

# NEOGENESIS OF WOOL FOLLICLES

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## Summary

The possible formation of new wool follicles in healing experimental wounds was studied in one adult sheep and in three new-born lambs. True neogenesis was considered to have occurred in the epithelium covering the scars, though relatively few follicles were observed. Attempts to prevent contraction of the scar tissue, so that formation of new follicles might be encouraged, were unsuccessful. This failure is possibly the reason for the paucity of new follicles. Several early-stage follicles, seen in association with the neck region of existing follicles surrounding the wound margin, were also considered to be newly formed.

## I. INTRODUCTION

In sheep, as in most mammals that have been examined, new hair follicles are not normally formed after the adult complement has been established. Exceptions to this are the annual new growth of skin and hair covering the antlers of deer (Billingham 1958; Billingham, Mangold, and Silvers 1959), and the development of new hair follicles at the end of each hair cycle in the bandicoot, *Perameles nasuta* (Lyne 1957). In the latter instance the follicles arise by branching from the outer root sheath of existing follicles, immediately below the sebaceous glands, and new dermal papillae are formed.

There is now a growing body of evidence, reviewed by Billingham (1958), suggesting that follicle and hair neogenesis can occur in certain circumstances, even in those species in which it is usually not seen. Breedis (1954) and Billingham and Russell (1956) reported the formation of new follicles in rabbit scars that had only partially contracted. Straile (1959), however, failed to reproduce Breedis' results. More recently, Joseph and Townsend (1961) reported the new formation of hairs in skin covering non-contracted wounds on the ears of rabbits. In man, Kligman and Strauss (1956) and Kligman (1959) have claimed the new formation of vellus hairs after dermabrasion of the face. Earlier observations by Brook, Short, and Lyne (1960) hinted at the possibility of neogenesis in sheep, and the present work is an extension of these observations.

The aim of the present investigations was to examine whether hindering wound contracture, by a method described for the rabbit (Breedis 1954), would lead to the formation of the new wool follicles in the sheep. The criteria used in deciding whether a follicle was new were its stage of development, its position, and the absence of a hair canal. Two types of new follicles were observed: one type developed directly from the epidermis, and the other from the outer root sheath of existing follicles.

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This latter process is akin to the branching normally seen in Merino sheep fetuses, where most of the follicles develop from the neck region of existing ones (Hardy and Lyne 1956).

## II. MATERIAL AND METHODS

Four Merino sheep (one adult and three new-born lambs) were used. Circles (each of 16 Indian ink spots), 35 mm in diameter in the adult and 30 mm in the lambs, were tattooed on the dorsal aspect of the trunk of each animal. The wool was closely clipped before tattooing.

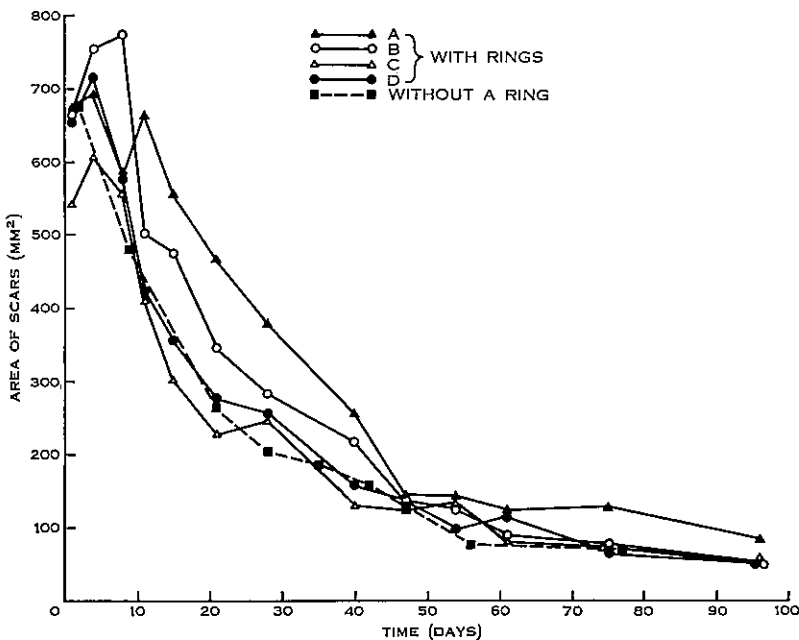


Fig. 1.—Changes in surface areas of scars in the dorsal skin of the adult sheep. Note that the wounds with rings contracted as quickly as the wound without a ring.

