STUDIES ON EXPERIMENTAL DERMAL CYSTS IN SHEEP

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Summary

The growth of seven experimental dermal cysts in four sheep has been studied for periods of up to 83 weeks. The similar growth behaviour of all cysts indicates the reliability of the technique for producing standard epithelium-lined cysts. This has been emphasized by producing the cysts in animals of different genotype.

Growth in surface area of the cysts is accompanied by a decrease in the number of wool follicles per unit area. Histological evidence and the constancy of the secondary/primary follicle ratio indicate that the decrease in density per unit area is the result of separation of follicle groups caused by the growth of interstitial connective tissue.

The lack of evidence of abnormality in the structure and metabolism of cyst skin suggests that the cyst environment has little effect upon the metabolism of the implanted skin. There is no evidence that pressure causes any detectable change in the cyst wall.

The value of epithelium-lined cysts as a biological system which can be used to study growth and metabolism is discussed.

I. Introduction

Epithelium-lined cysts are not uncommon pathological structures in man. Their aetiology and location vary considerably; for example, they may occur as developmental cysts at lines of embryonic closure, as implantation cysts at sites of mechanical injury, as sebaceous cysts arising from infection and blockage of ducts, and as ovarian cysts. They are common in the jaws, where they may be associated with neoplastic, developmental, and inflammatory lesions.

It is generally thought that epithelium-lined cysts tend to increase in size and that this growth is related to a positive intracystic pressure. This assumed relation has been the basis for several studies on the mechanism of cyst growth. For example, James (1926) and Toller (1948) measured the hydrostatic pressure in cysts of the jaws of humans by means of water manometers and found that the pressures were higher than capillary blood pressure. However, although high hydrostatic pressures have been recorded (Toller (1948) obtained an average value of 70 cm water from 51 cases of dental cysts, range 56·6–95·0 cm water), there is little histological evidence that pressures of such dimensions affect the cyst wall. Stokke (1956) discussed this question and suggested that the recording methods used by Toller and James were responsible for the high intracystic pressures.

The necessity for surgical removal of cysts in man generally prevents any extended studies aimed at relating intracystic pressures to cyst growth and cyst

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wall structure and therefore, in order that the behaviour of cysts could be studied over an extended period, dermal cysts were produced experimentally in sheep. In this study sheep were preferred as experimental animals to rodents, the usual laboratory animals, because:

(1) The size of sheep permitted a number of cysts to be implanted on each animal in accessible locations.

(2) Most wool follicles have a long growth phase and can be used as markers to indicate the state of the cyst environment as compared with that of normal skin. The extensive knowledge of wool follicles available (Fraser and Short 1960) is an additional advantage.

(3) Previous studies (Molyneux 1960) showed that the thickness of sheepskin, compared with that of rat and rabbit skin, facilitated the formation of cysts and that the presence of sweat glands in sheepskin contributed towards a fluid cyst content suitable for pressure recordings.

This paper reports observations made on sheepskin growing for up to 83 weeks as a cyst. The investigation was designed primarily to provide basic information on the behaviour and growth of artificially induced epithelium-lined cysts. It was thought that positive intracystic hydrostatic pressure might influence the structure and density of the wool follicles and other epidermal derivatives in the cyst wall and that this effect could readily be studied by comparing the histology of the implanted skin with that of surface skin. The basic premise of this approach is that the mechanism of growth of epithelium-lined cysts is similar regardless of location and that the results of studies of experimental dermal cysts would be of value in relation to pathological cysts.

II. Materials and Methods

Seven epithelium-lined cysts were experimentally implanted in four adult sheep. The animals were not selected with a view to homogeneity, but rather to provide diverse characteristics so that the efficiency of the implantation method could be assessed in various environments. The animals chosen were a Corriedale and three Merinos. Of the latter one had a high and one had a low follicle density while the third was a mutant without sweat glands (Short, personal communication). One of the reasons why sheep were selected as experimental animals was the presence of sweat glands which by secreting into the cyst contributed to the cyst contents and intracystic pressure. To examine the effect of lack of sweat secretion on cyst growth, a Merino without sweat glands was used.

The cysts were implanted on the midside region of the animals under local anaesthesia. The surgical technique developed for producing the cysts and the sampling method have been described (Molyneux 1960). At intervals after implantation the skin covering and surrounding the cysts was closely clipped and the size of the then-prominent cysts measured with calipers. When implanted the cysts were semicircular sacs (formed from a circular piece of skin 4 cm in diameter) but as growth occurred they tended to become spherical (Plate 1, Figs. 1 and 2). The size of the cyst, which included a double thickness of surface skin, was measured

across two normal diameters, the mean value being used to calculate the approximate surface area of the cyst, assuming it to be a sphere.

