaceous cells appear in the wall of the follicle, immediately below the sweat gland bud. Figure 4 shows a sebaceous gland in a PCOX follicle at stage 10*c*.

(ii) *PCOY Follicles*

At about 40 days after birth (Fig. 1), a new population of follicles, referred to as PCOY follicles, appears between the PCOX follicles. These follicles are much more numerous than the PCOX follicles (Fig. 6) and during the first hair cycle produce large medullated hairs (about 2·0 cm long). The most advanced PCOY follicles reach stage 8 at about 60 days and stage 10*c* at about 100 days after birth (Fig. 1).

The sweat glands of PCOY follicles do not have the dormant phase described for the PCOX follicles. When the most advanced PCOY follicles reach stage 8, the sweat glands extend almost to the upper level of the follicle bulbs.

The sebaceous glands of PCOY follicles develop at a relatively earlier stage than those of PCOX follicles and discharge material into the upper part of the follicle before the tip of the hair reaches this level. A hair canal in the neck of the follicle is formed by the disintegration of sebaceous cells. Within the epidermis above the follicle, another canal is formed by the keratinization of epidermal cells and the two canals join. Oil Red O preparations reveal sebaceous material in the neck region of the follicle as well as in the epidermal portion of the hair canal.

(iii) *PLO Follicles*

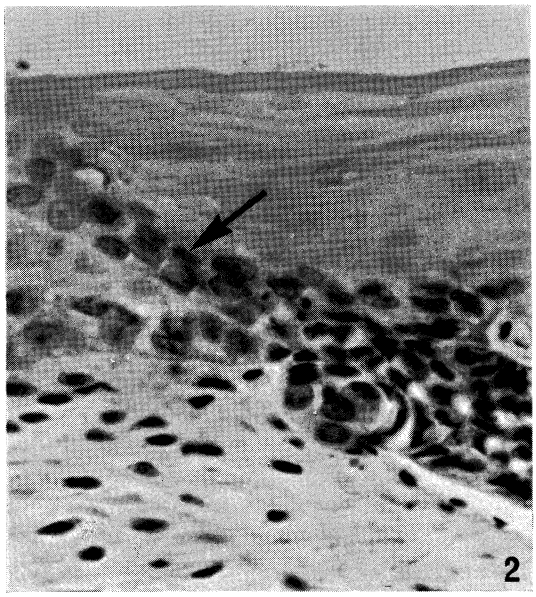
The stages in the development of these follicles, which appear on either side of both types of central follicles, are similar to those of the PCOY follicles. The first PLO follicles are seen at about 60 days after birth. Although their rate of development (Fig. 1) to stage 8 is about the same as it is for the PCOY follicles, they do not reach stage 10*c* until about 170 days after birth. Figure 7 shows a typical "trio" group with a PLO follicle on either side of a PCOX follicle. The presence of only one PLO follicle adjacent to a PCO follicle is rare. Sweat glands are usually formed in association with the PLO follicles and all of these follicles develop sebaceous glands.

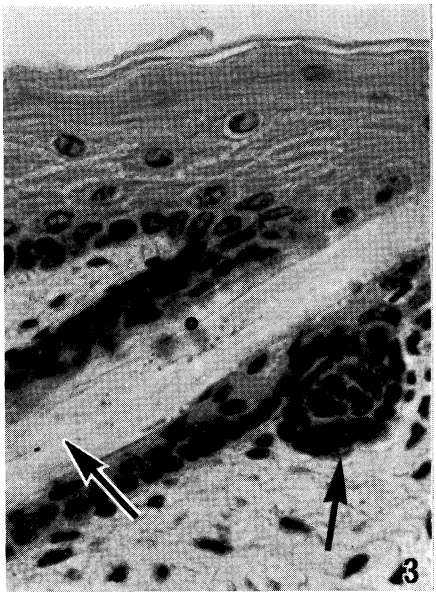
(iv) *SO Follicles*

These follicles are similar to the PLO follicles except that no sweat gland is formed. They first appear at about 80 days after birth and develop at about the same rate as the PLO follicles (Fig. 1). SO follicles are usually formed on the sweat gland side of the follicle group (Fig. 8). The first SO follicles are formed in association with the PCOX follicles. Initiation of SO follicles ceases about 100 days after birth.

