THE PRE-NATAL DEVELOPMENT OF WOOL FOLLICLES IN MERINO SHEEP

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Summary

A histological study has been made of wool follicles in skin from the trunk of 24 sheep foetuses ranging in age from 69 to 145 days of gestation. Fifteen of these foetuses are known to be entirely Merino in origin, and the remainder are believed to be wholly or predominantly Merino. Extensive use has been made of serial sections parallel to the axis of the wool follicles as well as those parallel to the skin surface. Skin from Merino lambs and adults has also been examined.

A series of stages in the development of hair follicles in mammals is defined. The special features of each stage in the development of primary and secondary wool follicles in the Merino are indicated. Secondary follicle development, not fully described hitherto for any breed of sheep, shows some distinctive features, the most important of which is the origin of later follicles by branching from earlier ones. Thus there are two types of secondary follicles, original and derived. Some preliminary information has been obtained about their development and structure in late pre-natal and early post-natal life.

Carter's (1943) proposed series of stages of wool follicle group development, based on the appearance of transverse sections of skin at the level of the sebaceous glands, are re-defined in the light of the present observations and accompanied by a table showing the stage of development of each type of follicle.

Attention is drawn to the need for further study of the way in which branching follicles contribute to the development of the wool fibre population in Merinos and other breeds of sheep. Some possible implications for current researches on this subject are discussed.

I. INTRODUCTION

At the present time there is widespread interest in the study of the development of the wool follicle population in sheep, and an awareness of its importance for the understanding of the adult fleece and the control of wool production. This is evident from the number of recent papers on post-natal wool follicle development, including those by Burns (1953, 1954*a*, 1954*b*), Fraser (1952*a*, 1952*b*, 1953, 1954), Fraser and Short (1952), Margolena (1954), Schinckel (1953, 1955*a*, 1955*b*), and Short (1955*a*, 1955*b*). Various features in the development of wool follicles during the pre-natal period have been described by Spöttel and Tänzer (1923), Duerden and Ritchie (1924), Tänzer (1926), Wildman (1932), Carter (1943), and Carter and Hardy (1947). Yet, when the present authors commenced a study of the development of foetal sheep skin and wool in tissue culture (Hardy and Lyne 1956*b*), they found that none of the published accounts was adequate for a comparison between the development of wool follicles *in vivo* and *in vitro*. The time seemed opportune for a more precise account of pre-natal follicle development in the Merino, including some processes which have

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hitherto received little attention. It is the aim of this paper to give such an account. A preliminary note concerning this work has already been published (Hardy and Lyne 1956*a*).

II. Material and Methods

(*a*) *Animals*

Twenty-four sheep foetuses from a number of sources provided material for this study. While 15 of these were known to be entirely Merino in origin, it was not possible to check on the alleged Merino origin of the nine others. However, the foetuses in the latter group were either wholly or predominantly Merino, and were similar in external features, body dimensions, and skin structure to those of known parentage of the same age, so they were included in the observations.

The G series of 12 South African Merino foetuses of known ages ranging from 69 to 145 days had already been used for wool follicle development studies by Carter (1943) and Carter and Hardy (1947), and is described in the latter paper. The KR series of seven undated foetuses believed to be wholly or predominantly Merino in origin, and foetus No. X401, a Merino foetus, were sampled by one of us (M.H.H.) and the skin sections were used in a previous study (Carter and Hardy 1947). The S series of four foetuses was used also for tissue culture experiments (Hardy and Lyne 1956*b*). Two undated foetuses were believed to be Merinos. The other two, 98 and 125 days old, were of fine-woolled Merino (non-Peppin) parentage.

A further estimate of the age of all dated foetuses from their body dimensions was made by a modification of Cloete's (1939) technique as described by Carter and Hardy (1947), and good agreement was obtained between estimated and true ages. The external features were compared with those described by Cloete as an additional check. Estimated ages obtained by the same means were used in all calculations for the undated foetuses.

In addition to the pre-natal material, skin samples from Merinos have been studied from birth to 7 days of age, at 2, 4, 5, 6, 18, and 19 weeks, and at 9 and 18 months.

(*b*) *Histological Methods*

Entire foetuses and most of the skin samples were fixed in formalin, but a few skin samples were fixed in Zenker's fluid. The sampling positions, all from the fleece-bearing area, were chosen according to the system described by Carter and Hardy (1947), and included a mid-side sample for every foetus. Serial sections at 7 or 8 μ, or occasionally thicker, were cut transversely through the wool follicles and, when possible, in the longitudinal direction as well. They were stained with haemalum and eosin, and, in all except the G series, picric acid was added to stain the wool fibres.

Many individual follicles and follicle groups were studied in complete serial sections in either the longitudinal or the transverse direction, and reconstructions were made from camera lucida drawings.

(*c*) *Terminology*

Reference will be made to primary follicles (*P*) and secondary follicles (*S*) as defined by Wildman and Carter (1939), and to follicle groups as defined by Carter

(1943). Types of secondary follicles will be defined in this paper. The following additional symbols for follicle types recently proposed by Hardy and Lyne (1956*c*) will be used: *PC* = central *P* of trio group; *PL* = lateral *P* of trio group; *PCX* = member of first-formed *PC*; *PCY* = member of later-formed *PC*; *PLx* = *PL* associated with *PCX*; *PLy* = *PL* associated with *PCY*; *SO* = original *S*; *SD* = derived *S*; *SOU* = unbranching *SO*; *SOB* = branching *SO*. The symbol *f* (follicle containing a fibre) is added where necessary to distinguish follicles which have reached this stage of development.

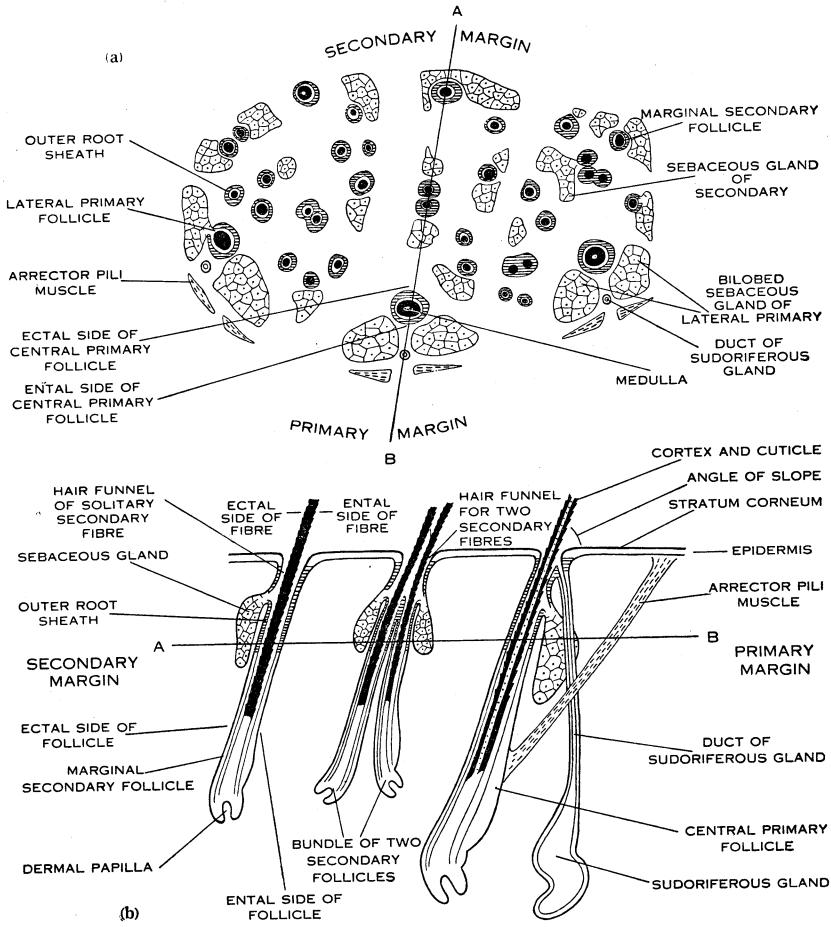

Fig. 1.—(*a*) Diagrammatic transverse section through an adult Merino wool follicle group at the level of the sebaceous glands. (*b*) Diagrammatic longitudinal section through the follicles and accessory structures in or near the plane passing through A–B shown in Figure 1 (*a*).

