Histological studies on wool follicles
by Mushtaq Ahmad Khan

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree
MASTER OF SCIENCE in Animal Science (Wool Technology)
Montana State University


Abstract:
In order to determine if supplemental feeding of sheep on winter range would favorably affect the wool follicles, histological observations were made in the skin of twenty-four mature animals on two feed levels both from Columbia and Rambouillet breeds. Six animals were put into each feed level from both the breeds. The high feed level group received two pounds of thirty percent protein pellet every second day, while the low fed group was maintained on range grazing only. Animals in the high feed level group gained weight while those in the low fed lost weight, especially those carrying twin lambs. A highly significant ($P<.01$) effect of feed treatment was observed on the live body weight of animals. There was no significant feed treatment effect on the number or size of wool follicles nor on the sebaceous glands. However, there was a highly significant ($P<.01$) difference between number of follicles among the two breeds.

Breed effect was also noted on the size of follicle ($P<.06$). Individual differences were also noted regardless of breed or treatment.

There was a highly significant correlation ($P<.01$) between the size of follicle and grease fleece weight, grease fleece weight and grade of wool and grade of wool and size of follicle. A highly significant negative correlation ($P<.01$) existed between the number of follicles and the grade of wool.

Also a negative correlation was obtained between the number of follicles and the grease fleece weight which was approaching significance at ' the 5 percent level. Body weight gain or loss did not show any significant effect on size or number of follicles.

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## ABSTRACT

In order to determine if supplemental feeding of sheep on winter range would favorably affect the wool follicles, histological observations were made in the skin of twenty-four mature animals on two feed levels both from Columbia and Rambouillet breeds. Six animals were put into each feed level from both the breeds. The high feed level group received two pounds of thirty percent protein pellet every second day, while the low fed group was maintained on range grazing only. Animals in the high feed level group gained weight while those in the low fed lost weight, especially those carrying twin lambs. A highly significant ($P < .01$) effect of feed treatment was observed on the live body weight of animals. There was no significant feed treatment effect on the number or size of wool follicles nor on the sebaceous glands. However, there was a highly significant ($P < .01$) difference between number of follicles among the two breeds. Breed effect was also noted on the size of follicle ($P < .06$). Individual differences were also noted regardless of breed or treatment.

There was a highly significant correlation ($P < .01$) between the size of follicle and grease fleece weight, grease fleece weight and grade of wool and grade of wool and size of follicle. A highly significant negative correlation ($P < .01$) existed between the number of follicles and the grade of wool.

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# INTRODUCTION

Sheep were among the first animals domesticated by man. This animal supplies wool for clothing, meat for food and in certain parts of the world milk for drinking. Continuous efforts have been made to improve both wool and mutton to give the greatest financial return to the sheep grower. The chief value of wool is its ability to be spun into yarn. Other animals i.e., camel, goat and alpaca, produce valuable textile fibers. For general purposes other fibers are not as useful as the wool because the character of the fiber is not adapted to as many uses as wool, and the production of these fibers is inadequate to meet the world's needs. Sheep are found in nearly every inhabitable part of the world (Darlow et al. 1934).

Because wool is the most valuable animal fiber, efforts by research workers should be made to improve the quality as well as the quantity. It is encouraging to note that in recent years the attention of research workers in this field has been diverted from the fiber itself to the follicle which produces it. Various workers have studied the different types of follicles, their pre-natal and post-natal development, density per unit area and the ratio of different types of follicles over different regions of the body. Most of the research work is directed toward morphological studies of the follicle and its accessories. Some workers have studied the effect of environments on wool growth but very little information is available on the effect of these factors on follicle growth and development.

Most of the work of histological observations of follicular growth and development has been conducted in Australia, New Zealand and England. No preliminary information could be found on histological studies of American breeds of sheep.

This study was initiated as a preliminary investigation on the histology of wool follicles and their associated structures in Rambouillet and Columbia breeds of sheep on two different feed levels.

## REVIEW OF LITERATURE

In addition to the feed required for growth and maintenance, sheep require energy for wool production. Other factors such as temperature, age and season also affect wool growth. An understanding of the structure and population ratios of the skin follicles are essential for a complete understanding of the type and number of fibers produced in developing and mature animals. Little attention has been given to follicle development beyond the limit of morphology. This is partly due to the technical difficulties and expense of using histological preparations. Making good preparations with sufficient ease and consistency has been the greatest obstacle in studying the follicle population and development. Within the last twenty years the work on skin preparations has advanced considerably and new techniques have been developed for biological research on fleece growth (Carter, 1955).

### Morphology

The skin of sheep is composed of minute cells and consists of two main parts; the first, an under-skin or dermis, and overlying this is an outer skin or the epidermis. These two parts of the skin retain their identity throughout the growth of the animal. The epidermis is formed by constant growing and dividing of a very active layer of cells called the basal layer. There is a constant wear on the outermost layer of the epidermis and this is replaced by cells derived from the basal layer. The initial stages of formation of the wool follicle take place in the basal layer. At certain positions in the skin, the basal layer thickens and by a process of very active cell division, invaginates into the dermis. This invagination forms the wool follicle with its accessories. As this tubular invagination grows downward, two outgrowths appear from it, usually on the same side. These are the sebaceous gland and the sweat gland or the sudoriferous gland. The bulbous base of the follicle becomes turned in and grows upward to form a dome like s-shaped asymmetrical body of actively dividing cells called the papilla. This part is well supplied with blood vessels. The basal layer is continuous over the dome of the papilla and the basal cells in this region are actively dividing. The cells thus divided gradually elongate and give rise to the wool fiber. The tip of the growing fiber together with its inner root-sheath is pushed upward through the follicle plug by pressure of new cells being formed continuously from the dome of the pappila. The arrector pilli muscle, which is attached at its lower end to the follicle sheath and at the upper end to the basal layer, is usually on the same side of the follicle as the sebaceous gland. Contraction of this muscle causes the hair to stand at its end in human and certain other animals. In sheep it is inserted too high up on the follicle and consequently is not functional. The sweat gland opens into the neck of the follicle just above the sebaceous gland. The structure of the human hair follicle differs from the wool follicle of sheep because the sweat gland in human opens directly on the skin surface having no association with the follicle. In sheep the sweat gland lies between the two main lobes of the sebaceous gland. The sebaceous gland of sheep secretes a complex mixture of esters called the sebum or wool grease which is an oily, semi-liquid material which gives wool a greasy touch (Anonymous, 1950).

<u>Terminology and classification</u>

Carter (1955) states that until the middle of the 19th century, it was customary to regard the arrangement of hair follicles in the mammalian skin in a disorderly fashion. A German worker, Nathusius (1866), demonstrated the existence of some form of group pattern which was later established by other German workers (Spottel and Tanzer, 1923). The clear terminology in the development of wool follicles was still lacking when in 1923 Spottel and Tanzer described the follicles as "Leithaar and Cruppenhaar" meaning primary and secondary. These two German authors traced the development of the follicles in the foetal stages and demonstrated the earlier origin of Leithaar or the primary follicles. Wildman (1932) has used these terms to distinguish between the early and the late developing follicles in the foetuses of various British breeds of sheep. Duerden (1936) in his report noted two major types, namely primary and secondary follicles.

A compromise in terminology was then put forward by Wildman and Carter (1939) which was accepted by workers in this field. The same classification is still in use; except when classifying follicles into various types the following symbols and terms like X, C, P, Y, x, L, y, U, O, S, B and D have also been used. This classification was suggested by Hardy and Lyne (1956). The various symbols like X (first formed), C (central), P (primary), Y (later formed), x (associated with PCX), L (lateral), y (associated with PCY), U (unbranching), O (original), B (branching), S (secondary), and D (derived), are easy to use to designate various types of follicles. Carter (1955), using the same terminology expressed total follicle population as P + S and any sample of skin population as $^{n}P + S$.

The follicles lie at an acute angle to the skin surface and usually have a common slope. In many breeds of sheep, especially the Merino, the earliest growing follicles are arranged in groups of three and such a grouping is called the trio arrangement. The follicles forming the "trio" are known as primary follicles and characteristically have all the accessory structures present with each follicle, i.e. sebaceous gland, sweat gland and arrector muscle. The secondary follicles differ from the primaries in having an incomplete set of accessory structures, the sweat gland and usually the arrector muscle being absent; the sebaceous gland may be present but it is smaller in size than that of primary follicles (Anonymous, 1950).

The grouping of these follicles has been shown to consist of one or two follicles per group by Narayan (1961). But the proportion of these incomplete trio groups was considered to be too small to make a difference in the primary lateral/primary central ratio. He found that in the hairy group (Malpura and Sonadi breeds) the PL/PC ratio, the mean S/P ratios and the percentage of trio groups are lower but the percentage of couplets and solitary groups is higher than in the carpet wool group (Chokla, Magra, Nali, Jaisalmere, and Marware breeds). It appears that in the hairy breeds the trio arrangement is not a predominant feature of the grouping of the primary follicles.

The origin of later secondary follicles by branching from earlier ones was first reported by Tanzer (1926) in Karakul sheep. Frolich, et al. (1929) without citing a reference indicated that additional observations had been made by Tanzer (1926) on follicle branching in other breeds including the Merino. Bundles of secondary follicles with common openings at the skin surface were found in the adults of many breeds of sheep examined by Spottel and Tanzer (1923). The number of bundles and the number of follicles per bundle increased progressively from the primitive Moufflon to the fine-wooled Merino. Duerden (1939) postulated that secondary follicles were formed from a wedge of unidentified epidermal tissue which later became divided laterally into separate follicles. In his illustration from a 16 week old Romney Marsh foetus he states, "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 (1939) in his description of the secondary follicle development. Hardy and Lyne (1956) reported the majority of these follicles arise by simple budding from other discrete secondary follicles which are already undergoing differentiation.

Lyne (1957) studied the skin samples from the mid-lateral region of the body of four medium-wooled Merino rams (age 20-21 months), two inbred Merino ewes (age seven years) and one Corriedale ewe (age more than seven years). He found the presence of a few bundles of primary wool follicles in all the sheep examined except one ram. The bundles consisted of two to six primary follicles with a common neck and opening at the skin surface. A normal sudoriferous gland and arrector pilli muscle were associated with each of the bundles. The follicles were usually separate at the level of the junction of sebaceous glands, although occasionally they were contained within a common outer root-sheath. The method of formation of bundles of primary follicles is unknown. Lyne (1957) hypothesized that they develop by branching in a manner similar to that described for secondary follicles by Hardy and Lyne (1956).