(b) Development of Derived Follicles

Further follicles arise as branches from the outer root sheath of the original follicles, just below the sebaceous gland, and are referred to as derived central primary (PCD), derived lateral primary (PLD), and derived secondary (SD) follicles. All PLO and SO follicles branch. Branching of the PCOY follicles is not uncommon whereas the PCOX follicles do not branch. The presence of an epidermal pad [see Section III(*d*)] is one of the features which distinguishes the PCOX follicles from the PCOY follicles. As derived follicles are very slender, particularly prior to the formation of the hair cone, and develop in close proximity to their original follicles (Fig. 9), they are not easily studied. However, the stages of development appear to be the same as for original follicles, except that they arise from the outer root sheath instead of from the epidermis. No additional sebaceous gland is formed.





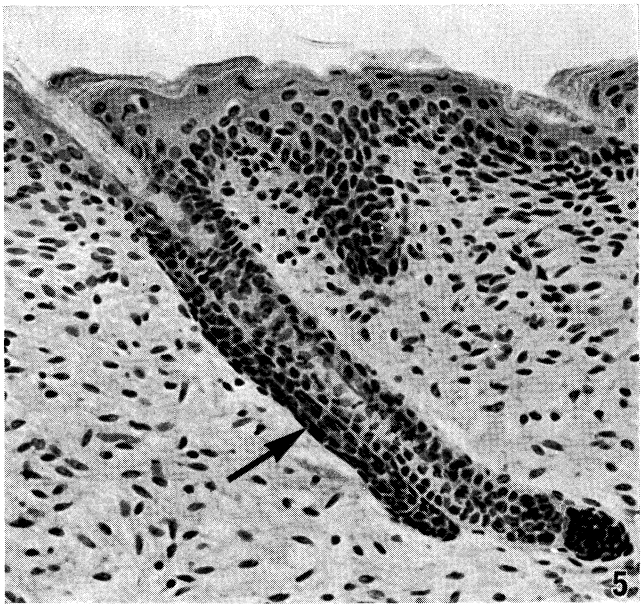


Fig. 2.—Longitudinal section of the upper part of a PCOX follicle at stage 5 in a 15-day-old animal showing a developing epidermal hair canal (arrow). Haematoxylin, eosin, and picric acid. ×620.

Fig. 3.—Longitudinal section of a PCOX follicle at stage 10*c* in a 15-day-old animal. Left arrow, club hair; right arrow, sweat gland bud. Haematoxylin, eosin, and picric acid. ×620.

Fig. 4.—Longitudinal section of the upper part of a PCOX follicle at stage 10*c* in a 24-day-old animal showing a developing sweat gland (left arrow) above a sebaceous gland (right arrow). Haematoxylin, eosin, and picric acid. ×560.

Fig. 5.—Longitudinal section of a PCOX follicle at stage 10*c* in a 54-day-old animal. The sweat gland (arrow) does not have a distinct lumen. DOPA plus alcoholic carmine. ×280.