The terms for parts of the follicle group used by Carter (1943) and additional terms used in this paper are illustrated in Figure 1. The *angle of slope* of an emergent fibre (or a follicle), is the acute angle which the axis of the fibre (or follicle) makes with its projection on the skin surface. Auber (1950) and earlier authors refer to the

ental side of an emergent *fibre* as that on the side of the acute angle of slope and the *ectal* side as the opposite one. Thus the *ental* side of a *follicle* is that which makes an obtuse angle with the skin surface and the *ectal* side is the one opposite. The *neck* of a developing or mature follicle is the epidermal part from the opening of the sebaceous gland to the base of the epidermis. The term *funnel* (Duerden and Ritchie 1924) indicates the cavity between the wool fibre and the follicle wall which extends from about the level of the opening of the sebaceous gland to the surface of the skin. A *follicle bundle* is an aggregation of two or more follicles which are joined over part of their length, whether by origin from a common epidermal downgrowth or by fusion of originally separate follicles.

III. The Development of Individual Follicles

The sequence of development of a mammalian hair follicle may conveniently be described by a series of stages. The following definitions of follicle stages are based on those proposed by Hardy (1949) for the mouse but are widened to make them applicable to other rodents which have been examined, to sheep, and possibly to a variety of mammals. Each stage number is prefixed by the letter *F* to distinguish it from the stages of follicle groups to be described later.

Stage F1: *Follicle plug.*—A plug of cells from the epidermis (or primitive ectoderm) extends into the dermis (or primitive mesoderm). This stage begins when a localized thickening of the basal layer of the epidermis can be recognized. There is frequently an aggregation of dermal cells beneath the epidermal plug.

Stage F2: *Pre-papilla.*—The base of the epidermal plug flattens prior to invagination.

Stage F3: *Papilla.*—The base of the epidermal plug is invaginated and a dermal papilla is formed. This stage begins when the base of the epidermal plug becomes recognizably concave.

Stage F4: *Hair cone.*—The elongated cells of Henle's layer of the inner root sheath are recognized, forming a cone which is directed along the axis of the follicle towards the skin surface.

Stage F5: *Advanced hair cone.*—The tip of the hair cone reaches the deepest level of the sebaceous gland, if present, or the deepest level of the sebaceous glands of adjacent follicles of the same type.

Stage F6: *Hair formation.*—The tip of a keratinized hair fibre appears inside the hair cone.

Stage F7: *Hair in epidermis.*—The tip of the keratinized hair reaches beyond the base of the epidermis above the follicle. A hair canal has usually been formed in the epidermis before the hair tip reaches this level.

Stage F8: *Hair emerged.*—The tip of the hair has penetrated the superficial layers of the epidermis.

The above stages can be recognized in both the primary and secondary follicles of sheep. The special features in the development of each type of follicle in the Merino will be considered in the next section.

The development of hair canals in association with wool follicles in sheep will be described in detail in a subsequent paper. Briefly, the hair canal is formed by two separate processes—keratinization of cells in the epidermis and disintegration of sebaceous cells which have migrated to the neck of the follicle. In the epidermal cells there is keratohyalin granulation and keratinization which is followed by the appearance of an intercellular space, while in the sebaceous cells there is cell degeneration followed by space formation. Eventually a continuous canal runs from the follicle neck to the upper part of the epidermis. The development of hair canals does not always take place during identical stages of follicle development, but in the following descriptions reference will be made to the average state of the hair canal at each follicle stage.

(*a*) *Primary Follicles*

Several authors have made observations on the development of primary follicles in sheep. Spöttel and Tänzer (1923) studied a few foetuses of the Merino and two other breeds. The descriptions of follicle development in the South African Merino by Duerden and Ritchie (1924) and in British breeds by Wildman (1932) clearly referred only to primary follicles, although the authors do not emphasize this point. Whilst Duerden and Ritchie and also Wildman studied follicles mainly in longitudinal sections, Carter (1943) based his description of primary follicles in the Australian Merino entirely on transverse sections.

A more complete picture of follicle development is made possible by the use of both longitudinal and transverse sections. Since most of the previously published observations were confirmed in the present study, the following account will be limited to the special features of *P* follicles in the Merino at each of the defined mammalian stages (Fig. 2). This description applies to both the central *P* (*PC*) and lateral *P* (*PL*) follicles, which were studied separately.

Stage F1.—At the beginning of this stage an aggregation of dermal cells appears beneath the epidermal plug, which usually has an acute angle of slope.

Stage F2.—The base of the epidermal plug flattens when it is still relatively short, the length of the plug being usually not more than twice its diameter. Near the beginning of this stage the sudoriferous gland rudiment appears as a solid bud from the ental side of the follicle (stage *F2a*). Later the bilobed sebaceous gland bud is formed on the ental side below the sudoriferous gland rudiment, and differentiated sebaceous cells can be recognized (stage *F2b*). These observations agree with those of Wildman (1932) and Carter (1943), and are contrary to those of Duerden and Ritchie (1924), who stated that in the Merino foetus the sudoriferous gland rudiment appeared later than that of the sebaceous gland.

Stage F3.—This stage can be subdivided according to the shape of the dermal papilla. At stage *F3a* the length of the papilla is less than its diameter, and at stage *F3b* its length is equal to or (rarely) greater than the diameter. Sometimes the solid sudoriferous gland rudiment shows a distal swelling, and the first trace of the arrector pili muscle may be found. Formation of the epidermal part of the hair canal may begin at *F3a*, and sebaceous cells have sometimes migrated to the follicle neck at *F3b*.

Stage F4.—This stage is more difficult to distinguish in the Merino than in some other mammals because Henle's layer is not yet refractile, but the elongated nuclei of this layer may be seen in the cells which form a cone in the deeper part of the hair