Wildman (1957) suggests that care should be exercised in identifying follicles as either primary or secondary during counts made to determine the S/P ratio. In follicle population studies, particularly in adult skin and in Merino skin, the classification of some follicles was difficult and he classed such follicles separately as "doubtful identity". He points out that further evidence is required in classifying follicles before Lyne's (1957) S/P determination can be established. The follicles may not have developed by branching as Lyne (1957) assumes; because they are smaller than the large obvious primaries, have no sweat glands and may be identified later than the original primary follicles. If this is so, the fact that these and the original primary follicles lead into a common neck loses its significance in assigning the smaller follicles to one category because they may have all the characteristics of secondary follicles. In his opinion, further observations in the differentiation of these follicles should be made to assess their significance in population studies.

Orwin (1961) has reported the occurrence of branching follicles in the skin from two-year-old New Zealand Romney ewes. Wildman (1961) points out that there appears to be some confusion in Orwin's (1961) communication concerning the evidence required to demonstrate conclusively whether follicles are branched, or are separate follicles which have coalesced. Orwin's (1961) photographs show more than one fiber in a common sheath at sebaceous gland level and at higher levels. Wildman (1961) indicates the evidence presented by Orwin (1961) is not adequate to conclude that one follicle has been derived from another by branching. These follicles may have been separately initiated and coalesced so the fibers come to lie in a common sheath and a common follicular mouth. He suggests that skin sections at lower levels than that of the sebaceous glands should be made. The follicles should then be traced up and observations confirmed by vertical sections before deciding whether a follicle has been derived from another one by branching or has coalesced with it. Wildman (1961) does not suggest that branching does not occur in breeds of sheep other than the Merino. In thousands of serial skin sections from English Romney sheep and lambs examined at all levels, he has found coalesced follicles but not true branched follicles.

Follicles with multiple fibers were ascribed by Flemming (cited by Pinkus, 1951) to "partial merging" of several embryonic papillae, rather than to "budding" of an originally normal papilla. This is considered to be an anomaly as suggested by Auber and Ryder (1955). Until the factors leading to the formation of these follicles have been determined, these considerations must remain a possibility (Orwin, 1961).

Development and factors affecting it

Different workers have studied the pre-natal and post-natal development of both types of follicles. Carter (1943) studied the embryological material from South African Merino foetuses and concluded that the primary follicles develop during the 35th to 85th day of pre-natal life (pre-trio and trio periods). The secondary follicles develop thereafter. In most breeds of sheep the follicle development in any but a few special regions of the skin is almost negligible during the first fifty days of pre-natal life. Table I gives details of different stages of follicle development and its accessory structures. He also reported that the primary follicles first appear at the poll and face of the foetus and then on the rest of the body.

TABLE I. GENERAL COURSE OF DEVELOPMENT OF A TYPICAL FOLLICLE GROUP. (Carter 1955).

| Phase | Period | Foetal age (days) | Pre-natal sequence (approximate): Follicle initiation | Pre-natal sequence (approximate): Gland initiation | Pre-natal sequence (approximate): Fibre and smooth muscle initiation | Post-natal sequence (approximate): Fibre types (juvenile) | Post-natal sequence (approximate): Fibre types (adult) | |
|---|---|---|---|---|---|---|---|---|
| Primary or Protophase | Pre-trio period | 50 | Primary (central) follicles initiate | | | Halo hairs ↓ Super-sickles | PRIMARY (outer coat) (LEITHAAR) | |
| | | 60 | | | | ↓ Hairy-tip-curly-tip | Bristle<br>Kemp<br>Outer hair | Stichelhaar<br><br>Mittelhaar |
| | | 70 | | Primary (central) tubular gg. | | ↓ | Over hair<br>Protective hair<br>Awn<br>Heterotype | <br><br>Grannenhaar<br>Stammhaar |
| | Trio period | 80 | Primary (lateral) follicles initiate | Primary (lateral) tubular gg.<br>Primary (central) acinous gg. | | Sickles ↓ Curly-tips | Transitional hair<br>Intermediate hair | <br>Seitenhaar |
| Secondary or Neophase | Post-trio period | 90 | Early ↑ | Primary (lateral) acinous gg. | | Curly-tips ↓ | | |
| | | 100 | | Early secondary acinous gg. | Primary (central) fibres and smooth muscle initiate<br>Primary (lateral) fibres and smooth muscle initiate | | SECONDARY (inner coat) (GRUPPENHAAR) | |
| | | 110 | Secondary follicles initiate | | Primary (central) fibres emerge<br>Primary (lateral) fibres emerge | | Under hair<br>Under fur<br>Down hair, etc. | Beihaar<br>Flaumhaar<br>Nebenhaar<br>Wollhaar |
| | | 120 | ↓ | | Early secondary fibres initiate | | | |
| | | 130 | | | Early secondary fibres emerge | | | |
| | | 140 | | | Later secondary fibres initiate | | | |
| | | 150 (birth) | Late | | | Histerotrichs | | |

The general pattern of differentiation results in an orderly array of follicles, differing in their size and the complexity of their structure according to the line of their initiation and the rate of growth in the following general order: central primary, lateral primaries, early secondaries, late secondaries. Structural distinctions within the group only persisted for periods of time with varying clarity according to the breed or genotype (Carter, 1955).

Carter and Hardy (1947) showed that at birth all the primary follicles have reached maturity as judged by keratinized fiber in the follicle. They were unable to find any evidence that would suggest the initiation of new primary follicles after the 85th day of pre-natal life. Barton (1951) discovered that it was not until 115 days after conception, or 33 days before birth, that the secondary follicles were visible. Secondary follicles continue to be produced for a short period of time after the lamb is born.

Pohle et al. (1945) examined fleeces of 69 lambs of Rambouillet, Corriedale, Targhee, and Columbia breeds that were classed as hairy at docking time. Samples from the fleeces of these lambs were examined by the cross-section method at monthly intervals until yearling age at the U. S. Experiment Station and Western Sheep Breeding Laboratory, Dubois, Idaho. They concluded that the number of hair constituted only a fraction of the total population and these diminished rapidly as the lambs increased in age, indicating that the primaries ceased producing. Fewer hair were found on the side than on the thigh (britch) or back. Fiber diameter decreased between 6 and 11 months of age, showing a slight increase thereafter. This may have been due to weaning and subsequent poor feeding or to inherent properties of the follicle. The yearling fleeces of the hairy lambs were compared to those classed as non-hairy at docking. No important differences were found among the yearling fleeces of hairy and non-hairy lambs in hairiness, mean fiber diameter and variability or percentage of medullation.

Grandstaff and Blunn (1944) reported that the average amount of kemp in the fleeces of Navajo lambs decreased from 15 percent at 28 days of age to 4.5 percent at 84 days and then maintained a fairly constant level until the lambs reached yearling age. They also found highly significant differences between sheep and between the position, the sample was taken in the percentage of wool, hair and kemp fibers. In another experiment, Grandstaff and Wolf (1947) found significant differences between ages, years and lambs within years in both breeds. They claim that a reasonably accurate selection for wool characters can be made at an age of 112 days when the fiber content of the fleece has reached a fairly constant level; meaning that maturation of secondary follicles has fully occurred.

A study of the follicle types, ratios and the populations was made in four different breeds of Indian sheep by Narayan (1960). He made comparisons between follicle and fiber counts per unit of the skin area on the shoulder, side and the britch regions of the body. He established that there was a significant breed difference in wool follicle densities in different regions of the body. He suggests that adverse pre-natal conditions adversely affect the growth of secondary follicles. He mentioned that any adverse condition influencing the initiation or maturation of secondary follicles, even to a small extent, will tilt the balance in favor of primary follicles and the adult fleece will then be composed of fibers from a higher percentage of primary follicles than the secondary follicles. The britch region had the highest percentage of total and secondary follicles with medullated fibers.

Schinckel (1955) suggests that in considering differences between follicle population densities, body size must be taken into consideration to determine whether differences lie in total follicle population on the whole sheep, or merely in mechanical changes in density due to changes in body size. He found that the total follicle population was the highest on the shoulder while the britch value was below that for the side but not significantly so. The density of primary follicles became less per unit area as the animal grew in age. Because all the primary follicles are fully developed and producing fibers at birth, the decrease is a direct result of skin expansion accompanying growth. The greatest decrease in follicle density took place between birth and one month of age. The major increase in skin expansion also took place up to four months of age and then small decrease in follicle density was measured up to sixteen months of age.

He also found that little follicle development occurred during the first weeks after birth and that this period of inactivity was longer than the usual 1 to 2 days a lamb takes before it starts growing. During the second and third week of age the greatest activity in the development of the secondary follicles was observed. After the third week the secondary follicles continue to develop but at a much reduced rate.

Schinckel (1953) also studied the wool follicle development in the early post-natal life. He took skin samples from the mid-side at various ages ranging from 10 to 426 days of age. Large differences in the S/P ratio were found at 10 days of age. He feels that this difference appears to be too large to have developed since birth, and strongly suggests that the late pre-natal development of secondary follicles may be affected by foetal environment.

His results also indicated that lambs from twin births have a retarded maturation of secondary follicles compared with single lambs. The S/P fiber ratio of twins was also permanently below that of singles. It was not determined to what extent the difference between single and twins resulted from post-natal nutrition. There is experimental data (Schinckel and Short, unpublished data, 1956) to show that restricted post-natal nutrition delays the maturation of secondary follicles but they are not sure if this is a permanent depression of the secondary follicle development.

Ferguson et al. (1956) found that depression of wool growth rate accompanied the depression of follicle maturation caused by thyroid deficiency. This did not appear to be a consequence of the lowered follicle population but rather it appeared to be independent of it. It is suggested that the two effects may be associated with different metabolic defects which occur to different relative extents in different lambs. Stephens (1940) claims poor nutrition leads to depression of secretion of thyrotrophic hormone and hence of thyroxine in the guinea pig, and this effect, if present in the sheep, may account at least in part for the delayed follicle development of lambs fed on a restricted diet.