A full-thickness disk of skin was removed from just inside the tattooed spots. The disk usually included the underlying panniculus carnosus muscle. The underside of the removed skin was examined with a dissecting microscope for any sign of cut follicles, but none were found.

Rings of 22-gauge stainless steel were then inserted into some of the wounds in an attempt to hinder wound contracture. Each ring had eight prongs projecting through the skin on the circle of tattooed spots. Perspex covers, placed on top of the prongs, were used to protect the wounds. The technique is described in detail by Breedis (1954).

The wounds were inspected at least once a week and the wool was clipped or shaved from around the wound margin and projecting prongs. Distances between opposite tattooed spots were also measured at intervals so that changes in the shape

TABLE 1  
STAGES OF DEVELOPMENT OF NEW FOLLICLES

The stages of development are those described by Hardy and Lyne (1956). +, New follicles seen in epithelium covering the scar tissue; —, no new follicles seen in this epithelium

Wound	Days after Wounding																			
	8	10	11	12	15	16	18	19	21	23	25	26	28	32	34	35	40	42	46	47
Lamb 1																				
A*	—	—				—				—						+	—	—		
B*										—						(3b,4)	—	—		
C*										—						—	—	—		
D*																—	—	—		
Lamb 2																				
A†								—				+			—					
B†												(1)								
Lamb 3																				
A†											—									
B†																				
Adult sheep																				
A*																				
B*																				
C*																				
D*																				
Control†																				

\* With stainless steel rings. † Without rings.

and size of the experimental areas could be followed. The surface areas of the scars were obtained from photographs taken at regular intervals.

Rectangular biopsies of part of the healing region plus several millimetres of adjacent normal skin (Plate 1, Fig. 2) were taken at intervals (Table 1), fixed in 5% formol-saline, and embedded in paraffin. At least 30 vertical serial sections, 8  $\mu$  thick, were cut from each sample, and stained with haemalum, eosin, and picric acid.

Five disks of skin were excised from the adult and rings with prongs were inserted into four of the wounds; the fifth (control) wound was left untouched. Four disks of skin were excised from lamb No. 1 and rings with prongs were inserted into each wound. Two disks of skin were removed from each of the other two lambs and no rings were inserted.

### III. RESULTS

#### (a) *Macroscopic Observations*

A typical wound with the ring inserted is shown in Plate 1. In the adult the wounds with rings contracted just as quickly as those without (Fig. 1). No similar comparison can be made for the lambs, because wounds with and without rings were made on different animals. There were, however, indications that the rings hindered the rate of wound contracture, since at 20 days the areas of wounds with rings were 30-40%, and those without rings about 10% of their original size; the scars were of similar size at the end of the experiment. The inward movement of the skin caused small scars to appear on the inner side of the prongs in both the adult and lamb No. 1. Inspection with the naked eye did not reveal any new fibres in the wounded region.

#### (b) *Microscopic Observations*

Sixty-six biopsies of the healing wounds were made at intervals (see Table 1) for microscopic examination. In the examination of the serial sections particular attention was given to the new epithelium covering the scar tissue. Fourteen follicles were seen in this region and were considered newly formed. Although these follicles were seen in only 9 out of the 66 biopsies taken, at least one was seen in each animal. All of these follicles were independent of old, pre-existing follicles. No new follicles were seen before 26 days after wounding.