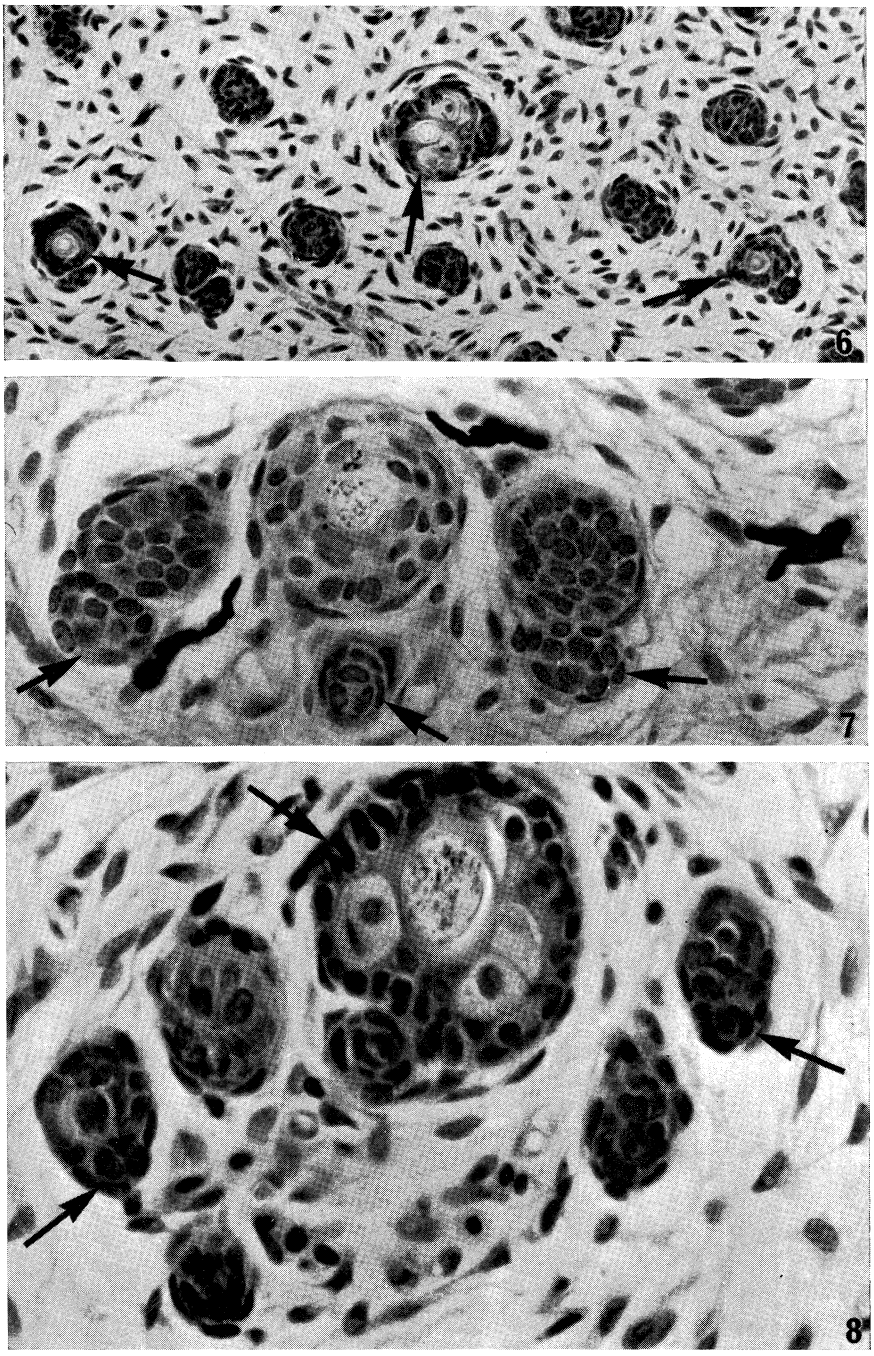


Fig. 6.—Section parallel to the skin surface showing the distribution of the follicles in a 60-day-old animal. Arrows indicate PCOX follicles; the remainder are PCOY follicles. Haematoxylin, eosin, and picric acid. $\times 220$.

Branching is first observed at about 110 days after birth (Fig. 1) and ceases at about 180 days. The most advanced derived follicles reach stage 8 at about 160 days after birth. This branching results in the formation of large bundles of follicles similar to those shown in Figures 10 and 11.

(c) *Development of the Follicle and Hair Population*

(i) *Follicle Groups*

The formation of follicle groups, as distinct from follicle bundles, is a prominent feature of the skin in *T. vulpecula*. Observations on the number of follicles per follicle group at different ages are summarized in Figure 16. The mean number of original follicles per group remains constant (around 4·6) in all samples from animals more than 100 days old. The largest follicle groups, containing up to 12 original follicles, are associated with PCOX follicles. Groups containing a PCOX follicle also contain the largest number of derived follicles. The number of follicles per group increases up to 10-fold with the development of the derived follicles.

(ii) *Density of the Follicles and Hairs*

Observations on the follicle population densities (number of follicles per 1 mm^2) are summarized in Figure 17. There is a sudden increase in the PCO follicle density between 40 and 50 days after birth due to the appearance of the PCOY follicles. The follicle density increases again when the PLO follicles appear at 60–70 days after birth, and the maximum density is reached at about 90 days during the period of initiation of the SO follicles. The final increase in the total follicle population density occurs when the derived follicles appear, and it is first shown in the counts (Fig. 17) at about 150 days after birth.