From Wallace's (1948) extensive studies on growth of lambs before and after birth in relation to nutrition, it appears that during the 56 to 84 day foetal stage, the specific growth rates of carcass, organs, and skin are very similar. During the 84 to 112 day interval, the specific growth rate of skin exceeds those of the carcass and organs, but from 112 to 140 days, the rates are again approximately the same. Between birth and 112 days, the specific growth rate of the skin is considerably lower than that of the carcass or organs but is considerably greater from 112 to 332 days after birth. During this period considerable growth of fleece takes place.

Short (1955) traced the maturation of secondary follicle population from birth to six months of age by comparing the mature fiber producing secondary to primary follicles. He found that in lambs the secondary follicles were present at birth. The rate at which the secondary follicles mature reaches a maximum at 7 - 21 days after birth, with approximately 65.0 percent of secondaries growing fiber by 28 days. In his opinion the adult secondary follicle population may be permanently affected in two ways. Firstly, the number of secondary follicles initiated to the time of birth may be restricted by adverse foetal environments. Secondly, the number of initiated secondary follicles which mature may be restricted by the early post-natal environments, particularly up to 21 days. In another experiment Short (1955) studied the effects of adverse maternal nutrition on the fleece structures. Although the post-partum feeding of ewes was ad libitum, restricted nutritional regime during gestation had a delayed impact on the post-natal growth of lambs, resulting in restricted final S/P ratio in the offspring of poorly fed ewes. However, at 200 days of age, the lambs with fewer mature follicles per unit area of skin grew fibers both longer and coarser than lambs with higher fiber densities. This evidence supports the observations that the wool production per unit area of skin is independent of the fiber density, and further substantiates the hypothesis of competition between follicles for the precursors of wool keratin. Stephenson (1959) studied the developmental effects influencing the rate of primary anlagen formed in the foetus. The major factor controlling the initiation of new primary follicle anlagen on different regions of the body was the rate of skin expansion. There is probably a competition effect among anlagen during this phase of development. It was also shown that during the period of primary anlagen initiation, foetuses with greater surface area have a greater total number of primary anlagen.

Davenport and Ritzman (1926) reported age and feed level had a modifying effect on fleece weight. They found that advancing age, state of health, level of subsistence and exposure to changes in weather conditions affect the growth of wool to such an extent that an unfavorable combination of these factors may vary the fleece weight by as much as 50 percent. Darlow _et al_. (1934) stated that a fattening ration acts as a stimulus to the sebaceous glands in the production of wax. They also suggested that the organs which are concerned with the secretion of wool fiber may be affected by such influences as drastic changes in the level of feeding for only a short period of time. The function of these organs is easily disturbed when sheep become abnormal in health. The growth of wool is reduced when ration is insufficient for maintenance and the amount of wool fiber produced seems to be affected to a greater extent than the body weight.

In another experiment they found appreciable differences in the changes of crimps per inch among the different lots but they did not know whether these differences could be attributed to the difference in rations. Results of this experiment also indicated that there is a relationship between breaking strength and stretch. They also found that the normal balanced ration will contain ample cystine for body maintenance and wool growth. Fiber diameter increases as the ration is increased and probably the increase in fleece weight is related to increase in diameter.

Some workers claim the organs concerned with wool production are not easily subject to such influences as levels of feeding especially for the period of 5 or 6 months if the sheep remain in normal health (Joseph, 1926). He concluded the quality of the fiber is not affected at all and the quantity may be modified only slightly. Graig (1891) fed two lots of grade Shropshires on high protein and high carbohydrate rations for 12 weeks and found very little difference in staple length and amount of clean wool produced. The sheep in the lot receiving the high protein feed produced 0.4 pounds more raw wool than those in the carbohydrate lot but the difference was due mostly to grease in the wool.

According to Baker (1929), wool keratin contains about 13.1 percent cystine, which in turn contains all of the sulphur found in the wool. Hart (1912) fed sulphur at different levels to four lots of sheep and reported that neither the average weight of fleece nor the proportion of pure wool fiber to the total weight was greater on high sulphur than on low sulphur rations. He concluded that normal dry rations of grain and hay contain ample sulphur for wool production and that additional sulphur seems to have no influence upon the proportion of pure wool fiber produced.

Morrison (1956), in his book "Feeds and Feeding", states that wool grease is important in protecting the fiber from injury by the weather. He further states that sickness, undue exposure to severe climatic conditions, or lack of feed will decrease the yield of wool, will produce smaller and weaker fibers and will sometimes even cause "BREAKS". He concludes that unless the under-nutrition is severe, the amount of yolk (wool grease plus suint) will be decreased more than the yield of scoured wool. Drummond et al. (1957) reported an experiment conducted by Moule (1955) at the Commonwealth Scientific and Industrial Research Organization Laboratory, Australia, in which one group of sheep was fed a daily ration of one ounce of digestible protein per head and the second group was given a daily ration of eight ounces per head. The second group produced two and one-half times more wool as compared to the first. The wool from the group on low protein ration was finer in diameter. Further studies revealed that the fiber diameter was not affected in the primary follicles by the lower level of nutrition; however, the fiber diameter in the secondary follicles was severely reduced by lowering the protein in the ration. The author also stated that if the condition became severe the secondary follicles may cease to produce. Austin (1943) describes that the scarcity of nourishment causes the secondary follicles to contract and produce a finer fiber; the primaries being of a relatively more robust growth may not be affected. He stated this may be the reason for the old ewes to produce a thin doggy fleece.

Auber (1950) and Chase (1954) found the medulla arises from basal layer cells of the papilla. Rudall (1934) points out that medulla formation is not a permanent feature of the follicle, but depends upon season, nutritional state and closeness of shearing. Rudall (1955) states that medullation, dependent upon genetic constitution, disappears when the ratio of bulb volume to papilla volume is increased and reappears when this ratio is decreased.

Schinckel and Short (1961) have studied the effect on body weight and wool production of high and low levels of nutrition during pregnancy in Merino ewes and from birth to four months of age in lambs. They claim husbandry methods that do not restrict the body weight and post-natal growth of lambs, greatly influence the initiation and early maturation of wool follicles. In another experiment Schinckel (1960) observed large differences among sheep in appetites and in the efficiency of conversion of feed to wool. He found high producing sheep tended not only to be more efficient but also to have greater appetites. This suggests that high level of production is associated with high level of general metabolic activity and relatively large appetites.

Coop (1953) made very intensive studies using wet and dry ewes grazing on pastures equivalent to super-maintenance and sub-maintenance feed levels. Wet ewes were also stall fed, a constant maintenance ration. He concluded that pregnancy reduced the rate of wool growth in winter, and lactation delayed the increase in spring. The maximum production of fiber occurred sooner in dry ewes than in wet ewes.

Carter et al. (1957) used different ages to study the skin follicle population in Merino sheep under different levels of nutrition. They also took into consideration the body condition of the animal at the time of sampling (i.e. whether with a full abdomen or relatively empty after some hours deprivation of food and water). Greater abdominal fullness and hence greater skin tension at sampling time lowered the population density values. Slightly less than average nutritional status at the time of sampling tended to enhance the population density. In another experiment they found considerable breed differences in population densities. The coarser breeds had less and finer breeds more follicle density.

Sugai (1954) taking skin samples from shoulder, side and thigh at 1, 3, 6 and 9 months of age after birth made studies on follicle density. He concluded that follicle density was the highest at 1 or 3 months and decreased until 9 months of age. Individual differences were found in mean follicle diameter which was the greatest at 3 or 6 months, and decreased thereafter. The largest diameters were found on the side and thigh and the smallest on the shoulder. Primary follicles tended to be larger than the secondary follicles at all ages and in all body regions. In another experiment Sugai (1955) used four two-year-old, four three-year-old and 4 lambs of Corriedale breed to study follicle density, ratio of primary to secondary follicles and shedding of fiber from the follicles. There was a little difference between the adult sheep and lambs in the morphology of wool follicles. New follicles with fibers showing incomplete keratinization were present in lambs but occurred in the adult sheep only where there was regrowth of fibers from the shed follicles. The follicles and their associated sudoriferous and sebaceous glands tended to increase in size as the age of sheep increased from 1 to 3 years. Age had a slight effect on the S/P ratio. This ratio was higher in the shoulder region than on the belly and thigh. Mean follicle density was different in different parts of the body. It was lower in the two and three-year-old groups.

Auber and Burns (1947) studied several breeds of sheep ranging in age from one month to five years and found empty follicles from which the fibers had been shed. Examination of the follicles in horizontal and vertical serial sections revealed the frequent occurrence of a type of shedding in which the old fiber was lost from the follicle when the new fiber was in its very early stages of development. It appeared that there was no possibility of the new fiber either growing alongside the old one or mechanically forcing it out.

Climatic factors and the seasonal changes have important influences on wool production. Studying the length changes of unclipped staples from pen-fed crossbred and Rambouillet ewes, several workers (Bowstead and Larose, 1938; Larose and Tweedie, 1938 and Sackville and Bowstead, 1938) found that humidity differences of 25-30 percent and temperature differences of up to 52° F had no effect on wool growth.

On the other hand, Ferguson, Carter and Hardy (1949) found variations in the wool production of sheep kept on a constant diet. They showed correlation between the wool growth and the atmospheric temperature and deduced that the high temperatures increased the wool growth-rate by causing vasodilation and a consequent flow of nutrients to the follicle. To test this, Ferguson (1949) performed an unilateral thoracic sympathectomy which caused vasodilation of the cutaneous blood vessels on one side of the sheep. Wool growth-rate showed an increase of 36 percent over the control side for a period of ten weeks, after which there was no difference in wool growth-rate for the two sides. The difference disappeared just at the onset of warmer weather.

Light seems to have a favorable effect on wool growth in sheep. Wodzicka (1960) measured the monthly wool growth of three groups of five rams for a period of one year. The first group was kept under natural daylight and was shorn monthly. The second group was subjected to a 7:17 light:dark rhythm and was shorn once in winter and once in summer; and the third group was kept in natural daylight and was similarly shorn at six-month intervals. Although food intake was greater in winter than in summer, the wool production of all groups was greater in summer. The winter wool production of group 2 was no greater than that of group 3. The differences in shearing frequency had no significant effect on wool growth. The author states, the available evidence indicates that temperature controls the seasonal rhythm of wool growth.