Plate 2, Figure 1, shows a new follicle with very few pre-papilla cells. Probably the best example of a new follicle is the short, broad, immature follicle in the healing area of a lamb, 67 days after wounding, and which is illustrated in Plate 2, Figure 4. The epidermal plug has started to invaginate and an aggregation of dermal cells can be clearly seen below. This interesting follicle, in common with other early stages, is without a hair canal.

No newly formed sebaceous glands were observed with the possible exception of a rudiment seen in the adult 96 days after wounding (Plate 3, Figs. 1 and 2). No sebaceous cells could be detected. No apocrine sweat glands or arrectores pilorum muscles were seen in association with any of these new follicles. A few epithelial buds (Plate 2, Fig. 2) were observed. As these structures were without an aggregation of pre-papilla cells they were not classified as follicles.

The epithelium covering the wounds was usually greatly thickened with well-defined spinous, granular, and cornified layers. Plate 3, Figure 3, shows two new follicles developing from a greatly thickened epithelium in a lamb, 35 days after wounding.

In addition to the new follicles described above, several early-stage follicles were seen in association with the epidermis (Plate 2, Fig. 3), or the upper part (neck region) of existing follicles (Plate 4, Figs. 1 and 2) surrounding the wound margin. Plate 4, Figure 3, shows a very early-stage follicle bud associated with a possibly regenerating follicle. Immature follicles (regenerating or new), similar to those shown in Plate 4, Figure 4, were not uncommon in the immediate vicinity of the scars.

#### IV. DISCUSSION

The method used to inhibit wound contracture, which was similar to that used by Breedis (1954) and Straile (1959), was ineffective in the adult sheep. Though the rings may have slowed the rate of wound contracture in the lamb, in both the adult and lamb the contracting skin moved past the prongs leaving obvious scars on their inner sides. In this respect our findings are in complete accord with those of Straile (1959).

Failure to prevent wound contracture may well be the cause of the scarcity of new follicles; nevertheless, their presence confirms the ability of sheep skin to initiate follicle development postnatally. The age of the animal did not appear to matter in this respect.

Injury was probably the stimulus for the formation of new follicles from existing ones adjacent to the wound margin. This type of neogenesis has apparently not been reported before.

Although not many new follicles were seen in the sections, we believe that true neogenesis occurred in these sheep. If neogenesis could be shown to occur freely then it will be of immense help in the study of follicle initiation. In the small laboratory animals that have been used in investigations of neogenesis, the hair cycles have been a complicating factor. This disadvantage does not apply to the domestic sheep since wool follicles have virtually no cyclic activity. The difficulty of preventing the contracture of scars in the trunk skin of sheep might be overcome by selecting a region such as the external ear which is probably covered with immobile skin similar to that described by Joseph and Townsend (1961) in the rabbit.

#### V. ACKNOWLEDGMENTS

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## EXPLANATION OF PLATES 1-4

## PLATE 1

- Fig. 1.—Circular full-thickness wound in the dorsal skin of the adult sheep. The photograph was taken immediately after the insertion of a stainless steel wire ring. The tattooed spots between the eight projecting prongs cannot be clearly seen in this photograph.
- Fig. 2.—Appearance of the healing region after 21 days. The tattooed spots in this figure (and in Figs. 3 and 4) were darkened with Indian ink before photography. The shape of a typical biopsy is indicated.
- Fig. 3.—The appearance of the healing region after 47 days.
- Fig. 4.—The appearance of the healing region after 96 days. The inward movement of the skin and elongation of the tattooed spots is obvious.