During the period up to about 40 days after birth, follicle formation balances skin expansion so that the number of follicles per unit area remains almost constant. The decrease in the density of the PCO follicles after about 60 days is proportional to the increase in skin surface area (Lyne, unpublished data). This increase in skin area also accounts for the decreases in the number of PLO and SO follicles per unit area.

The relation between age and the density of the hairs is shown in Figure 18. The hair density remains fairly constant up to about 60 days after birth when hairs first appear in the PCOY follicles. The increase in hair density during the period from 60 to 90 days after birth is due to the maturation of PCOY follicles. The hair density continues to increase as the PLO, SO, and derived follicles mature. In samples taken after 250 days all follicles have reached maturity (i.e. they contain keratinized hairs).

Fig. 7.—Section parallel to the skin surface (immediately below the sebaceous gland of a PCOX follicle) in a 78-day-old animal showing a "trio" group. The central arrow indicates the sweat gland duct of the PCOX follicle. The outer arrows indicate the sweat gland buds of the PLO follicles. DOPA plus alcoholic carmine. ×560.

Fig. 8.—Section parallel to the skin surface in an 80-day-old animal showing a follicle group associated with a PCOX follicle (central arrow). The two outer arrows indicate sweat gland buds contiguous with still developing PLO follicles; the remainder, lacking any evidence of a sweat gland, are SO follicles. Haematoxylin, eosin, and picric acid. ×560.

(*d*) *Innervation of the Skin and Follicles*

At 14 days after birth, the youngest animal examined using the silver-impregnation technique, the skin is free of nerves other than a few at the level of the panniculus carnosus. At 21 days there are some nerves in the dermis but no nerve networks around the PCOX follicles, the most advanced of which have reached stage 10*c*. At 54 days there is a well developed dermal nerve network in the mid-region of the dermis (Fig. 12) and branches lead to the PCO follicles, all of which develop nerve networks (Fig. 13) at a level immediately below the sebaceous gland openings. The nerve networks of the PCOX follicles are larger than those of the PCOY follicles. Nerve networks do not appear to be formed around the lateral and secondary follicles. This means that only one innervated follicle develops per follicle group.

A thickened epidermal pad (Figs. 14 and 15) forms on one side of each PCOX follicle. There is abundant alkaline phosphatase immediately below the dermo-epidermal junction of the epidermal pad (Fig. 14). A little enzyme activity also appears to be located in the basal layer cells of this region. The first epidermal pads are seen at 41 days after birth. In samples from older animals, a distinct layer of Merkel cells can be recognized near the base of the epidermal pad (Fig. 15). The region below the pad becomes well supplied with nerves, the first of which appear at about 61 days after birth.

IV. Discussion

Many features in the development of the hair follicles in *T. vulpecula* are similar to those described in the bandicoot *Perameles nasuta* (Lyne 1957*a*). However, follicle initiation from the epidermis extends over a period of almost 100 days in the former compared with a period of about 30 days in the latter. This slow development in *T. vulpecula* compared with *P. nasuta* is also apparent in body growth studies (Lyne and Verhagen 1957; Lyne 1964).

T. vulpecula and a few other marsupials are exceptional in that practically the whole of the skin is covered by a coat of hairs before the vibrissae emerge (Lyne 1959). Gibbs (1938) drew attention to this unique character of hair formation in *T. vulpecula* and suggested that it is probably associated with the development of the young in the pouch. Gibbs was not, however, aware that only few marsupials show this early development of the first coat hairs. Moreover, the rate of development of the first-formed follicles in *T. vulpecula* is much faster than it is for follicles formed later. The PCOX's are so small that the hairs grown during the first cycle reach a total length of only about 0·5 mm. The function of these small hairs is unknown.

Another unique feature of the formation of the PCOX follicles is that their sweat glands appear to remain stationary at an early stage of development during the

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Fig. 10.—Section parallel to the skin surface in a 6-year-old animal showing a follicle group with a bundle of central primary follicles (lower arrow), two bundles of lateral follicles, and one bundle of secondary follicles (upper arrow). Haematoxylin, eosin, and picric acid. ×220.

Fig. 11.—Section parallel to the skin surface in a 3⅓-year-old animal showing a follicle group with a PCO follicle (upper arrow) and five bundles of follicles (lateral and secondary). Lower arrows indicate sweat gland ducts. Haematoxylin, eosin, and picric acid. ×220.