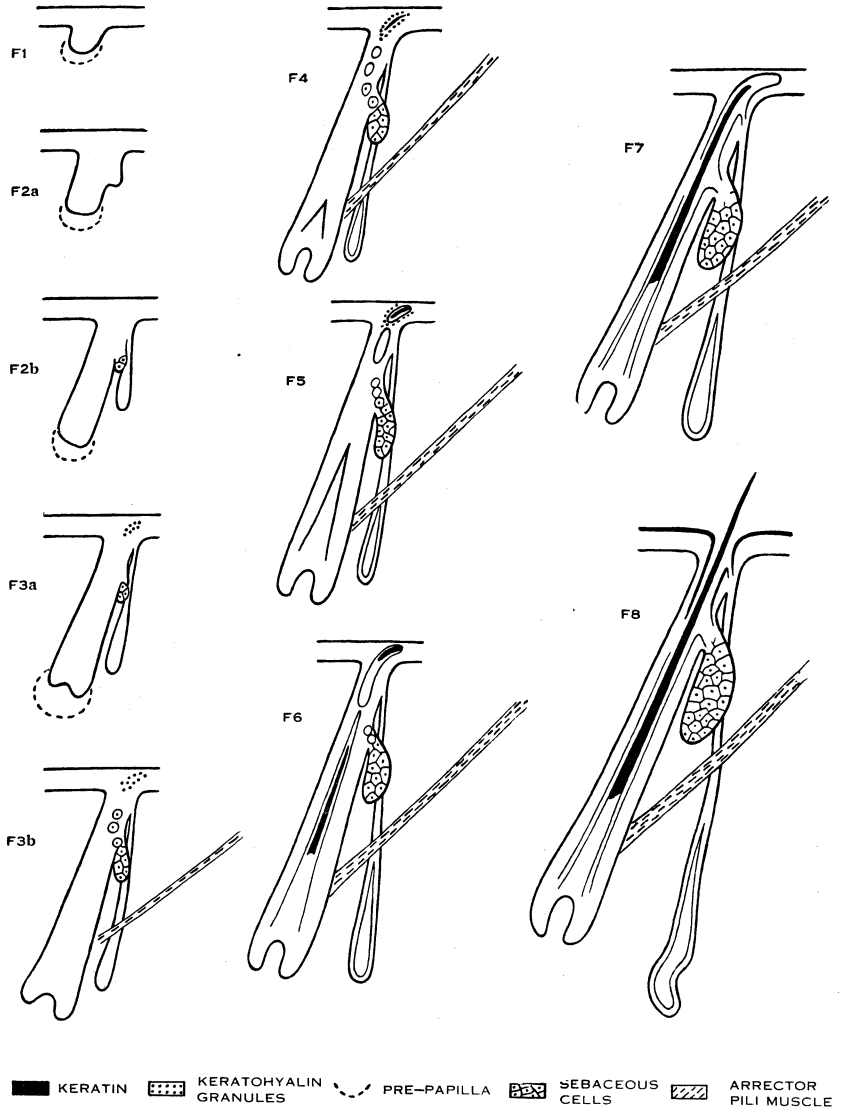

Fig. 2.—Diagram of stages *F*1–*F*8 in the development of primary wool follicles in the Merino foetus.

follicle. The length of the dermal papilla is at least equal to its diameter, and is frequently greater. The deepest part of the sudoriferous gland sometimes has a small lumen. The arrector pili muscle is always present, usually as two strands of smooth muscle fibres extending from the upper part of the dermis to the ental side of the wool

follicle at a deep level. In this region the outer root sheath sometimes has a marked ental swelling, as noted by Duerden and Ritchie (1924) and Auber (1950), but in many Merino foetuses a swelling is not apparent at this or later stages. The hair canal has developed further.

Stage F5.—The hair cone is now refractile and reaches the level of the base of the bilobed sebaceous gland, which is well developed. Inside the cone Huxley's layer and the developing hair cuticle and cortex can be recognized. The sudoriferous gland sometimes has a small lumen. There is frequently a hair canal space in both the epidermis and the follicle neck.

Stage F6.—The tip of the hair cone is solid and refractile, and lies at or above the level at which the sebaceous gland opens. The keratinized tip of the hair lies inside the cone. At this stage all the follicle layers can be recognized. The sudoriferous gland has usually begun to widen below the level where it passes between the two strips of the arrector pili muscle. Hair canal formation is completed.

Stage F7.—The tip of the wool fibre has emerged through the inner root sheath layers and now lies at the epidermal level in the hair canal on the ental side of the follicle. The uppermost part of the canal is usually bent, forming an obtuse angle with the axis of the follicle and lying almost parallel to the skin surface. The inner root sheath layers split and fragment as they reach the hair canal.

Stage F8.—The hair has pierced the uppermost layer of the epidermis (the periderm) and emerged.

(*b*) *Secondary Follicles*

There appears to be no complete account in the literature of the development of *S* follicles in sheep. Spöttel and Tänzer (1923) reported that in addition to the lack of sudoriferous glands and arrector pili muscles, there were several minor ways in which *S* follicles differed from *P* follicles in their development in Merino and English Blackface foetuses. However, they failed to report the arrangement of *S* follicles in bundles from the beginning of their development, which has been noted in the present study. In the Merino foetus most of the later *S* follicles, here to be referred to as derived secondary follicles (*SD*), were found to originate by simple branching from a few original secondary follicles (*SO*). A later paper by Tänzer (1926) on the Karakul contains what seems to be the only reference to a similar branching origin of *S* follicles in any breed.

The following is a brief account of the stages of development of the original secondary follicles, which are illustrated in Figure 3 and Plates 1 and 2.

Stage F1.—At the beginning of this stage an aggregation of dermal cells appears beneath the epidermal plug, which continues to elongate and retains its rounded base (Plate 1, Fig. 1; Plate 2, Fig. 1).

Stage F2.—The *SO* follicle is usually more elongated than the *P* follicles when its base begins to flatten (Plate 1, Fig. 2). A sudoriferous gland rudiment is not formed at this or any later stage. The sebaceous gland rudiment does not usually appear until later.

Stage F3.—As with *P* follicles, this stage can be subdivided into *F3a*, when the length of the papilla is less than its diameter (Plate 2, Fig. 2), and *F3b*, when its length

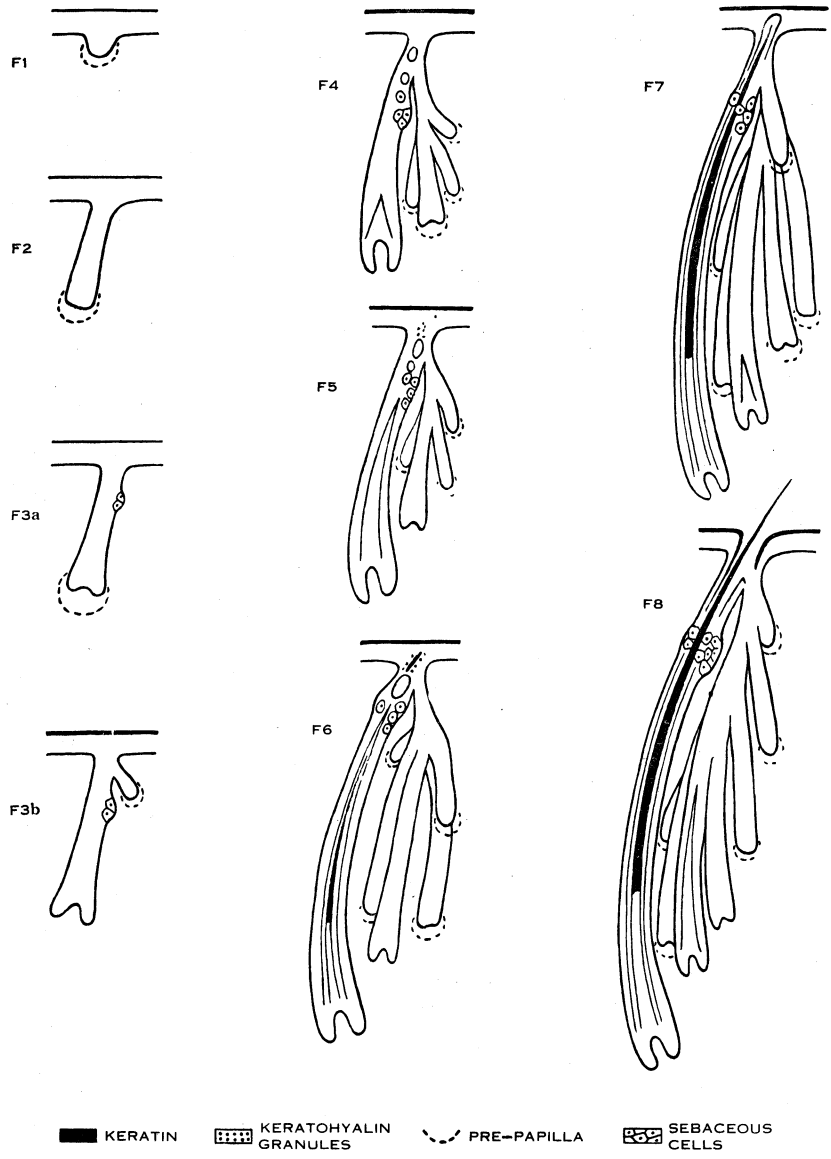

Fig. 3.—Diagram of stages *F*1–*F*8 in the development of original secondary follicles in the Merino foetus. Some stages in development of derived secondary follicles are also shown.