## PLATE 2

The stages of development are those described by Hardy and Lyne (1956)

- Fig. 1.—Vertical section of the epithelium covering the scar tissue in lamb No. 2, 26 days after wounding. A few pre-papilla cells (PP) can be seen below the new follicle which is at stage 1. The epidermis is greatly thickened with well-defined spinous, granular, and cornified layers.
- Fig. 2.—Vertical section of the epithelium covering the scar tissue in lamb No. 1, 91 days after wounding. The epithelial bud projecting into the scar tissue is without an aggregation of dermal cells and is not regarded as a hair follicle.
- Fig. 3.—Vertical section of a new follicle arising from the epidermis adjacent to the wound margin in the adult, 96 days after wounding. An aggregation of pre-papilla cells (PP) can be seen below the rounded base of the follicle plug.
- Fig. 4.—Vertical section of the epithelium covering the scar tissue in lamb No. 3, 67 days after wounding. The new follicle is at stage 3a. Invagination of the epidermal plug has begun and the dermal papilla cells (DP) can be clearly seen beneath.

## PLATE 3

The stages of development are those described by Hardy and Lyne (1956)

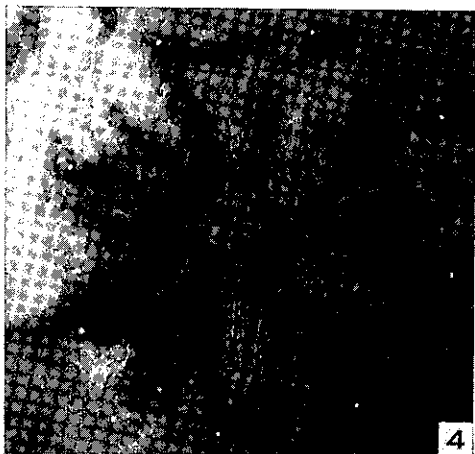
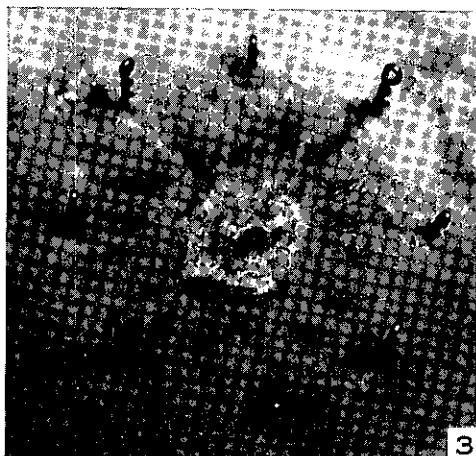
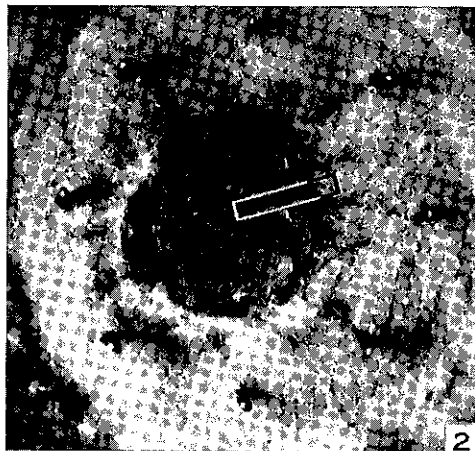
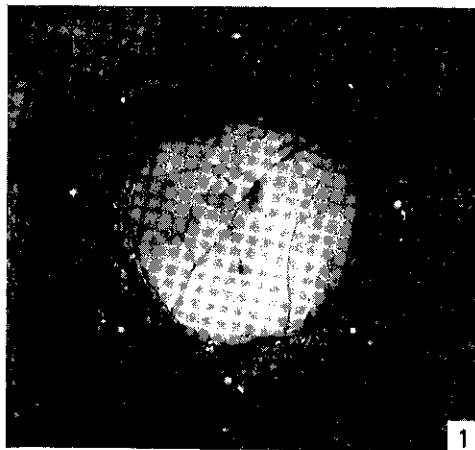
- Fig. 1.—Vertical section of the epithelium covering the scar tissue in the adult, 96 days after wounding. The scar, not previously sampled, is shown in Plate 1, Figure 4. The solitary follicle is probably a new structure.
- Fig. 2.—An enlargement of the follicle seen in Plate 3, Figure 1. A keratinized fibre (*H*), surrounded by inner root sheath cells (*IRS*), has reached the level of the epidermis. The small bud, just below the epidermis, appears to be a sebaceous gland rudiment (*SG*). There are no sebaceous cells.
- Fig. 3.—Two new follicles developing from a greatly thickened epithelium covering a scar in lamb No. 1, 35 days after wounding. The sausage-shaped structure on the right (*A*), is a follicle at stage 3. The dermal papilla is formed but it is not seen in this photograph. The odd-shaped structures on the left (*B*) all belong to one follicle at stage 4. Portion of a developing hair canal (*HC*) can be seen in the epidermis above this follicle. Neither follicle has a sebaceous gland.