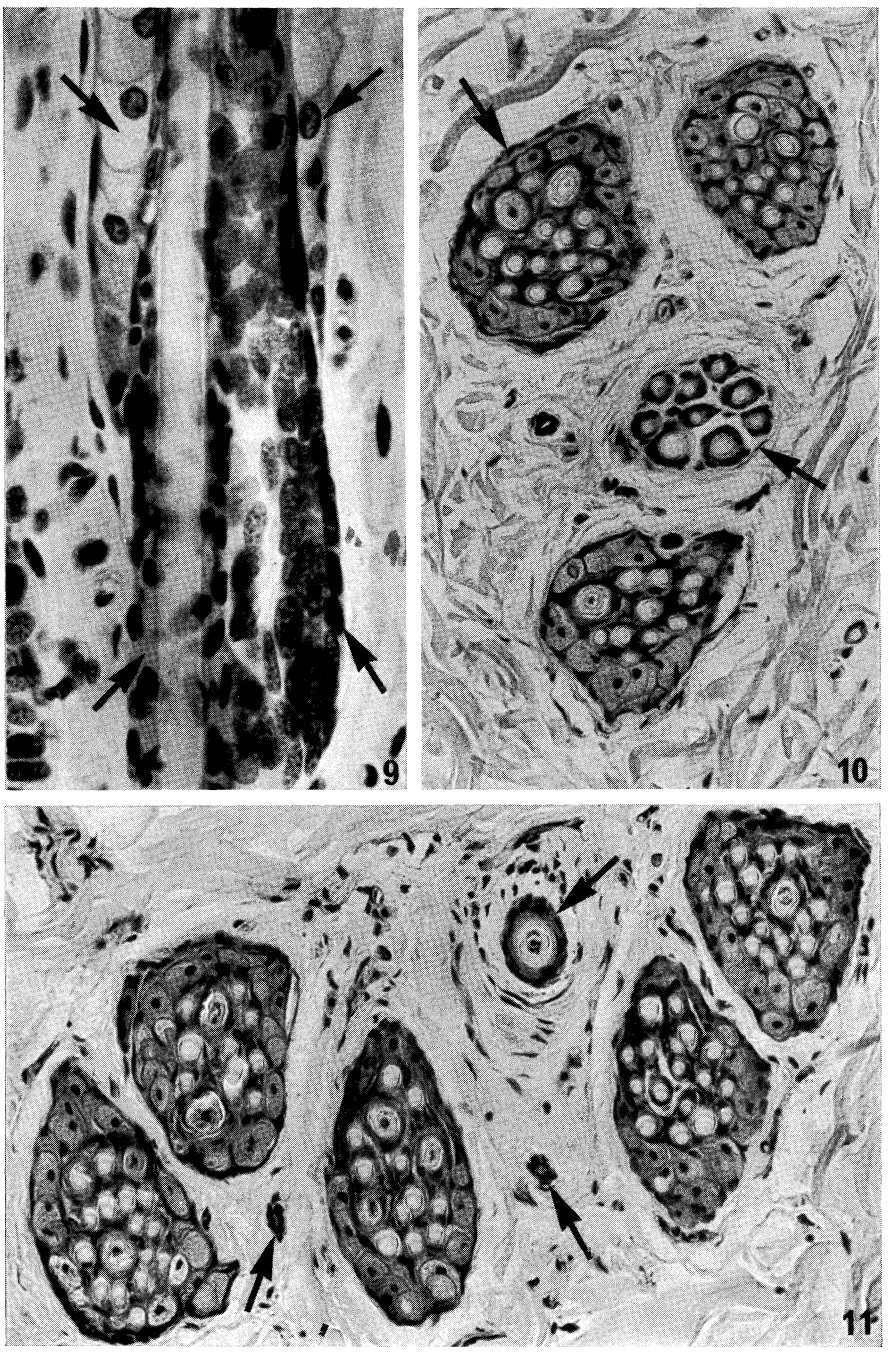

Fig. 9.—Longitudinal section of a bundle of follicles (primary lateral or secondary) with a derived follicle (lower right arrow) at advanced stage 1*b* in a 178-day-old animal. Lower left arrow indicates original follicle. Two upper arrows indicate the sebaceous gland. Examination of serial sections did not reveal a sweat gland. Haematoxylin, eosin, and picric acid. $\times 560$.