is equal to or (rarely) greater than its diameter. The rudiment of the sebaceous gland, with differentiated sebaceous cells, usually appears at *F3a* (Plate 1, Fig. 3). No arrector pili muscles are formed at this or any later stage. At *F3b* a new (*SD*) follicle

sometimes arises from the *SO* follicle, frequently as a lateral branch above the level of the sebaceous gland. Each *SD* follicle has at first a rounded base and would be classified as *F*1.

Stage F4.—The hair cone (Plate 2, Fig. 4) is similar to that of *P* follicles. Many differentiated sebaceous cells are present, but the gland rudiment does not protrude very far from the follicle wall, and is seldom bilobed. Sebaceous cells may migrate to the neck of the follicle prior to hair canal formation. An ental swelling of the outer root sheath, if present, is less conspicuous at all stages than in the *P* follicles. Frequently by this stage many *SD* follicles have formed as branches from the *SO* follicle or from the first *SD* follicle.

Stage F5.—The hair cone is refractile and reaches the level of the base of the sebaceous gland. Huxley's layer and the developing hair cuticle and cortex can be recognized inside the hair cone. The formation of the epidermal hair canal begins, frequently in the upper neck region of the follicle, and sebaceous cells are degenerating in the lower neck of the follicle. Further formation of *SD* follicles may occur.

Stage F6.—This stage is similar to that in *P* follicles except that hair canal formation is less advanced. There is usually a canal space in the lower neck of the follicle.

Stage F7.—The tip of the keratinized wool fibre has emerged through the inner root sheath and lies in a completed hair canal which is common to the bundle of follicles. The characteristic bending of the hair canal of *P* follicles was not observed in *SO* follicles. The inner root sheath layers are undergoing fragmentation as they reach the hair canal.

Stage F8.—The hair has emerged from the skin surface (Plate 1, Fig. 8).

The derived secondary follicles go through the same stages of development as the original ones, except for the following differences:

(i) Stage *F*1 is a budding from an *SO* follicle (Plate 1, Figs. 2 and 3; Plate 2, Fig. 3) or an *SD* follicle, and not from the epidermis.

(ii) The sebaceous gland may not form as early as stage *F*3. If it is not present before stage *F*5, the criterion for the latter stage would be the appearance of the hair cone at the deepest level of the sebaceous gland belonging to the *SO* follicle.

(iii) The hair canal into which the *SD* fibre enters at stage *F*7 is the common canal for the follicle bundle which was formed in advance of the tip of the fibre from the *SO* follicle.

The different types of branching of *S* follicles are best illustrated by diagrams. Some of the *SO* follicles, particularly some of the earliest to develop, never have branches (Fig. 1 (*b*)). These are called the unbranching original secondary follicles (*SOU*) to distinguish them from other branching original secondary follicles (*SOB*). Some branching appears at first as bifurcation, although lateral branching is more common (Fig. 3, *F*3*b*). Branching is usually at or above the level of the sebaceous gland (Figs. 4 (*a*) and 4 (*b*); Plate 1, Figs. 3 and 6), but is occasionally below it (Fig. 4 (*b*)). The largest bundles are usually those in which the *SOB* follicle is at the

secondary margin of the follicle group, and the *SD* follicles arise in sequence as lateral branches from the *SOB* follicle or from one another, mainly in the direction of the

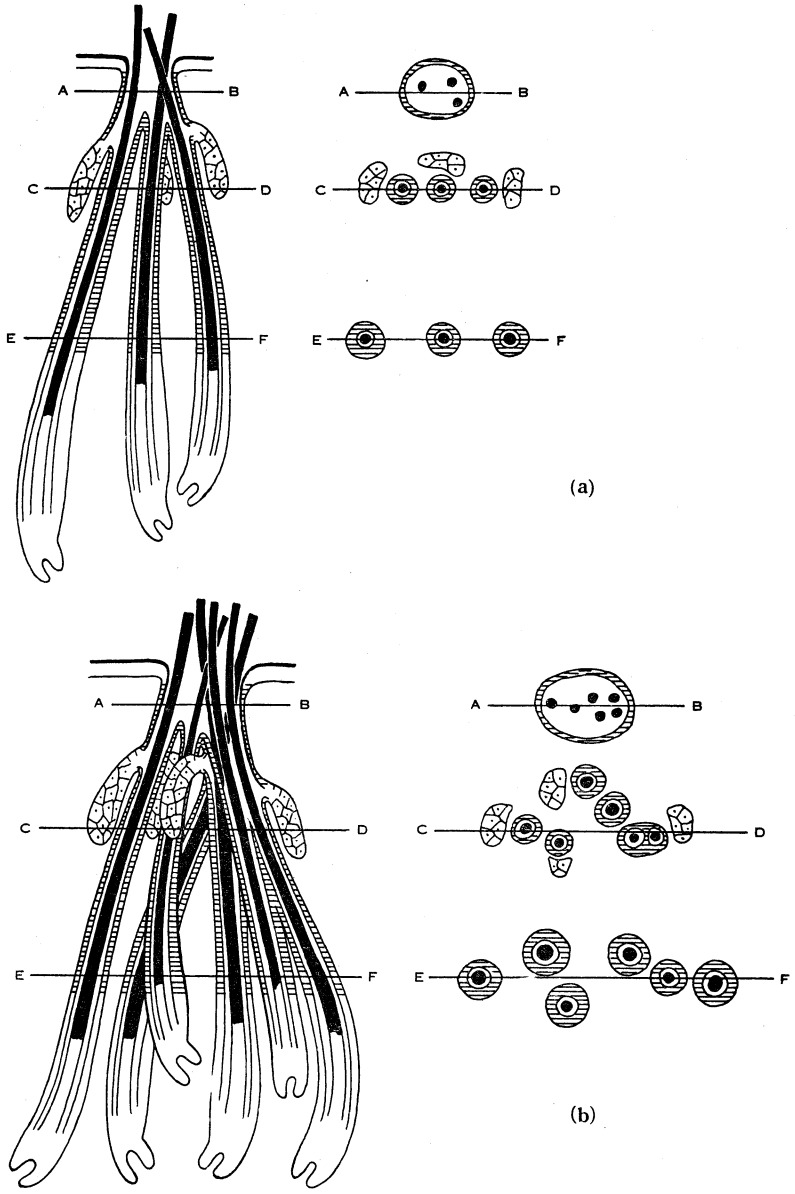

Fig. 4.—(*a*) Diagrammatic longitudinal and transverse sections through a bundle of three fully developed secondary follicles in the Merino. (*b*) Diagrammatic longitudinal and transverse sections through a bundle of six fully developed secondary follicles in the Merino.

primary margin of the group. Other bundles, in which the *SOB* follicle is nearer the centre of the group, tend to have fewer follicles.