Hart (1961) studied the effects of various light treatments on wool growth in New Zealand, using Corriedale ewes. He compared monthly wool growth with that of the controls having normal daylight hours. He concluded that in the controls the peak of wool growth occurred during November-January with long daylight hours, and the minimum during June-August with short daylight hours. He stated that the increase in wool weight was associated with increased wool fiber length, but not thickness. He also found the effect of a ratio of 2-hours light to 4-hours dark repeated throughout 24 hours was greater on wool growth than the 8-hours light to 16-hours dark ratio. In another experiment Morris (1961) took three groups of uniformly fed, mature, non-pregnant ewes. A control group was kept under normal sub-tropical environmental conditions and the second group remained under the same environments but was subjected to an accentuated reversed rhythm of seasonal changes in length of day. The third group was subjected to normal hours of daylight but was exposed to a somewhat reversed seasonal temperature-cycle. Wool growth was recorded for two years at six-week intervals from a 100 sq. cm. area tattooed on the mid-side. The results indicated that the control group had a minimum wool growth-rate in winter and maximum in summer. The seasonal variation in wool growth was greatest in the reversed day-length group which according to the author may be reasonably attributed to the greater seasonal variation in day-length experienced by this group. Upsetting the normal seasonal temperature cycle in the third group had no observed effect on the wool growth cycle.

Burns _et al_. (1949) made observations on the dimensions of follicles of a lamb aged one month, one ram aged two and one-half years, and two eight-year-old ewes of Romney Marsh breeding. The results indicate that in the lamb, follicles occupied a relatively greater volume of skin than in the adult animals, although their actual volume was much less. They report that medullation was independent of follicle size. The area at the skin surface occupied by a trio group was approximately twice as great in the adults as in the lamb. However, the volume of the follicle per unit area of skin surface was similar in the lamb and the ram; it was less in the ewes, presumably because of the high proportion of short and abnormal follicles in these animals.

## CONDITIONS OF THE EXPERIMENT

### Experimental animals

The sheep used in this study were grade Rambouillet and Columbia ewes owned by the Montana Agricultural Experiment Station. They were wintered at the Red Bluff Ranch near Norris, Montana. The ages varied from four to six years except one animal which was taken from the two-year-old group in order to make sufficient animals.

### Description and location of the range

The range grazed by these animals was on the Red Bluff Ranch, Norris, Montana. The general description of the topography, soils and vegetation of the range was previously reported by Hoxsey (1959).

The elevation varies on portions of the range from approximately 4650 feet up to 5400 feet. Most of the range was characterized by relatively steep slopes dissected by deep drainages. Slopes vary from approximately 0 to 34 percent. Portions of the winter range were largely on the south-facing slopes, whereas much of the fall range was on gentle north-facing slopes. Much of the spring range was on relatively steep, south-facing slopes.

Soils were largely light-textured with heavier textures occurring at the lower elevations and more gentle sloping portions of the range. The higher portions of the area grazed were characterized by rockiness and uneven topography.

Water was available from springs and Hot Springs Creek. In general, the water was of good quality and was plentiful for the sheep. In addition, snow was available during portions of the late fall and winter months. The sheep were not trailed excessive distances to secure water.

The general vegetation on the ranch was dominated by bluebunch wheatgrass (Agropyron spicatum). Other grasses of considerable importance on the winter range were needle and thread (Stipa comata), junegrass (Koeleria cristata), western wheatgrass (Agropyron smithii), bluegrasses (Poa spp.), and sedges (Carex spp.). Forbs on the winter range were unimportant as a source of feed. The only forbs grazed to any extent were the dried twigs, leaves, and pods of several of the locos (Oxytropis spp.), lupines (Lupinus spp.), and milkvetches (Astragalus spp.). An important half shrub common on the winter range and highly palatable to the sheep was fringed sagewort (Artemisia frigida). Shrubs of importance included bitterbrush (Purshia tridentata), rabbitbrushes (Chrysothamnus spp.), and scattered plants of the big sagebrush and silver sagebrush (Artemisia tridentata and Artemisia cana).

The spring range was characterized by essentially the same species as the winter range, with the exception of bitterbrush. Spring range was perhaps steeper with sharper draws. Along the bottom of the draws and the slopes, there were extensive stands of various browse plants, including shunkbrush sumac (Rhus trilobata), ninebark (Physocarpus malvaceus), squaw current (Ribes cereum).

Most of the fall range was comparatively free of browse plants. It was dominated by the grasses common on the winter and spring ranges, and, in addition, at the higher elevations were considerable stands of Idaho fescue (Festuca idahoensis). The forbs common on the winter range were present on the fall range with extensive stands in certain locations of white pointloco (Oxytropis serecia)and the prairie milkvetch (Astragalus striatus).

These ranges were open to grazing most of the winter except when there was a heavy accumulation of snow.

## METHODS AND PROCEDURE

Twelve mature animals ranging in age from four to six years (except one two-year-old Columbia) were selected from each breed, Rambouillet and Columbia. Six sheep from each breed were taken from a high feed treatment and six from a low feed treatment.

The winter trials started at the Red Bluff Ranch, Norris, Montana, on December 29, 1961. Before putting the animals on trial, they were weighed and scored for condition. The high feed treatment was group fed two pounds of a 30 percent protein pellet every other day. The composition of the supplemental pellet is presented in Table II.

TABLE II. FEED COMPOSITION OF THE SUPPLEMENTAL PELLET.

| Ingredients | % |
|---|---|
| Barley | 10.0 |
| Cottonseed meal | 20.0 |
| Linseed meal | 20.0 |
| Soybean meal | 22.5 |
| Dehydrated alfalfa meal | 20.0 |
| Molasses, cane | 6.3 |
| Salt | 1.0 |
| Trace mineral | 0.2 |
| Vitamin A & D1/ | X |
| | 100.0 |

1/ To provide approximately 2,000 I.U. of vitamin A and 500 I.U. of vitamin D.

Both high and low feed treatments were maintained on the same range except that the low treatment group did not receive any supplement. During severe storms grazing was not possible, therefore, both groups were fed hay.

The feed treatments were discontinued on April 2, 1962 and the animals were weighed and scored for condition. After this date all the animals were fed alike.

Skin samples were taken within a week after lambing and preserved in Bouin's fluid for histological preparations.

Sampling technique

The sheep was laid on its left side in a convenient position. The sample was taken from the right side. The position of sampling was at the intersection of two imaginary lines, one tangent to the dorsal border of the scapula and the other tangent to the posterior border.

When sampling the first few sheep, the skin was washed with soapy water and the wool closely shaved off. A local anaesthetic (procaine hydrochloride) 3 c.c. was injected subcutaneously at two points adjacent to the area to be sampled to reduce pain. The instruments were clean, but not surgically sterile. The wounds were left open and an antiseptic solution applied. The use of this method was time consuming, caused some bleeding, and left swelling of the tissue.

The remainder of the sheep were sampled by a method whereby a small lock of wool over the area to be sampled was pulled until the skin was stretched quite tight. A sharp pair of scissors were used to snip a section about one-fourth inch below the point of the skin. A sample about one-half inch in diameter was obtained quickly and with little or no damage to muscle tissue.

Fixation and preservation of the samples

The samples were put into bottles containing Bouin's fluid (picro-formalacetic) and labelled with the sheep eartag number and the date of sampling. Each sample was left in the fixative for about the same length of time to ensure uniform shrinkage.

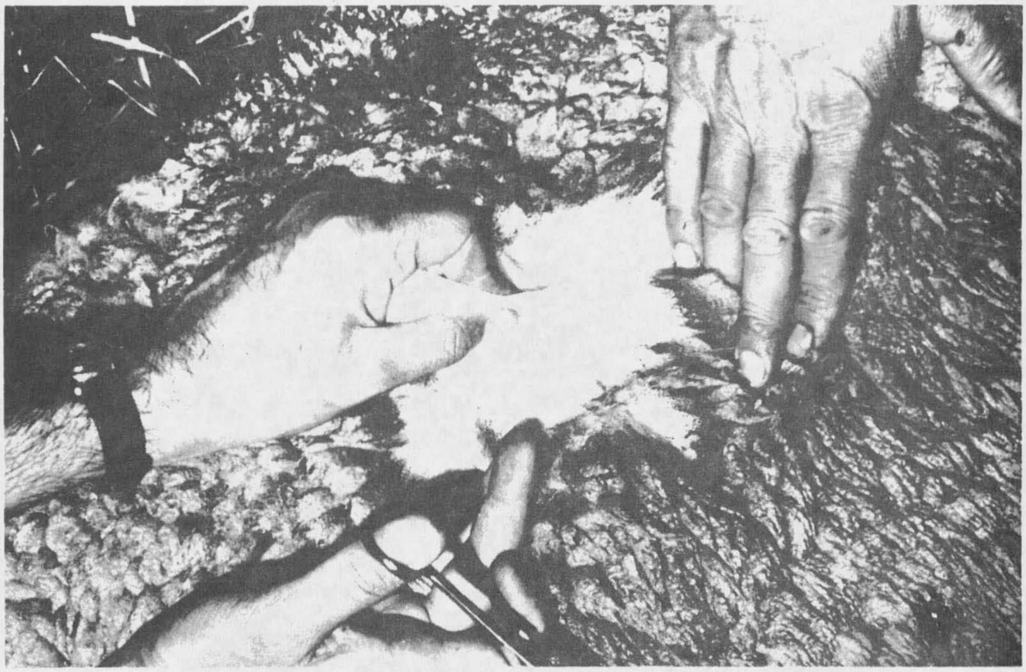

Figure 1. Stretching skin in preparation to take sample.

Figure 2. The site after removal of sample.

According to Galigher (1934) it is a rapid fixer, but materials may be left in it for long periods of time without being injured. Bouin's fluid is generally used as a fixing and preserving reagent. All stains give good results with this reagent but it is particularly favorable to the Haematoxylin - Eosin staining method which was used in this study.

Preparation of skin samples

The preparation of skin sections for histological study was similar to those described by Carter (1939) and Clarke (1960).

The epithelial surface was clipped with fine scissors to reduce any residual hair to minimum and remove any loose fibers likely to injure the paraffin ribbon at a later stage. Fragments of skeletal muscle or ragged fascia on the subcutaneous surface were trimmed off to ensure flat embedding.