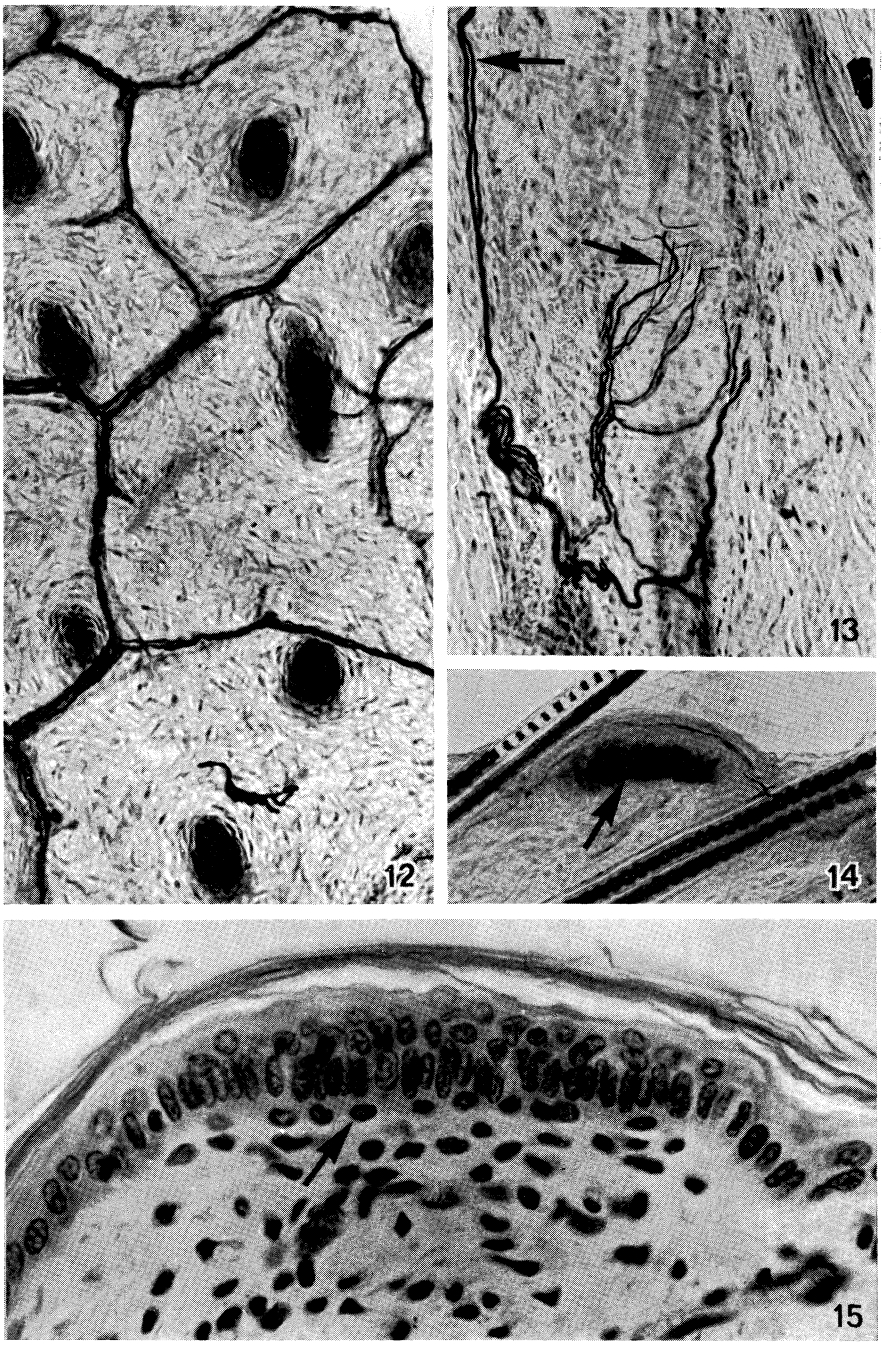

Fig. 12.—Section parallel to the skin surface in a 54-day-old animal showing a network of nerves in the mid-region of the dermis. Silver impregnation. $\times 250$.

Fig. 13.—Longitudinal section of the upper part of a PCOX follicle in a 187-day-old animal showing a follicle nerve network (lower arrow). Upper arrow indicates a nerve leading to an epidermal pad (not shown). Silver impregnation. $\times 220$.

first hair cycle. Further development of the sweat glands takes place during the first period of follicle quiescence.