By the end of pre-natal life at approximately 150 days, the branching of *S* follicles is extensive. As many as nine follicles have been found in one bundle with a common opening, and sometimes more than one of these is at stage *F*8, that is, a few *SD* follicles may have produced fibres. A full description of post-natal development will not be given here, but bundles of varying numbers of *S* follicles with a common funnel have been found at all ages examined, and these would seem to originate from the *SOB* follicles. In older sheep the common funnels were sometimes shorter than in foetuses and lambs, and sometimes had fewer fibres emerging from them. Shallow depressions on the skin surface with a large number of follicles opening into them were frequently observed.

IV. The Development of Follicle Groups

The description of 18 stages in the development of the follicle group in the Merino given by Carter (1943) was based mainly on the appearance of the groups in transverse sections at the level of the sebaceous glands. In the present work, Carter's sequence of group stages was confirmed in general, but since other levels and planes of section were studied, a more precise description of group stages is now possible.

The following, then, is a re-definition of the first 18 group stages proposed by Carter. The prefix *G* is added to the stage numbers to distinguish them from the *F* stages of individual follicles described above. The criteria for distinguishing each stage, printed in italics, are those which Carter and Hardy (1947) actually employed, although they did not state the fact. This description should be read in conjunction with Table 1, which indicates the *most advanced* stage of development which was *usually* found in each type of follicle at each group stage.

Stage G1: *Central primary* X *follicles* (PCX) *present.*—These are more or less equally spaced on the skin surface.

Stage G2: *Central primary* X *and early central primary* Y *follicles* (PCY) *present.*—*PCX* follicles have increased in length but are still at *F*1; they already have a characteristic slope, and later follicles within one region usually grow with approximately the same angle of slope. The *PCY* follicles, which are found in the spaces between the *PCX* follicles, are smaller than the latter.

Stage G3: *Sudoriferous gland buds formed with central primary* X *follicles.*—*PCX* follicles are at *F*2*a*.

Stage G4: *Trio groups formed by the addition of a lateral primary* x *follicle* (PLx) *on each side of some of the central primary* X *follicles.*—These trios of primary follicles constitute the primary margins of the groups that will form later.

Stage G5: *Further trio groups formed by the addition of some lateral primary* y *follicles* (PLy) *on either side of the central primary* Y *follicles, as well as trios with the central primary* X *follicles.*

Stage G6: *Sudoriferous gland buds formed in association with all types of primary follicles.*—*PCX*, *PCY*, *PLx*, and *PLy* follicles are at *F*2*a*. The linear arrangement of follicles is now clear. In addition to trios, some solitary (*PCX* or *PCY*) or couplet (*PCX*, *PLx*; or *PCY*, *PLy*) groups are found.

Stage G7: *Sebaceous gland buds formed on the ental side of central primary follicles* (PCX *and* PCY).—*PCX* follicles are at *F*3*a* or *F*2*b*, and *PCY* follicles at *F*2*b*.

Stage G8: *Secondary follicle formation begun.*—The first plugs (*F*1) of *SO* follicles appear between the *PC* and *PL* follicles on their ectal side.

Stage G9: *Sebaceous gland buds formed on the ental side of some lateral primary follicles* (PLx *and* PLy).—*PLx* follicles are at *F*3*a* or *F*2*b*, and *PLy* follicles at *F*2*b* or *F*2*a*. There is an increased number of *SO* follicles. The first-formed *S* follicles tend to define the secondary margin of the group, and later *S* follicles form between them and the *P* follicles.

TABLE 1

SUMMARY OF STAGES IN THE DEVELOPMENT OF FOLLICLE GROUPS AND THEIR RELATION TO THE GROWTH OF INDIVIDUAL FOLLICLES

Mean Age of Foetus* (days)	Follicle Group Stage	Most Advanced Stages Found in Each Follicle Type†				
		PCX	*PCY*	*PLx*	*PLy*	*SO*
64	*G*1	**F1**	—	—	—	—
69	*G*2	*F*1	**F1**	—	—	—
71	*G*3	**F2a**	*F*1	—	—	—
74	*G*4	*F*2*a*	*F*2*a*	**F1**	—	—
76	*G*5	*F*2*a*	*F*2*a*	*F*2*a*, *F*1	**F1**	—
79	*G*6	*F*2*a*	*F*2*a*	**F2a**	**F2a**	—
82	*G*7	*F*3*a*, **F2b**	*F*2*b*	*F*2*a*	*F*2*a*	—
86	*G*8	*F*3*b*, *F*3*a*	*F*2*b*	*F*2*a*	*F*2*a*	**F1**
90	*G*9	*F*3*b*, *F*3*a*	*F*3*b*, *F*3*a*	*F*3*a*, **F2b**	*F*2*b*, *F*2*a*	*F*1
95	*G*10	**F4**	*F*4	*F*3*b*, *F*3*a*	*F*3*a*	*F*2
97	*G*11‡	*F*5	*F*5	*F*3*b*	*F*3*a*	*F*2
98	*G*12	*F*6	*F*6, *F*5	*F*5, **F4**	*F*4, *F*3*b*	*F*2
99	*G*13	*F*6	*F*6	*F*5	*F*5, *F*4	*F*3*b*, **F3a**
101	*G*14	**F7**	*F*7	*F*7, *F*6	*F*7, *F*6	*F*3*b*, *F*3*a*
102	*G*15	**F8**	*F*8	*F*7	*F*7, *F*6	*F*3*b*
107	*G*16	*F*8	*F*8	**F8**	*F*8, *F*7	*F*4, *F*3*b*
115	*G*17	*F*8	*F*8	*F*8	*F*8	*F*7, **F6**
133	*G*18	*F*8	*F*8	*F*8	*F*8	**F8**

* From the study of the relation between age of foetus and stage of development by Carter and Hardy (1947). These figures may be interpreted also as representing the approximate age for each stage on the mid-side region.

† Heavy type indicates those follicle stages which define the group stages. Where two follicle stages are shown for a follicle type at a single group stage, some skin samples contain the more advanced follicle stage indicated, while others contain only the slightly less advanced stage.

‡ *G*11 is defined not by a follicle stage but by a stage in hair canal formation.

Stage G10: *Hair cones in some central primary follicles* (PCX *and* PCY).—Some *PCX* and *PCY* follicles are at *F*4, with rudimentary arrector pili muscles. *PLx* follicles are at *F*3*b* (some with rudimentary arrector pili muscles) or *F*3*a*.

Stage G11: *Hair canal spaces formed in the epidermis above central primary follicles* (PCX *and* PCY).—*PCX* and *PCY* follicles are at *F*5 (with arrector pili muscles) and the *PLx* follicles are at *F*3*b*, some with arrector pili muscles.

Stage G12: *Hair cones in lateral primary follicles* (PLx *and* PLy).—Some of the *PC* follicles are at *F*6. In material stained with picric acid, the appearance of keratinized fibres in these follicles is the best criterion for this stage. The *PLx* follicles are at *F*5 or *F*4 with arrector pili muscles, and the *PLy* follicles at *F*4 (with arrector pili muscles) or *F*3*b*.