Dehydration, clearing and paraffin impregnation were then pursued in the following routine:

(a) Dehydration: The tissue was kept in the following dilutions of alcohol for the time shown. Seventy percent alcohol does not harm the tissue so it may be left overnight if necessary.

Alcohol 70% - - - - - - - - - overnight

Alcohol 95% - - - - - - - - - one hour

Alcohol 100% (absolute) - - one hour

(b) Clearing: Dioxine - two hours (change once). Precaution was taken not to inhale fumes of this reagent in too large a quantity because they are poisonous.

(c) Paraffin impregnation or infiltration: Three paraffin dishes were placed in a thermostatically controlled oven where the tissue was allowed to stay in each dish for 40 minutes. The oven was maintained at a temperature of 60 degrees centigrade.

(d) Embedding: After the tissue had been impregnated with molten paraffin, it was embedded in the following manner-- A paper block mold was made to hold the paraffin in a block. Melted paraffin was poured into this mold and was allowed to cool for 3 to 4 minutes. When a hard layer of paraffin appeared at the bottom of the mold, the tissue was placed in the center in an upright position. The mold was carefully immersed in a beaker containing cool water. After five minutes, it was transferred to another beaker containing ice cold water to harden the block uniformly.

Sectioning

Paraffin blocks with the tissue embedded in them were trimmed and mounted on wooden blocks using heat. The tissue was embedded horizontal to ensure cross sectioning of the follicles. Before the blocks were mounted on the Spencer Rotatory Microtome, they were cooled in ice water. After preliminary trimming, serial sections were cut between 8 and 9 microns thick. All the tissue was sectioned to ensure that there was enough material for making observations at different levels.

Mounting sections on slides

The slides were cleaned and a thin film of Mayer's fixative was applied to the surface.

These slides were dried and then placed on a hot plate regulated to a constant temperature of 40 degrees centigrade. Two or three drops of distilled water were put on the slide to help the sections flatten and attach to the surface.

The mounted slides were left for 24 hours for drying to ensure complete attachment of the sections. Five to ten serial sections were mounted on each slide. The slides were serially numbered and the sheep eartag number indicated on the label.

Staining

Haematoxylin and eosin staining method was used according to the following schedule. The staining rack was arranged in an order according to what each jar contained. The following steps show the time allowed in each jar with the name of reagent used:

1. Toluene - 5 minutes (first bath)
2. Toluene - 5 minutes (second bath)
3. Absolute alcohol (100%) - 1 minute
4. Alcohol 95% - 1 minute
5. Alcohol 70% - 1 minute
6. Alcohol 50% - 1 minute
7. Alcohol 35% - 1 minute
8. Haematoxylin - 15 to 30 seconds
9. Wash in $H_2O$ (distilled) - 30 minutes
10. Alcohol 35% - 1 minute
11. Alcohol 50% - 1 minute
12. Alcohol 70% - 1 minute
13. Alcohol 95% - 1 minute
14. Eosin - 5 to 10 seconds

15. Rinse in 95% alcohol - 1 to 2 minutes

16. Alcohol 95% - 1 minute

17. Absolute alcohol (100%) - 2 to 3 minutes

18. Toluene - 5 minutes (first bath)

19. Toluene - 5 minutes (second bath)

The sections were mounted in "Canada balsam". Efforts were made to remove all air bubbles from under the cover slip.

The slides were then ready for microscopic examination except for final cleaning and drying. These were properly labelled and kept in serial order in slide boxes of twenty-five each. Separate slide boxes were used for each individual animal.

Microscopic examinations and measurements

Measurements were made to determine the differences in population density, size of the sebaceous gland and the size of the follicle. Slide number five was first examined in which the first and the last sections were carefully examined to see if the sebaceous gland was increasing or decreasing in size. Two tracings were made of the sebaceous gland at its greatest diameter. The follicle tracings were also made at this level. A magnification of 500 times was used for all observations. One tracing was made on each of the three sections on each side of the section of the greatest sebaceous gland diameter. A total of eight tracings were made of each of the structures. The outer boundary of the follicle was not clear, therefore, the follicle was traced at the boundary of the inner root-sheath. Follicles and sebaceous glands which had more than one-half their area on the sheet of paper were traced and counted.

Follicle density was obtained by counting the number of follicles on each sheet for individual animals. The sebaceous gland tracings were marked with an "S" to distinguish them from the follicle tracings.

For measuring the area of follicle and the sebaceous gland, a planimeter was used. A starting point was marked on the traced boundary of the follicle or the sebaceous gland and with the point of the planimeter-needle on this mark, the initial reading was taken. The needle was moved over the pencil-line until the starting point was reached. The final reading was recorded and the difference between the two readings gave the area in square inches. One hundred follicles were measured, taken from alternate tracings for area.

Data on the body weights of the experimental animals was also obtained. Information on type of birth (lambs carried) and grade of wool along with its weight was recorded.

Fleeces were graded and weighed at the Montana Wool Laboratory. American grades were used and mean for each grade was obtained in microns for statistical analyses.

## RESULTS AND DISCUSSION

### Effect of feed treatment on body weight and condition score

The body weight as influenced by feed treatment is shown in Table III. The information on condition score and type of birth (lambs carried) is also included. A highly significant ($P < .01$) effect of feed treatment on the body weight was observed (Appendix Table V). Most of the animals on the low feed treatment lost weight during the trial. All the animals gained weight in the high feed treatment group. A similar trend was observed on the condition score. It is possible that the method of determining condition scores is not standardized or refined enough and the condition determined by these scores may be questioned. If the type of birth and the condition of the animal are compared, the animals with twin births have higher condition scores showing they lost condition. This may be due to the heavy stress of nourishing the twins in the foetal stage. Some animals which have gained weight in spite of low feeding level may possess better appetites and feed efficiency. Three out of the six animals in low fed Columbias carried twins and lost weight due to the excessive stress of nourishing two foetal lambs. The same number of animals in the low fed Rambouillets had twins and one of them gained one pound but the condition score was high, indicating the animal lost condition. The two years old Columbia in low feed treatment, (No. 0006 V) was the youngest sheep on the experiment and lost the most weight. It is postulated that this condition was due to continuing growth and development of the ewe and the stress of the first pregnancy.

### Effect of feed treatment on the follicles and sebaceous glands

Data presented in Table IV shows the average number and size of follicles, and the average number and size of the sebaceous glands.

TABLE III. BODY WEIGHTS AND CONDITION SCORES OF ANIMALS ON AND OFF THE EXPERIMENT AND TYPE OF BIRTH, (LAMBS CARRIED).

| Sheep No. | Weight (lbs.) On | Weight (lbs.) Off | Condition Score On | Condition Score Off | Type of Birth (Single or twin) |
|---|---|---|---|---|---|
| **Rambouillets** | | | | | |
| High | | | | | |
| 1100 F | 159 | 165 | 1.7 | 2.0 | Twins |
| 1053 J | 149 | 156 | 2.3 | 2.7 | Twins |
| 1061 J | 139 | 145 | 2.3 | 2.3 | Single |
| 1004 H | 145 | 148 | 2.3 | 2.3 | Single |
| 1256 H | 124 | 142 | 3.0 | 2.3 | Single |
| 1342 H | 162 | 170 | 2.0 | 2.0 | Single |
| Avg. | 146 | 154 | 2.3 | 2.3 | |
| Low | | | | | |
| 1006 F | 160 | 161 | 2.7 | 4.0 | Twins |
| 1148 H | 154 | 159 | 2.0 | 3.3 | Twins |
| 1215 H | 140 | 136 | 2.3 | 3.7 | Twins |
| 1050 J | 143 | 141 | 3.0 | 4.0 | Single |
| 1223 J | 130 | 134 | 2.7 | 3.3 | Single |
| 1389 J | 145 | 136 | 3.0 | 4.0 | Single |
| Avg. | 145 | 145 | 2.6 | 3.7 | |
| **Columbias** | | | | | |
| High | | | | | |
| 6155 V | 152 | 159 | 2.3 | 2.7 | Single |
| 7546 V | 141 | 147 | 3.3 | 2.7 | Single |
| 7642 V | 170 | 174 | 2.7 | 3.3 | Single |
| 7660 V | 148 | 167 | 2.3 | 3.0 | Twins |
| 8516 V | 171 | 183 | 2.0 | 2.7 | Single |
| 8633 V | 120 | 127 | 2.3 | 3.0 | Single |
| Avg. | 150 | 160 | 2.5 | 2.9 | |
| Low | | | | | |
| 0006 V | 154 | 135 | 2.3 | 3.3 | Single |
| 6079 V | 139 | 130 | 3.3 | 4.0 | Twins |
| 6096 V | 133 | 131 | 3.0 | 3.3 | Twins |
| 8505 V | 140 | 134 | 2.7 | 3.7 | Single |
| 8578 V | 114 | 120 | 3.0 | 4.0 | Single |
| 8673 V | 142 | 140 | 2.3 | 3.0 | Twins |
| Avg. | 137 | 132 | 2.8 | 3.6 | |

TABLE IV. THE EFFECT OF BREED AND FEED TREATMENT ON WOOL FOLLICLES AND SEBACEOUS GLANDS.

| | Follicles (No.) | Follicle Size X 500 (Sq. In.) | Sebaceous Gland (No.) | Sebaceous Gland size X 500 (Sq. In.) |
|---|---|---|---|---|
| | | Rambouillet | | |
| High | 242 | .260 | 34 | 2.09 |
| Low | 223 | .186 | 28 | 1.93 |
| | | Columbia | | |
| High | 145 | .360 | 40 | 2.29 |
| Low | 147 | .295 | 26 | 2.04 |
| | | Breeds | | |
| Rambouillet | 232** | .223 | 31 | 2.01 |
| Columbia | 146 | .327 1/ | 32 | 2.16 |
| | | Treatments | | |
| High | 193 | .310 | 36 | 2.19 |
| Low | 185 | .240 | 26 | 1.98 |

** Significant at 1% level.
1/ Significant at 6% level.

Averages for each treatment group within each breed are also shown. There appears to be a trend toward an increase in number and size of the sebaceous glands, and also the size of the follicles in the high feed treatment group. The trend is not statistically significant which may be due to the large variation among animals and small sample size. These values could also be affected by the type of birth of the animal used. Those born as singles may have more follicles than those born as twins. Schinckel (1953) has shown that the lambs born as twins have a retarded maturation of the secondary follicles when compared to singles but the extent of this effect is not well established. Sheep number 6096 V, a Columbia, was born a twin and was on the low feed treatment, but had the highest number of follicles compared to the other Columbia sheep in either treatment. The other two Columbias born as twins had the lowest number of follicles (excluding the two-year-old animal). The size of sebaceous gland and follicle does not seem to be affected by the type of birth in this experiment. There was no significant treatment effect but there was a highly significant ($P < .01$) breed effect on the number of follicles. The Rambouillet ewes had a greater number of follicles than the Columbias. There was no significant treatment effect on the size of the follicle, (Table IV), but there was a breed effect which was significant ($P < .06$) at 6 percent level. The analysis of variance on number of follicles appears in Appendix Table I.