The hair canals of the PCOX follicles differ from those of later formed original follicles in that sebaceous material is not present in the neck of the follicle before the tip of the hair reaches this level. In the sheep (Lyne 1957*b*), the hair canals of both the primary and secondary follicles are formed by the keratinization of epidermal cells, and the migration and degeneration of sebaceous cells in the upper part of the follicle. In the mouse (Hardy 1949), however, no association between sebaceous cells and hair canal formation has been described. In *T. vulpecula*, the tip of the PCOX hair must penetrate a plug of epidermal cells which forms above the follicle, whereas a continuous canal is formed prior to hair emergence in other original follicles.

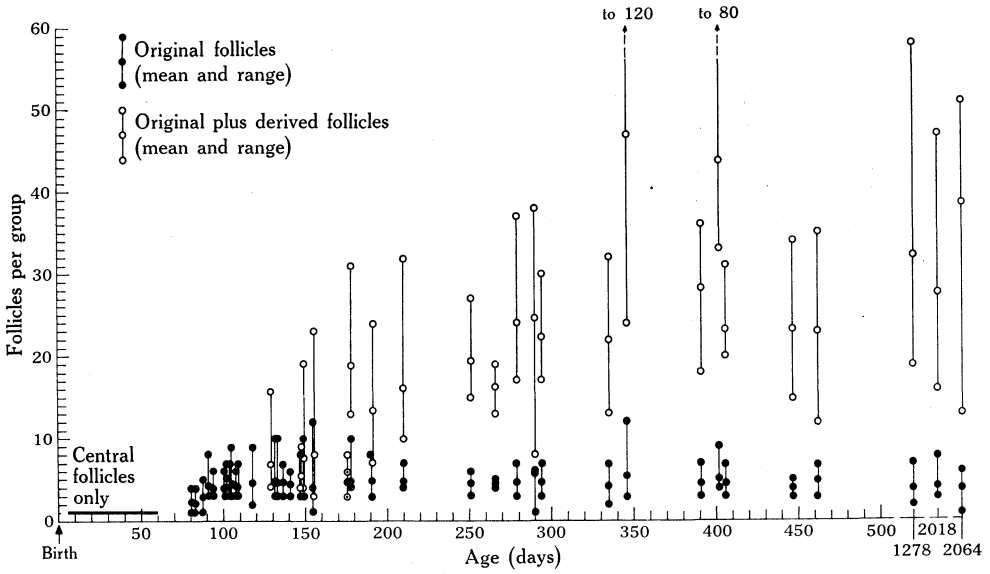

Fig. 16.—Relation between age and the size of the follicle group in 39 samples from 10 animals. Each mean and range is based on counts of 13–40 groups.

The sensory epidermal pad which forms near the orifice of each PCOX follicle is similar to that described in other species (Mann 1969; Straile 1969). The American opossum *Didelphis virginiana* (Mann 1968) resembles *T. vulpecula* in that the epidermal pad of the developing tylotrich follicle is not clearly seen until after the hair emerges through the skin surface.

The large bundles of follicles formed by branching in *T. vulpecula* are similar to those described in other species (Lyne 1966). Although Gibbs (1938) examined

Fig. 14.—Vertical section of the upper part of the skin of a 149-day-old animal showing an alkaline phosphatase-positive region (arrow) below an epidermal pad. × 220.

Fig. 15.—Vertical section of an epidermal pad in a 195-day-old animal. The arrow marks one of the Merkel cells visible as a distinct layer near the base of the epidermal pad. Haematoxylin, eosin, and picric acid. × 590.