Stage G13: *Sebaceous gland buds formed on some of the secondary follicles.*—The first-formed *SO* follicles, which are at *F*3*b* or *F*3*a*, are the first to have sebaceous gland buds. *SD* follicles may appear at this stage. There is sometimes a hair canal space in the epidermis above the *PL* follicles.

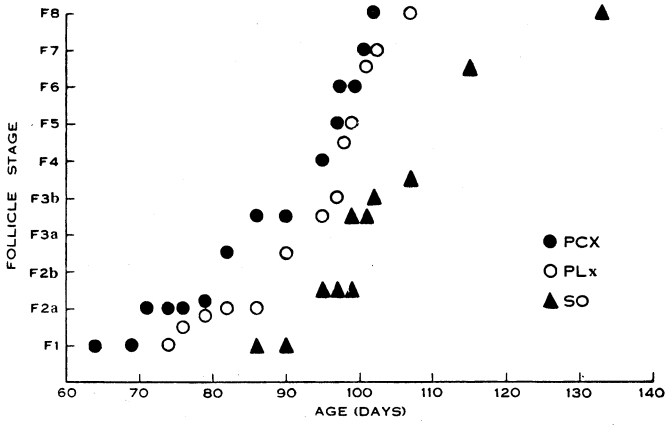

Fig. 5.—Graph showing the relation between the mean age for each group stage and the most advanced follicle stage found among central primary *X* (*PCX*), lateral primary *x* (*PLx*), and original secondary (*SO*) follicles respectively. The mean age for each group stage was obtained from the data of Carter and Hardy (1947). The group stage at each of 12 sampling points on 12 foetuses was plotted against the age of each foetus and from a smoothed curve the mean age for each group stage was read.

Stage G14: *Central primary follicles have fibres in hair canals at the level of the epidermis.*—*PCX* and *PCY* follicles are at *F*7.

Stage G15: *Central primary fibres have pierced the periderm and emerged from the skin surface.*—*PC* follicles are at *F*8. *SO* follicles show much branching.

Stage G16: *Lateral primary fibres have pierced the periderm and emerged.*—All types of *P* follicles have reached *F*8 except the *PLy* follicles which are sometimes not beyond *F*7.

Stage G17: *Early secondary fibres formed.*—All types of *P* follicles have reached *F*8. *SO* follicles have reached *F*6, and sometimes *F*7. Bundles of up to nine *S* follicles are found. The ratio of the number of *S* to the number of *P* fibres (*Sf*/*Pf*) at the level of the sebaceous glands is less than 2·0.

Stage G18: *Early secondary fibres emerged.*—The tips of the first *SO* fibres have passed through a hair canal and emerged. The *Sf*/*Pf* ratio is between 2·0 and 2·9.

By making use of the mean age for each group stage, estimated from 12 sampling points on the fleece-bearing area in the G series of foetuses by Carter and Hardy (1947, p. 12 and Fig. 2), the approximate times and rates of development of the different follicle types can now be compared (Table 1).

The relation between the *mean* age for each group stage and the *most advanced* follicle stage found among *PCX*, *PLx*, and *SO* follicles respectively is shown in Figure 5. In this graph the arbitrary follicle stages and sub-stages are placed at equal intervals along the ordinate so that the actual shape of a curve fitting the

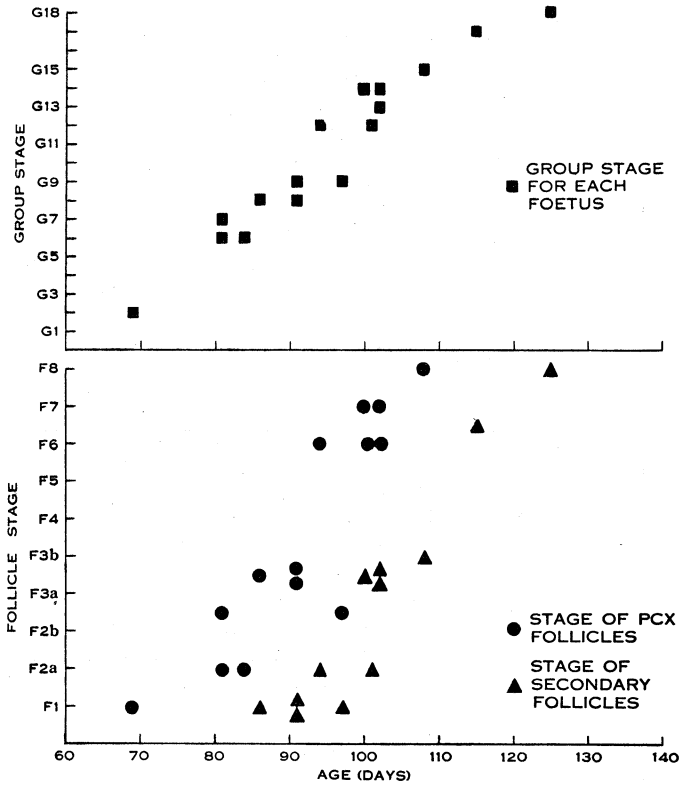

Fig. 6.—Graph showing the relation in the dorsal posterior thoracic region (see text) of (i) stages in development of follicle groups, and (ii) stages in development of central primary *X* (*PCX*) and original secondary (*SO*) follicles, to the actual age of each foetus in the G and KR series.

points plotted is not of biological significance in itself. The shape of the "stage-age curve" is similar for *PCX* and *PLx* follicles. The *PCY* follicles begin slightly later than *PCX* follicles but after about the 90th day of gestation their development almost coincides. Similarly the *PLy* follicles begin a little later than *PLx* follicles but soon catch up. The stage-age curve for the *earliest SO* follicles is similar in shape to those for the *P* follicles in the initial stages of development; the apparent slowing between stages *F*6 and *F*8 is probably spurious, due to the inaccurate estimates of mean age resulting from a small number of older foetuses in the G series.

Figure 6 shows the stages of development of follicle groups and of *PCX* and *SO* follicles on the dorsal posterior thoracic region (D1–D2, Carter and Hardy 1947) in relation to the *actual* (recorded or estimated) age of each foetus in the G and KR series. No marked difference in shape between the stage-age curves of *PCX* and *SO* follicles could be detected. However, it must be emphasized that the present observations are restricted to the earliest *SO* follicles, and that later *S* follicles may not have the same rate of development.

V. Discussion

From the data of Carter and Hardy (1947) and the present study (Figs. 5 and 6) it was estimated that the development from stage *F*1 to stage *F*8 in *P* follicles occupied about 38 days, and in the first *SO* follicles occupied about 40 days. Less is known of the rate of development of *SD* follicles. Most of the secondaries are probably initiated by about 135 days of gestation (Carter and Hardy 1947) and the remainder before birth (Schinckel 1955*b*; Short 1955*a*). As the majority have reached at least *F*6 by 28 days after birth (Fraser 1954; Schinckel 1955*a*; Short 1955*a*), it is probable that most *SD* follicles take between 30 and 45 days to develop from *F*1 to *F*8. However, the small number which do not produce fibres until 4 or 6 months after birth (Schinckel 1955*a*; Short 1955*a*) must take considerably longer to develop.