Snedecor (1956) stated that counts of variables, such as the number of follicles, tend to be distributed in Poisson fashion, which may result in non-additive effects. To determine if the analysis of variance of the data could be made, Tuckey's (1949) test for additivity was made. The "F" value of .0047 was not significant and gave assurance that the analysis of variance of the data was valid.

There was a significant ($P < .06$) breed effect, Columbia having larger follicles than the Rambouillets. An analysis of variance table of the size of follicle appearing in Appendix Table II shows no breed and treatment interaction.

Analysis of variance tables of the size of sebaceous gland and of the number of follicles versus lambs carried have been included as Appendix Tables III and IV respectively. There was no significant breed or treatment effect or interaction on the size of sebaceous gland. The number of follicles versus lambs carried had no statistical significance. It is postulated that feed treatment or number of lambs carried would have little effect on factors studied because the animals were mature.

A number of correlations were calculated to determine if there were any relationships between the fleece values and the follicles. Table V gives information on weight gain or loss, grease fleece weight and the fiber diameter. The grade measurements in microns were not available for the individual fleeces, therefore, the average of the micron spread for each grade was used. The following measurements represent the various grades of wool:

| | |
|---|---|
| Fine | 20.6 Microns |
| Half-blood | 23.5 " |
| 3/8-blood | 26.4 " |
| 1/4-blood | 29.4 " |

Table VI presents the correlation coefficients between the various measurements. There was no significant correlation between weight loss or gain and the number of follicles. This indicates that the level of feeding did not significantly affect the number of follicles. There was no significant correlation between gain or loss of body weight and size of the follicle. A negative correlation was obtained between the number of follicles and the grease fleece weight which was approaching significance at the 5 percent level. This may be explained by the fact that the fiber length and diameter exert a major influence on fleece weight. A highly significant ($P < .01$) correlation existed between the grade of wool and size of the follicle. This may be the reason for heavier fleece weight in Columbia than in the Rambouillet which produce finer wool. A significant ($P < .05$) correlation was found between the number of sebaceous glands and the grease fleece weight. This supports the theory that a greater number of sebaceous glands will secrete more sebum (wool wax) resulting in heavier fleece weight.

TABLE V. BODY WEIGHT CHANGE AND FLEECE MEASUREMENTS.

| Sheep No. | Body weight Gain or loss (lbs.) | Grease fleece weight (lbs.) | Avg. Diameter of wool (Microns) |
|---|---|---|---|
| High | | Rambouillets | |
| 1100 F | 6 | 7.5 | 20.6 |
| 1342 H | 12 | 11.6 | 20.6 |
| 1053 J | 7 | 11.4 | 23.5 |
| 1256 H | 18 | 10.1 | 23.5 |
| 1061 J | 6 | 10.0 | 20.6 |
| 1004 H | 3 | 9.0 | 23.5 |
| Low | | | |
| 1223 J | 4 | 9.4 | 20.6 |
| 1050 J | 2 - | 8.3 | 20.6 |
| 1215 H | 4 - | 9.4 | 20.6 |
| 1389 J | 9 - | 9.6 | 23.5 |
| 1148 H | 5 | 8.2 | 20.6 |
| 1006 F | 1 | 7.8 | 20.6 |
| High | | Columbias | |
| 7660 V | 19 | 11.4 | 26.4 |
| 8516 V | 12 | 14.0 | 26.4 |
| 8633 V | 7 | 9.1 | 23.5 |
| 7642 V | 4 | 15.7 | 29.4 |
| 7546 V | 6 | 10.6 | 26.4 |
| 6155 V | 7 | 9.1 | 23.5 |
| Low | | | |
| 8578 V | 6 | 8.5 | 23.5 |
| 6079 V | 9 - | 10.0 | 26.4 |
| 0006 V | 19 - | 11.1 | 23.5 |
| 6096 V | 2 - | 10.1 | 26.4 |
| 8505 V | 6 - | 10.1 | 29.4 |
| 8673 V | 2 - | 12.5 | 26.4 |

TABLE VI. CORRELATION CO-EFFICIENTS BETWEEN VARIOUS OBSERVATIONS.

| Observations | r | No. of animals |
|---|---|---|
| Size of follicle vs grease fleece weight | .661** | 24 |
| Number of follicles vs grease fleece weight | -.356 1/ | 24 |
| Number of sebaceous glands vs grease fleece weight | .437* | 24 |
| Size of sebaceous gland vs grease fleece weight | .084 | 24 |
| Number of follicles vs grade of wool | -.526** | 24 |
| Grease fleece weight vs grade of wool | .641** | 24 |
| Grade of wool vs size of follicle | .710** | 24 |
| Size of follicle vs size of sebaceous gland | -.09 | 24 |
| Gain or loss vs number of follicles | .270 | 24 |
| Gain or loss vs size of follicle | .241 | 24 |

1/ Approaching significance at 5% level.
* Significant at 5% level.
** Significant at 1% level.

It is interesting to note that some individual animals had a greater number of sebaceous glands than others. Feed treatment did not seem to have any effect on the number or size of the sebaceous glands. There was no significant correlation between the fleece weight and the size of sebaceous gland.

Table VI also shows a highly significant ($P < .01$) correlation between the size of the follicle and the grease fleece weight. It was also found in this study that grease fleece weight and grade of wool were highly correlated ($P < .01$) indicating that increase in fiber diameter is associated with increase in fleece weight.

A highly significant ($P < .01$) negative correlation existed between the number of follicles and the grade of wool. This is in agreement with the data presented by Batu et al. (1962) who worked with Turkish breeds of sheep. It is postulated that there is more area for the follicles to grow in size and also the nourishment will be comparatively better than in the skin with higher follicle density. In other words, there is less competition for keratin precursors and consequently more vigorous growth of wool.

During the course of these observations, many other interesting features were noted. Follicles with multiple fibers were observed at various levels. It appeared that these fibers were not emerging from separate follicles. There are conflicting reports in the literature on the branching of follicles. Branching has been described in some breeds of sheep by various workers (Tanzer 1926; Hardy and Lyne 1956; Orwin 1961). Differences in findings among various workers suggest that there should be more supporting evidence before concluding that branching takes place. To supplement the information on branching, vertical sections were made from skin samples of those animals which showed evidence of multiple fibers in a single follicle. Observations of both vertical and cross sections were made. It appeared that there was evidence of follicular budding. In cross sections, follicles containing more than one fiber were followed downward and were found to join together at lower levels.

The angle of a follicle in relation to the skin surface was not found to be uniform. The follicles were embedded at different levels of the skin, some being at deeper levels than others.

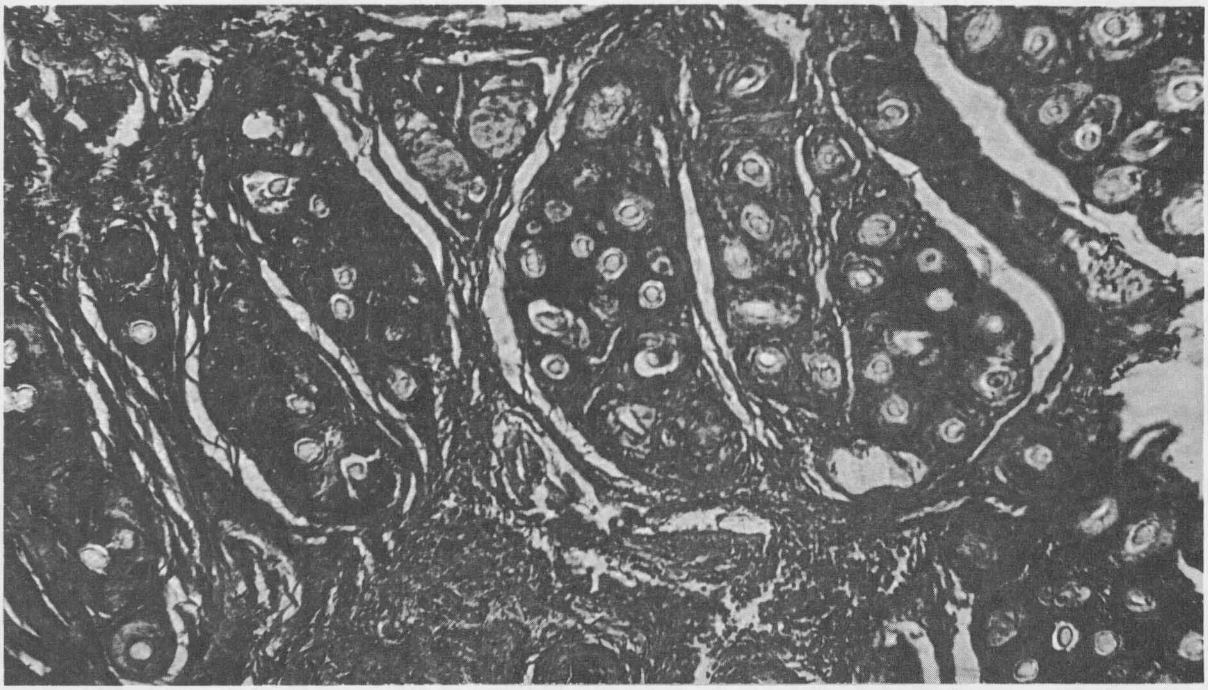

Figure 3. A skin section showing the arrangement of follicle bundles. 180 X.