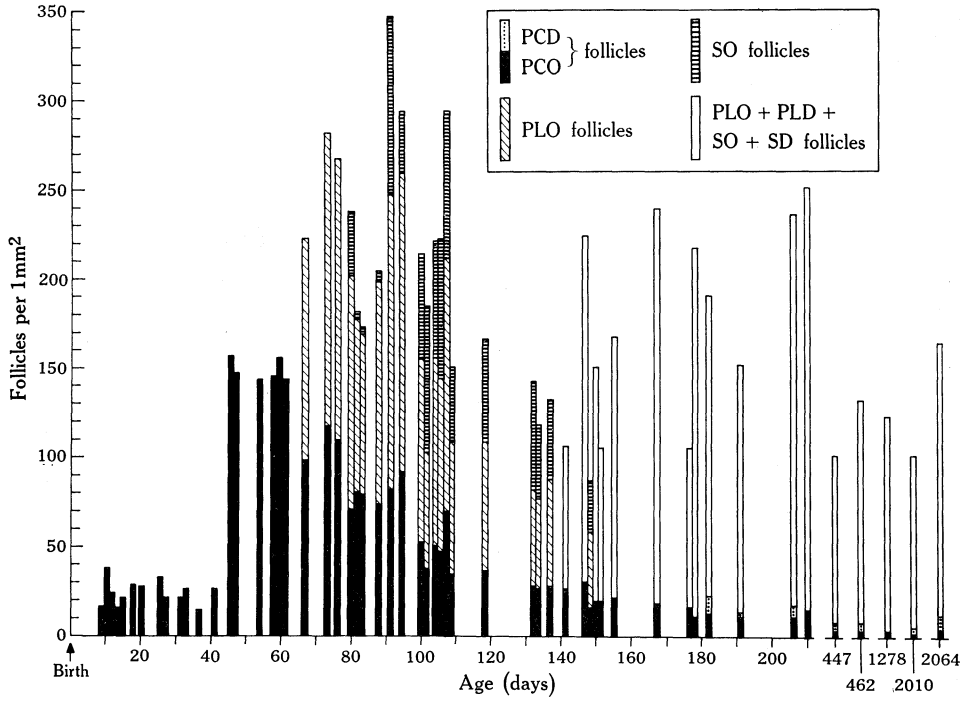

Fig. 17.—Relation between age and number of follicles per 1 mm² in 56 samples from 20 animals. The counts of the follicles in six samples (109, 141, 147, 149, 151, and 191 days) do not include all the immature follicles. In all samples except one taken after 140 days, the primary lateral and secondary follicles were difficult to identify and have been included together.

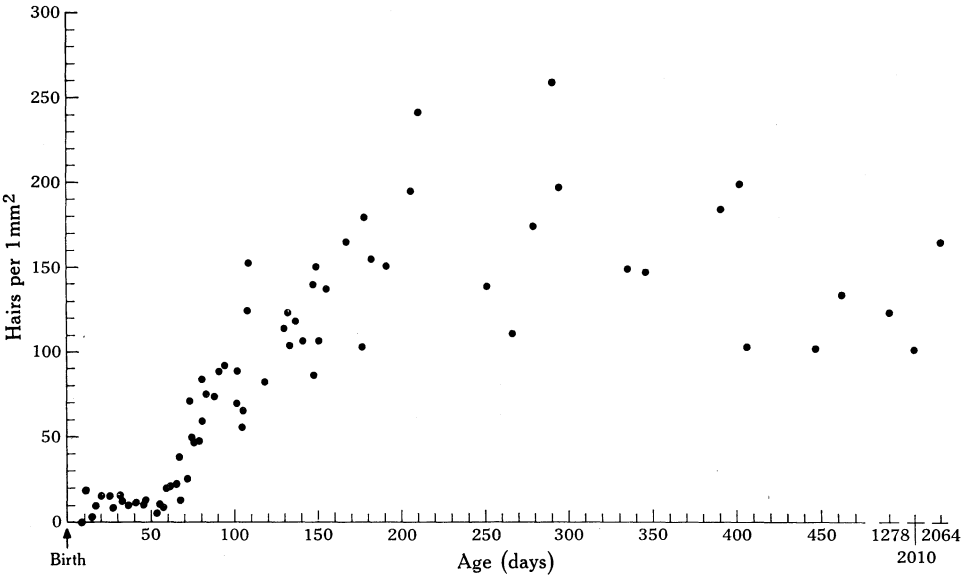

Fig. 18.—Relation between age and the number of hairs per 1 mm² in 71 samples from 26 animals.

skin samples of *T. vulpecula* ranging in age from about 12 days to 6 months she did not refer to follicle branching.

Hardy's (1947) description of the group arrangement of the follicles in adult *T. vulpecula* has been confirmed in the present study, and further details, such as the association of sweat glands with bundles of primary lateral follicles, have been added.

V. Acknowledgments

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