The origin of later *S* follicles by branching from earlier ones was first reported in the Karakul by Tänzer (1926). Frölich, Spöttel, and Tänzer (1929), without citing a reference, indicated that additional observations had been made by Tänzer on follicle branching in other breeds, including the Merino. None of the later writers referred to Tänzer's work, which contradicted the previous conclusions of Spöttel and Tänzer (1923). Duerden (1939) considered that *S* follicles were formed from a wedge of undifferentiated epidermal tissue which later became divided laterally into separate follicles. In the legend to his Plate 1, Figure 5, illustrating skin from a 16-week Romney Marsh foetus, he expressly stated "The follicles of a group appear as if arising by branching or budding; this is not so, but each group contains several closely adjacent follicles." Carter (1943) followed Duerden in his description of *S* follicle development. Although it is possible that some of the later formed *S* follicles may arise in the manner described by Duerden, there is strong evidence from the present study that the majority arise by simple budding from other discrete *S* follicles which are already undergoing differentiation. This conclusion was reached independently of that of Tänzer, whose findings were not available to the present authors until the completion of their observations.

It is remarkable that none of the more recent studies of the development of follicle populations have included any reference to branching follicles, although they are apparent in new-born Merino lambs; perhaps this is due to preoccupation with transverse sections through the follicles at the level of the sebaceous glands. In the following paragraphs the relevance of this finding to the current studies will be outlined.

It is generally agreed that wool fibre density (that is the number of fibres per unit area), which is strongly inherited, is one of the most important components of fleece structure. In the Merino the total number of *P* fibres is fairly constant at

different ages and between individuals (Carter and Hardy 1947; Schinckel 1955*a*), but the total number of *S* fibres varies greatly between individuals and strains. Thus the ratio *Sf*/*Pf*, which may range from 10 to 46 (Carter and Clarke, personal communication) is the most useful measure of differences in fibre numbers. The ratio of the total numbers of secondary follicles to primary follicles, or *S*/*P*, which is a measure of the potential number of wool fibres that can appear under favourable conditions, seems to be maximal at birth (Carter and Hardy 1947; Schinckel 1955*b*; Short 1955*a*). Variations in this most important determinant of wool fibre density in the adult Merino are now known to be largely due to two factors; these are the number of *SO* follicles and the number of *SD* follicles formed from them. There is no reason to assume *a priori* that the two processes are controlled in exactly the same way, and they need to be examined separately. No correlation has been found between *S*/*P* and birth weight (Schinckel 1955*b*; Short 1955*a*) and no significant difference between *S*/*P* values in singles and twins (Schinckel 1955*b*). Adverse maternal nutrition also failed to influence this ratio (Short 1955*b*). Thus overcrowding of follicles in small lambs does not seem to limit the branching activity which occurs during the latter part of the gestation period when differences in body size become marked, in British breeds at least (Barcroft 1946).

Reported values for *Sf*/*Pf* in Merinos at birth range from 0·9 to 7·3 (Carter and Hardy 1947; Fraser 1954; Schinckel 1955*b*; Short 1955*a*), but are usually between 2 and 4. These are of the same order as the values for *SO*/*Pf* at birth, and it may be deduced that the majority of *S* fibres present are from *SO* follicles. Within a flock, *Sf*/*Pf* at birth, unlike *S*/*P*, was highly correlated with birth weight (Schinckel 1953, 1955*b*; Short 1955*a*) and was lowered by adverse pre-natal nutrition (Short 1955*b*) so that these factors must affect the rate at which *SO* follicles, and perhaps the first *SD* follicles, mature. The development of the rest of the *SD*, which comprise most of the immature follicles present at birth, is apparently affected by growth and nutrition in early post-natal life, since *Sf*/*Pf* in adults was positively correlated with birth weight (Schinckel 1955*b*; Short 1955*a*) and growth from birth to 1 month (Schinckel 1955*b*) and was lowered by poor nutrition in the period immediately after birth (Short 1955*b*). A qualitative and quantitative study of *SO* and *SD* follicles in association with such investigations should be fruitful.

The distinction between *SO* and *SD* follicles may also throw some light on differences between breeds and strains. Branching is known to occur prenatally in the Karakul (Tänzer 1926) as well as the Merino, and Duerden's (1939) illustration suggests to the authors that it may occur in the Romney. *S* follicle bundles with common openings were found in the adults of all the many breeds examined by Spöttel and Tänzer (1923), but there was a progressive increase in the number of bundles and the number of follicles per bundle from the primitive Mufflon to the fine-woolled Merino. Some *S* follicle bundles in lambs or adults were also noted in the Romney by Burns and Clarkson (1949), in the South African Merino by Duerden and Ritchie (1924), and in the Corriedale as well as the Merino by Hardy and Lyne (unpublished observations). Although Spöttel and Tänzer attributed the increase in bundle formation from the Mufflon to the Merino to the increasing convergence of follicles at the skin surface, with consequent adhesion and fusion, it may well be due

rather to an increased amount of branching in pre-natal life. Since the high *Sf*/*Pf* ratio is the most outstanding feature which distinguishes the Merino from other breeds (Carter 1942, 1955), it is important to know how far this is due to an increase in the number of *SO* follicles and how far to an increase in the extent of branching. The same argument applies to the differences between strains within the Merino breed. Other important distinctions between strains and breeds are concerned with the types of fibres produced by different types of follicles (Carter 1955). Although the *S* fibres at the margin of a group, which are usually the first to be formed, tend to be thicker and more frequently medullated than those towards the centre (Carter 1943), and their follicles may produce different fibre types in lambs (Fraser 1952*a*), there has hitherto been no clear anatomical basis for distinguishing two types of *S* follicles. Fraser (1953) postulated that two waves of *S* follicle development, occurring before and after birth respectively, may have different levels of efficiency in fibre production. Making use of a theory of competition for "fibre substrate" (Fraser 1951; Fraser and Short 1952) and the concept of different efficiency levels for *P*, first-wave *S*, and second-wave *S* follicles, Fraser (1953) sought to explain the differences in fleece structure between breeds. His two efficiency levels for *S* follicles may apply to the *SO* and *SD* follicles respectively; this is another promising subject for investigation.

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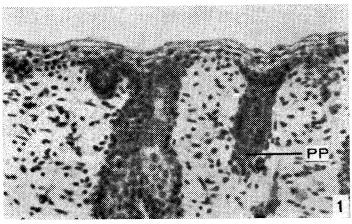
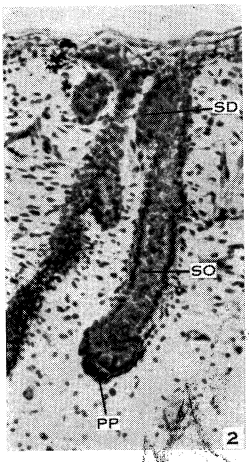
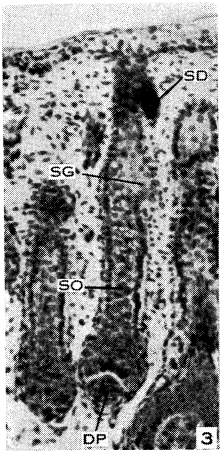
EXPLANATION OF PLATES 1 AND 2

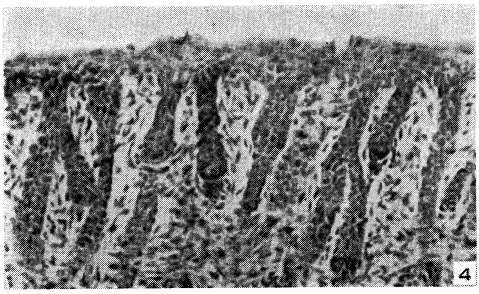
All figures are of sections through skin from the trunk of Merino foetuses. The staining is with haemalum, eosin, and picric acid, except Plate 1, Fig. 4, which is without picric acid. *DP*, dermal papilla; *G*, stratum germinativum; *H*, hair; *HN*, hair cone; *P*, periderm; *PP*, prepapilla; *S*, stratum spinosum; *SD*, derived secondary follicle; *SG*, sebaceous gland; *SO*, original secondary follicle.