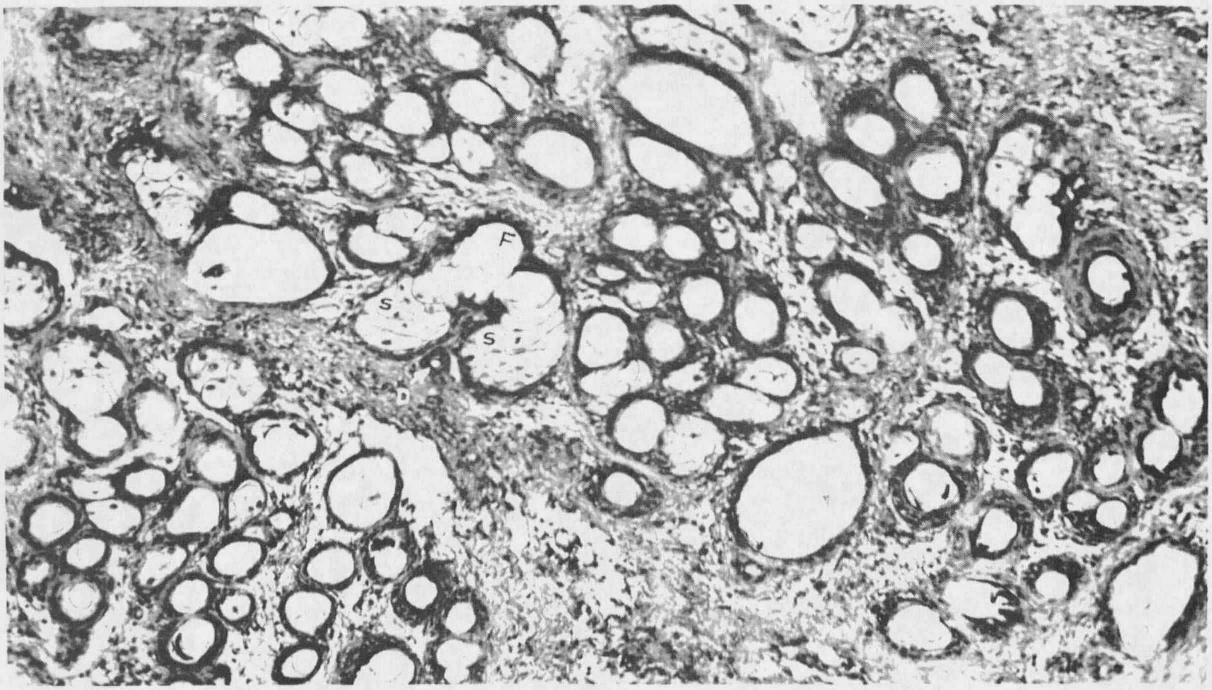


Figure 4. Greater follicle (F) density and smaller size in Rambouillet vs Columbia (Fig. 5); sebaceous gland (S); sudoriferous duct (D). 180 X.

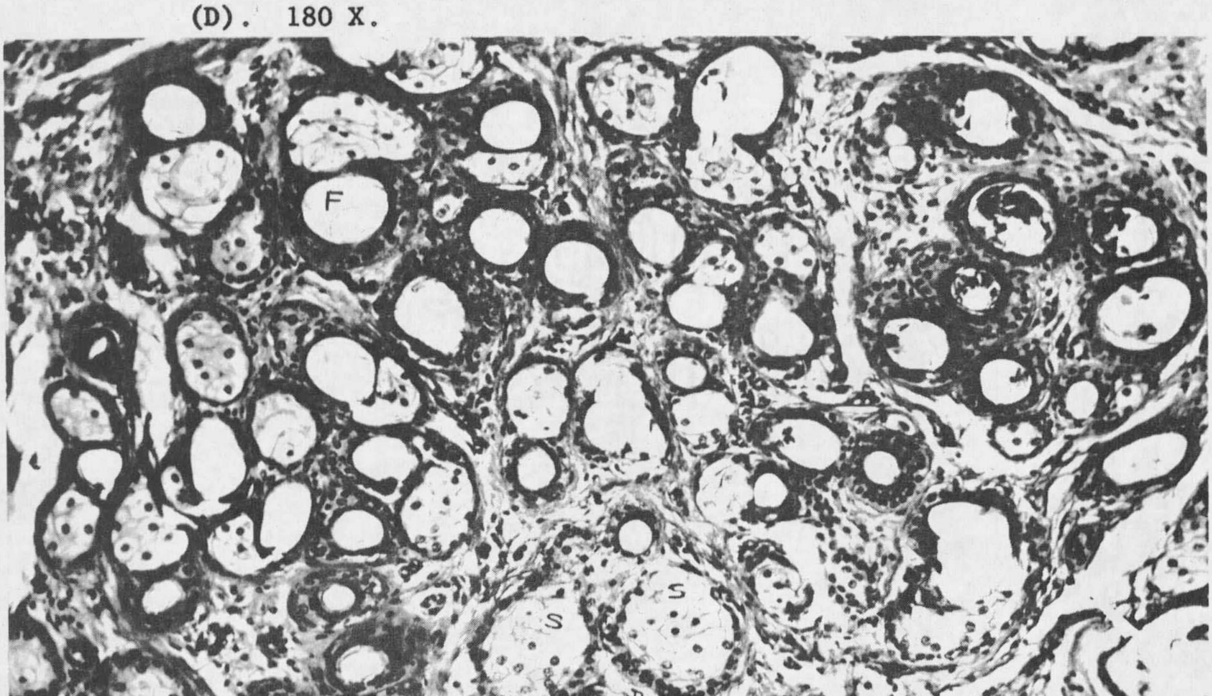


Figure 5. Lesser follicle (F) density and larger size in Columbia vs Rambouillet (Fig. 4); sebaceous gland (S); sudoriferous duct (D). 180 X.

Figure 6. Vertical section of a wool Follicle. 500 X.


Figure 7. Cross section of a convoluted sudoriferous gland (S). 500 X.

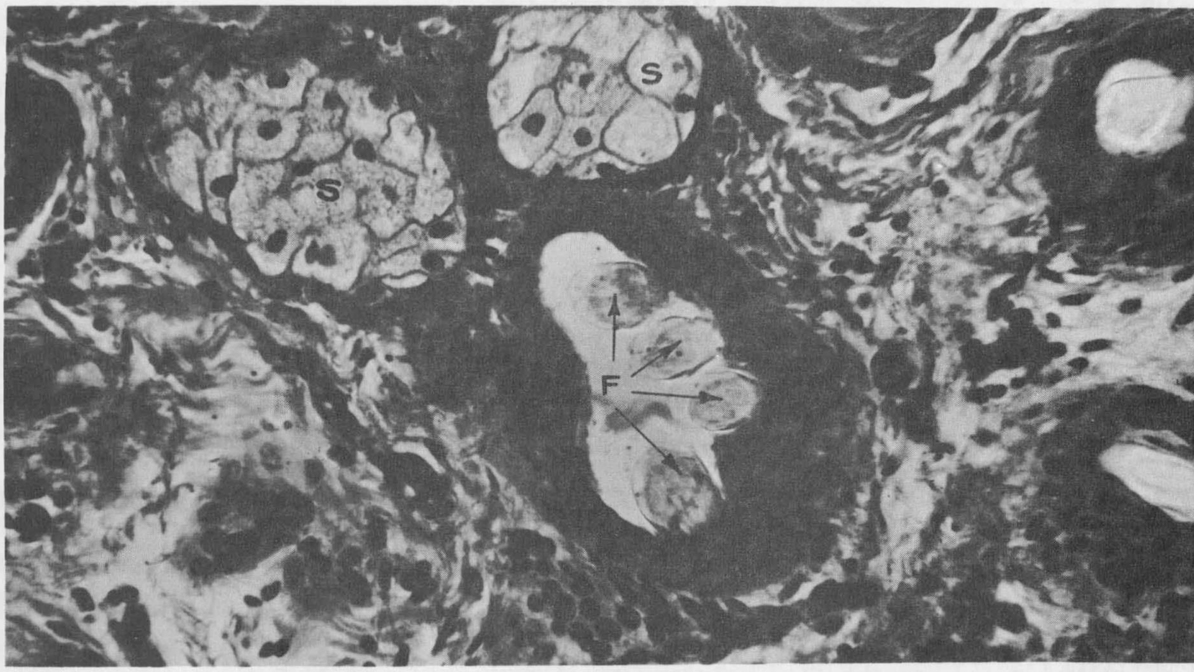


Figure 8. A single follicle with four fibers (F) and a bi-lobed sebaceous gland (S) in a Rambouillet. 500 X.

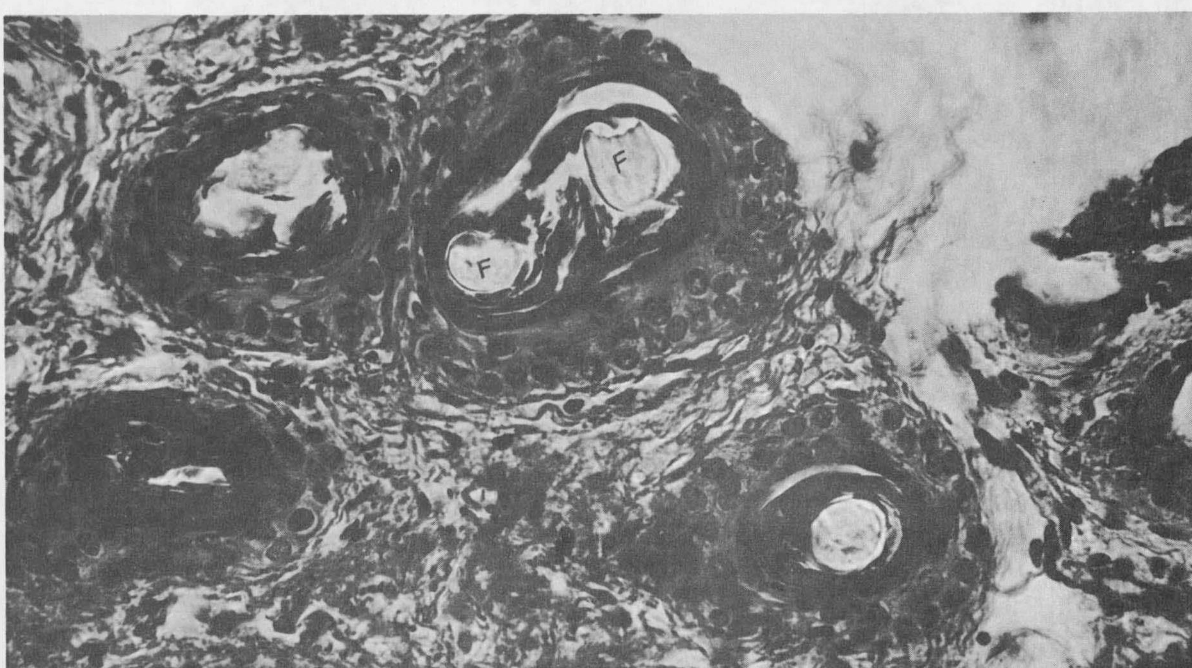


Figure 9. A single follicle with two fibers (F) in a Columbia. 500 X.