## PLATE 4

The stages of development are those described by Hardy and Lyne (1956)

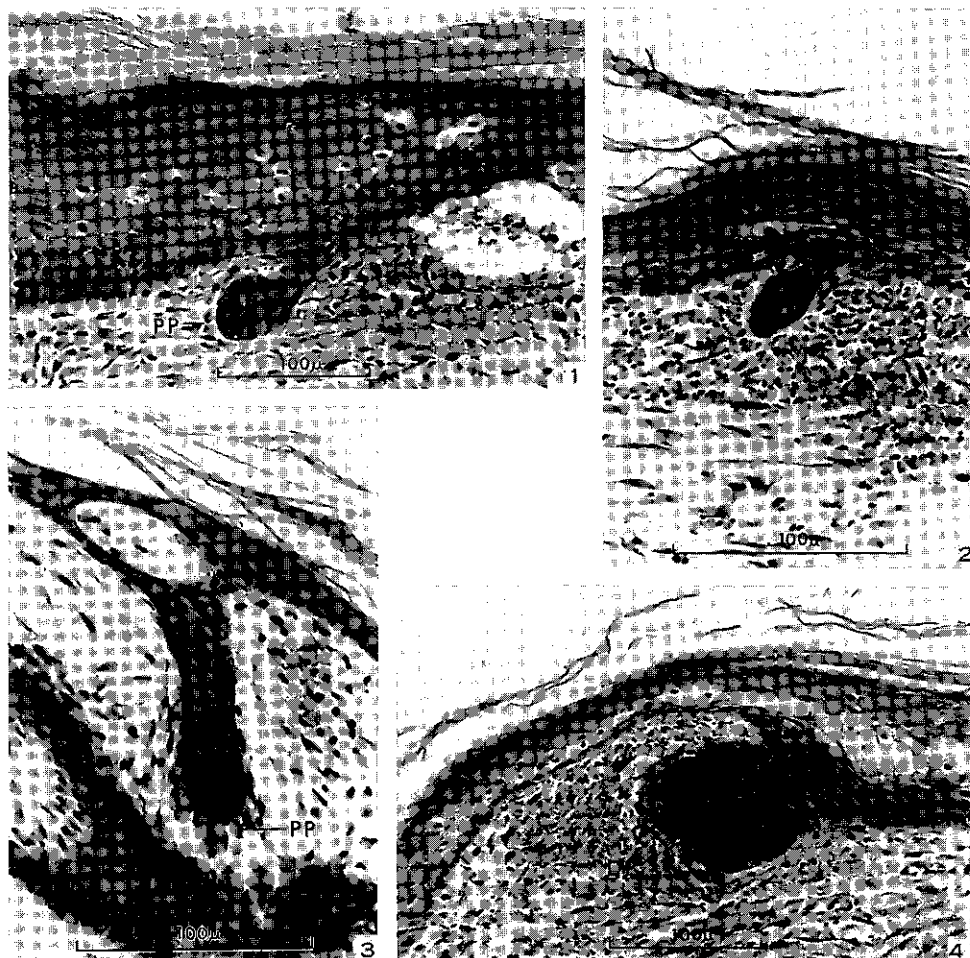
- Fig. 1.—Longitudinal section of a new follicle (*NF*) at stage 1 which has arisen by branching from the neck region of an existing follicle near the wound margin in the adult, 75 days after wounding. Pre-papilla cells are present but not clearly seen.
- Fig. 2.—Longitudinal section of a possible new follicle developing from the neck region of a bundle of two existing follicles near the wound margin in the adult, 75 days after wounding. Although the new follicle appears to be at stage 1, a hair cone (not shown in this section) has started to form and the follicle has reached stage 4.
- Fig. 3.—Vertical section of a possibly regenerating follicle (*RF*) and a new follicle (*NF*) bud at stage 1 near the wound margin in the adult, 75 days after wounding. A distinct cap of pre-papilla cells (*PP*) surrounds the new epithelial plug.
- Fig. 4.—Vertical section of two regenerating or new follicles in the skin near the wound margin in the adult, 66 days after wounding. The follicle on the right is at stage 2 and the one on the left is at stage 3a.