DEVELOPMENT OF WOOL IN MERINO SHEEP









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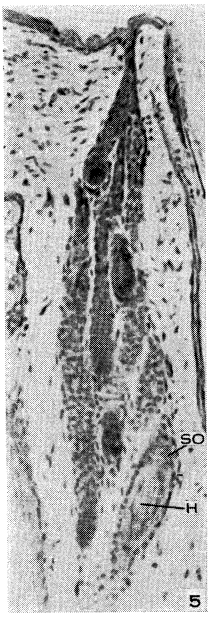
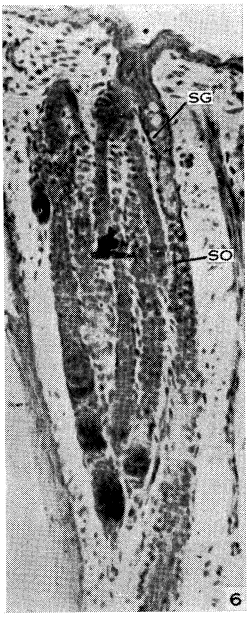
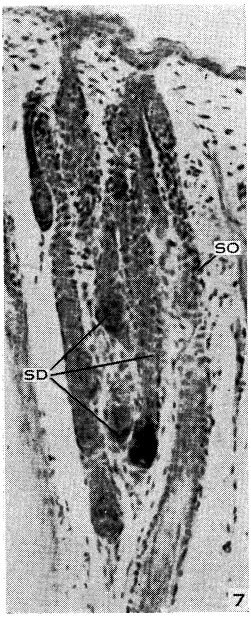
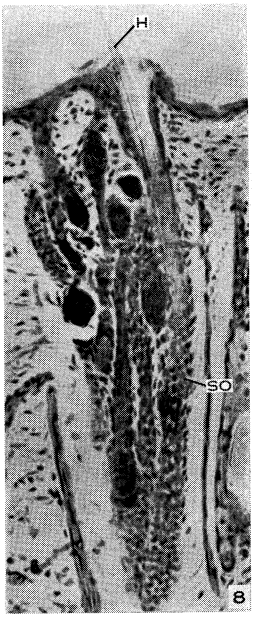
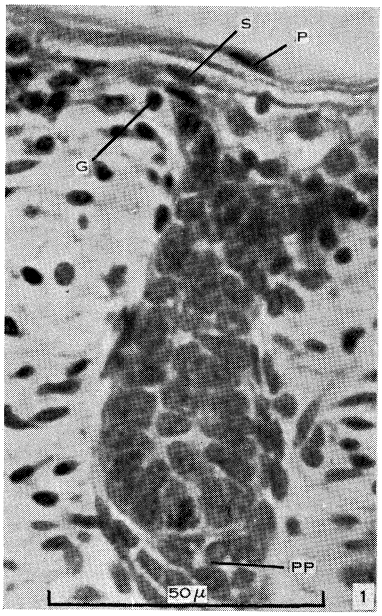


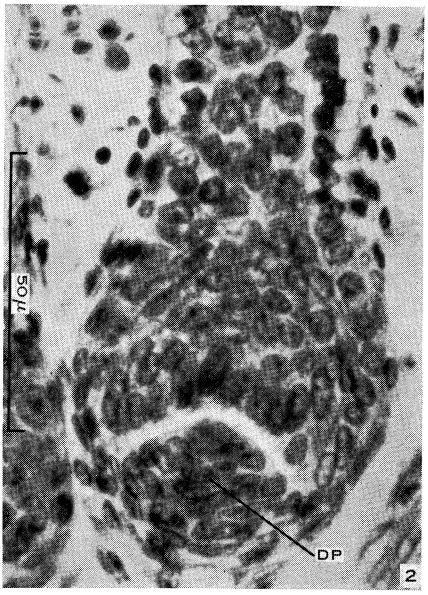


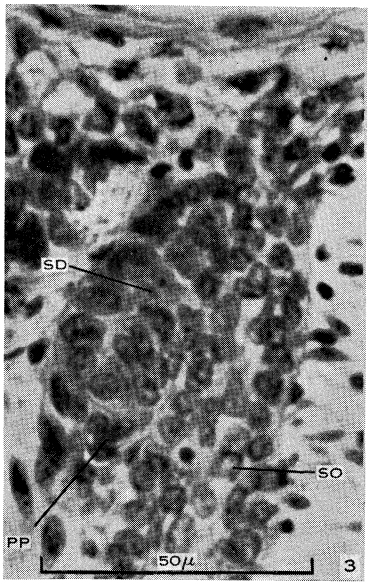




DEVELOPMENT OF WOOL IN MERINO SHEEP







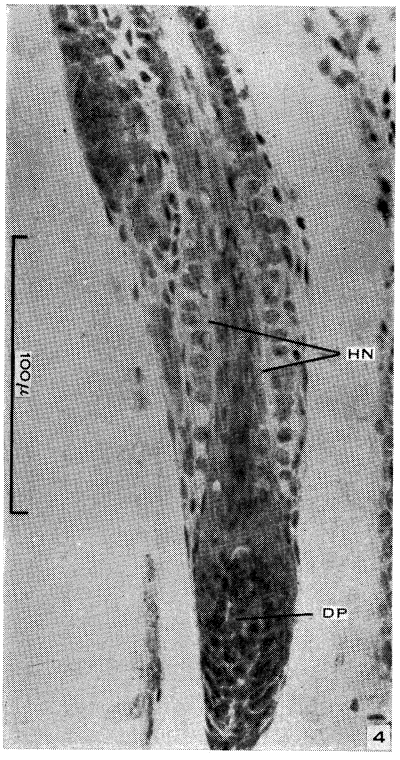


Plate 1

Development of branching secondary follicles

Fig. 1.—Vertical section of skin from 98-day foetus with two *S* follicles at stage *F*1.
Fig. 2.—Vertical section of skin from 98-day foetus with an *SO* follicle at stage *F*2 and an *SD* follicle at stage *F*1.
Fig. 3.—Vertical section of skin from 98-day foetus with an *SO* follicle at stage *F*3*a* and an *SD* follicle at stage *F*1.
Fig. 4.—Oblique section of skin from 102-day foetus with *S* follicles which show branching.
Fig. 5.—Longitudinal section of bundle of *S* follicles from 125-day foetus showing the narrow neck of the bundle. Oblique section of portion of *SO* follicle at stage *F*6.
Fig. 6.—Longitudinal section of two adjacent bundles of *S* follicles from 125-day foetus. *SO* follicle of bundle on right at stage *F*6.
Fig. 7.—Section adjacent to that shown in Figure 6.
Fig. 8.—Longitudinal section of bundle of *S* follicles from 125-day foetus with *SO* follicle at stage *F*8. Three *S* follicles belonging to another bundle are seen on the left.

Plate 2

Development of secondary follicles

Fig. 1.—Longitudinal section of an *SO* follicle at stage *F*1 from 98-day foetus.
Fig. 2.—Base of *SO* follicle at stage *F*3*a* shown in Plate 1, Figure 3.
Fig. 3.—*SD* follicle at stage *F*1 shown in Plate 1, Figure 2.
Fig. 4.—Longitudinal section of lower part of an *SO* follicle at stage *F*4 from 98-day foetus.