## SUMMARY

Histological observations were made on the skin of twenty-four mature animals of Rambouillet and Columbia breeds on two different levels of nutrition. These animals were part of a range band of ewes owned by the Montana Agricultural Experiment Station and were wintered at the Red Bluff Ranch, Norris, Montana. Each animal in the high feed treatment received two pounds of thirty percent protein pellet every other day and the low treatment group was maintained on range grazing only except in stormy conditions when hay was fed.

Standard histological techniques were used which have been described elsewhere. Serial sections were cut at all levels and each section was mounted on the slide for future examination. Follicles and sebaceous glands were magnified and traced on an 8½ X 11 inch sheet of paper. A planimeter was used to measure areas of the follicles and the sebaceous glands.

The feed treatment had a very highly significant ($P < .01$) effect on the live body weight of the animals. There was no significant effect of feed treatment on the number and size of follicles and number and size of sebaceous glands. However, there was highly significant ($P < .01$) breed effect on the number of follicles. Breed effect was also significant ($P < .06$) on the size of follicle, the Columbia breed having larger follicles.

A highly significant ($P < .01$) negative correlation was obtained between the number of follicles and the grade of wool (the fewer the follicles, the coarser the wool). A negative correlation existed between the number of follicles and the grease fleece weight (the fewer the follicles, the heavier the weight) which was approaching significance at 5 percent level. Body weight gain or loss did not show any significant correlation

between the size or number of the follicles.

There was a highly significant correlation ($P < .01$) between the size of the follicle and the grease fleece weight (the larger the follicle, the heavier the fleece weight). Grease fleece weight was also highly correlated ($P < .01$) to grade of wool (coarser wool produces heavier fleeces). Similarly grade of wool and size of follicle were highly correlated ($P < .01$) (larger follicles produce coarser wool).

One to five fibers were observed in a single follicle. Budding follicles were also observed in cross and vertical sections.

# APPENDIX

APPENDIX TABLE I. ANALYSIS OF VARIANCE OF NUMBER OF FOLLICLES.

| Source of variation | Degrees of freedom | Sum of squares | Mean square | F |
|---|---|---|---|---|
| Breeds | 1 | 44980 | 44980 | 31.2** |
| Treatments | 1 | 442 | 442 | N.S. |
| B X T | 1 | 672 | 672 | N.S. |
| Error | 20 | 28824 | 1441 | |
| Total | 23 | 74918 | | |

** Significant at 1% level.

APPENDIX TABLE II. ANALYSIS OF VARIANCE OF SIZE OF FOLLICLE.

| Source of variation | Degrees of freedom | Sum of squares | Mean square | F |
|---|---|---|---|---|
| Breeds | 1 | .066 | .066 | 4.12 1/ |
| Treatments | 1 | .029 | .029 | 1.81 |
| B X T | - | ---- | ---- | ---- |
| Error | 21 | .347 | .016 | |
| Total | 23 | .442 | | |

1/ Significant at 6% level.

APPENDIX TABLE III. ANALYSIS OF VARIANCE OF THE SIZE OF SEBACEOUS GLAND.

| Source of variation | Degrees of freedom | Sum of squares | Mean square | F |
|---|---|---|---|---|
| Breeds | 1 | .15 | .15 | N.S. |
| Treatments | 1 | .26 | .26 | N.S. |
| B X T | 1 | .01 | .01 | N.S. |
| Error | 20 | 7.14 | .35 | |
| Total | 23 | 7.56 | | |

APPENDIX TABLE IV. ANALYSIS OF VARIANCE OF NUMBER OF FOLLICLES VS LAMBS CARRIED.

| Source of variation | Degrees of freedom | Sum of squares | Mean square | F |
|---|---|---|---|---|
| | | Rambouillet | | |
| Type of birth (Lambs carried) | 1 | 36 | 36 | N.S. |
| Error | 10 | 18146.3 | 1814.63 | |
| Total | 11 | 18182.3 | | |
| | | Columbia | | |
| Type of birth (Lambs carried) | 1 | 913 | 913 | N.S. |
| Error | 10 | 10843 | 1084.3 | |
| Total | 11 | 11756 | | |

APPENDIX TABLE V. ANALYSIS OF CO-VARIANCE OF FEED TREATMENTS ON BODY WEIGHT OF THE ANIMALS.

| Source | Degrees of freedom | Sum of products $\Sigma x^2$ | Sum of products $\Sigma xy$ | Sum of products $\Sigma y^2$ | Degrees of freedom | Adjusted $\Sigma y^2$ | Mean square | F |
|---|---|---|---|---|---|---|---|---|
| Total | 23 | 4792.5 | 4743.0 | 6350.0 | | | | |
| Breeds | 1 | 28.1 | 49.0 | 88.2 | | | | |
| Treatments | 1 | 1722.0 | 1500.0 | 2404.0 | | | | |
| Error | 22 | 3042 | 3194 | 3858 | 21 | 505 | 24 | |
| Treatments & error | 23 | 4764 | 4694 | 6262 | 22 | 1637 | | |
| Treatments adjusted | | | | | 1 | 1132 | 1132 | 47.1** |

** Significant at 1% level.

APPENDIX TABLE VI. THE EFFECTS OF BREED AND FEED TREATMENT ON THE WOOL FOLLICLES AND SEBACEOUS GLANDS.

| Sheep No. | Follicles present (No.) | Avg. size follicle X 500 (Sq. In.) | Follicles Measured (No.) | Sebaceous gland (No.) | Avg. size of S.G. X 500 (Sq. In.) |
|---|---|---|---|---|---|
| High | | | Rambouillet | | |
| 1100 F | 248 | .126 | 100 | 25 | 2.09 |
| 1342 H | 240 | .309 | 100 | 44 | 2.07 |
| 1053 J | 217 | .346 | 100 | 31 | 1.45 |
| 1256 H | 230 | .340 | 100 | 50 | 1.81 |
| 1061 J | 241 | .220 | 100 | 34 | 1.80 |
| 1004 H | 278 | .224 | 100 | 22 | 3.32 |
| Avg. | 242 | .260 | | 34 | 2.09 |
| Low | | | | | |
| 1223 J | 192 | .263 | 100 | 29 | 2.89 |
| 1050 J | 177 | .245 | 100 | 18 | 2.21 |
| 1215 H | 171 | .155 | 100 | 34 | 1.98 |
| 1389 J | 261 | .227 | 100 | 44 | 1.97 |
| 1148 H | 311 | .116 | 100 | 25 | 1.41 |
| 1006 F | 227 | .113 | 100 | 18 | 1.14 |
| Avg. | 223 | .186 | | 28 | 1.93 |
| High | | | Columbia | | |
| 7660 V | 128 | .265 | 100 | 44 | 2.21 |
| 8516 V | 154 | .331 | 100 | 34 | 3.08 |
| 8633 V | 136 | .267 | 100 | 45 | 2.32 |
| 7642 V | 144 | .748 | 100 | 46 | 1.28 |
| 7546 V | 194 | .272 | 100 | 37 | 2.31 |
| 6155 V | 115 | .282 | 100 | 33 | 2.58 |
| Avg. | 145 | .360 | | 40 | 2.29 |
| Low | | | | | |
| 8578 V | 143 | .237 | 100 | 31 | 1.85 |
| 6079 V | 141 | .261 | 100 | 23 | 1.47 |
| 0006 V | 130 | .249 | 100 | 20 | 2.70 |
| 6096 V | 223 | .189 | 100 | 22 | 1.58 |
| 8505 V | 104 | .586 | 100 | 33 | 1.97 |
| 8673 V | 142 | .252 | 100 | 25 | 2.68 |
| Avg. | 147 | .295 | | 26 | 2.04 |

APPENDIX TABLE VII. COMPOSITION AND PREPARATION OF VARIOUS STAINS AND RE-AGENTS.

1) Haematoxylin Solution - (Harris' formula)

| | | |
|---|---|---|
| A- | Haematoxylin | 0.5 gram |
| | Absolute alcohol | 5.0 c.c. |
| B- | Potassium alum (cryst.) | 10.0 grams |
| | Distilled water | 95.0 c.c. |

Heat B to boiling and add solution A, then add the oxidizer, Mercuric oxide 0.25 grams. Stir the mixture and allow it to continue to boil for a minute or two, then cool quickly by immersing the container (pyrex flask) in cold water. Allow it to stand several hours to overnight; filter and store in a tightly stoppered bottle.

Modifications of this formula found in the literature consist of making the solution in 50 percent alcohol, longer boiling, less (sometimes more) alum and more mercuric oxide. Also the addition of acetic or hydrochloric acid is optional. When acetic acid is added 3 - 5 c.c. in the formula above are recommended. Acidification is said to improve the keeping qualities of the solution and to inhibit the staining of cytoplasm.

2) Eosin Solution:

| | |
|---|---|
| Eosin | 0.5 gram |
| Alcohol 95% | 100 c.c. |

APPENDIX TABLE VII. (CONTINUED).

3) Bouin's Fluid:

| | |
|---|---|
| Picric acid, saturated aqueous solution | 75 c.c. |
| Formalin | 25 c.c. |
| Glacial acetic acid | 5 c.c. |

One gram of picric acid crystals will saturate about 75 c.c. of water.

4) Mayer's Fixative:

Equal parts of glycerin and egg-white. a bit of salicylate of soda (one gram to 50 c.c.) or thymol is added to prevent putrefaction.

Various concentrations of alcohols were prepared in the following manner:

The graduated cylinder was filled to the mark whatever concentration was needed and then distilled water was added up to 100 c.c. because Absolute Alcohol (100%) was used for making these concentrations.

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