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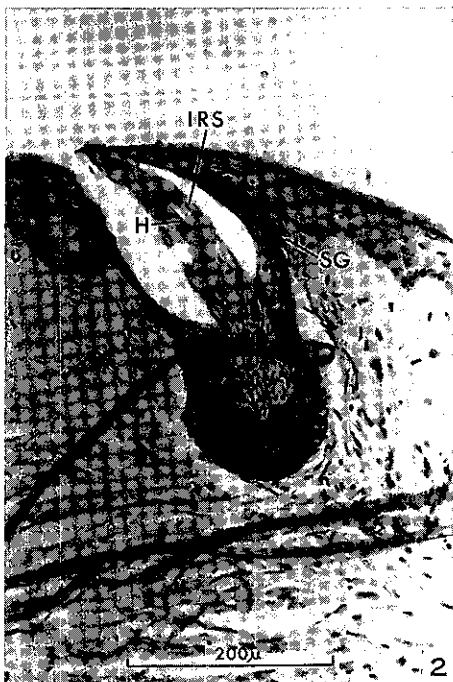
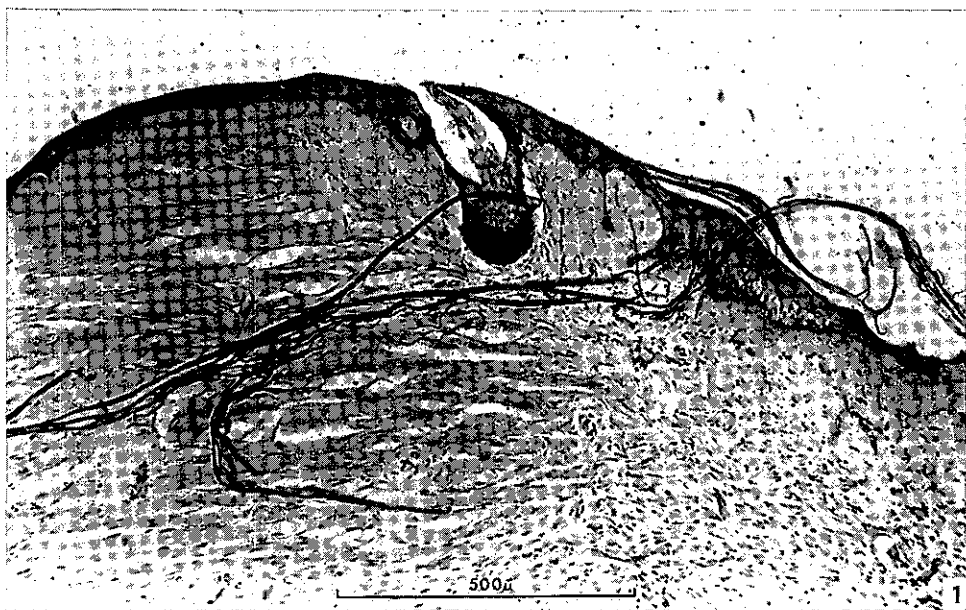




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