The cysts were then exposed by an incision through the surface skin and the fluid contents were aspirated into a sterile syringe. Skin samples of cyst walls and surface (control) skin were then obtained with a trephine similar to that described by Carter and Clarke (1957). Samples of intracystic wool were also collected. Biopsies of the cysts were made at approximately 5-week intervals after implantation. Multiple cysts were implanted in two animals and the growth of two cysts was observed for up to 83 weeks.

Examination of Skin Samples

The skin samples were fixed in 5% formol saline and, after paraffin embedding, serial sections (8 μ) were cut parallel to the skin surface. The cyst samples were sectioned entirely but most of the control samples were sectioned only to the mid-sebaceous gland level. Some entire sections of each cyst were also prepared (Plate 1). Most sections were stained with haemalum, eosin, and picric acid. To study the distribution of sulphydryl groups, selected samples were fixed in 1% trichloroacetic acid and stained by the dihydroxydinaphthyldisulphide (DDD) procedure of Barrnett and Seligman (1952). Sulphydryl and disulphide groups together were indicated by reduction of disulphide bonds with sodium thioglycollate at pH 8 followed by the application of DDD as described by Barrnett and Seligman (1954).

Counts of the follicle density (number per unit area) were made with a microprojector at a magnification of $\times 215$ on four to six fields, each 1 mm^2 in area at the mid-sebaceous gland level. The counts were corrected for shrinkage in a manner similar to that described by Carter and Clarke (1957). The following counts were recorded: number of primary (P) plus secondary (S) follicles per mm^2, number of P follicles per mm^2, and the S/P ratio.

Sections from superficial levels were also examined to study the thickness of the epidermis and at a deeper level to study the histology of the sweat glands.

III. Results

Biopsies of the cysts (see Table 1) were made at intervals and entire cysts were removed for examination 42–83 weeks after implantation. All cysts increased in diameter; the surface area of the largest had increased more than four times when removed at 83 weeks. In some cases the first measurements of cyst diameter were less than the diameter of the semicircular sac implanted. This can be explained by (1) the initial contraction of skin when released from its usual state of tension; or (2) the inflation of the implanted skin by cyst contents before growth occurred.

Examination of the cyst wall removed at biopsy showed that burying sheepskin had apparently not affected either the structure or the function of wool follicles, sebaceous glands, or sweat glands. Established cysts contained wool fibres and fluid, and volumes up to 12 ml were aspirated. In some cases the syringe plunger was forced back when aspirating the fluid contents. Plate 2, Figure 1, shows sweat glands lined by active epithelium in a cyst wall 83 weeks after implantation.

TABLE 1

TIMES OF SAMPLING AND SIZE OF CYSTS

Measurements of two diameters are given in centimetres. S, biopsy of cyst and control skin; *R*, cyst removed

Breed	Cysts Implanted	Time (weeks) after Implantation										
		5	8	13	18	23	28	42	47	55	78	83
Merino B432	A			S 2·8 by 2·2	3·2 by 2·5		S 3·5 by 2·7		S 5·0 by 3·7			SR 6·0 by 4·7
	B	S		S 2·7 by 2·2	2·8 by 2·2		S 3·2 by 2·2		S 4·2 by 3·0			SR 5·2 by 4·0
Merino B195	A		S 4·0 by 2·4	S 4·4 by 2·5		S 5·5 by 3·2		S 6·5 by 3·7			SR 6·4 by 4·1	
	B			S 4·2 by 2·2		S 4·8 by 2·5		SR 6·5 by 4·0				
	C							S 5·5 by 3·0			SR 5·3 by 4·0	
Merino A445 (sweat glandless mutant)	A			S 2·2 by 2·2	S 3·5 by 2·7					SR 4·0 by 3·0		
Corriedale A459	A	S		S			S 4·0 by 3·2		SR 5·5 by 4·0			

To assess the effect of the cyst environment on the function of wool follicles, the rate of wool growth in the Corriedale cyst was determined by the method of Downes and Lyne (1959) using [^{35}S]cystine. Forty-seven weeks after implantation the rate of growth of the cyst wool, 0·46 mm per day, was similar to that of surface wool.

The distribution of sulphydryl and disulphide bonds corresponded with that of control sections, indicating that normal keratinization probably occurred in the cyst environment (Molyneux, unpublished data).

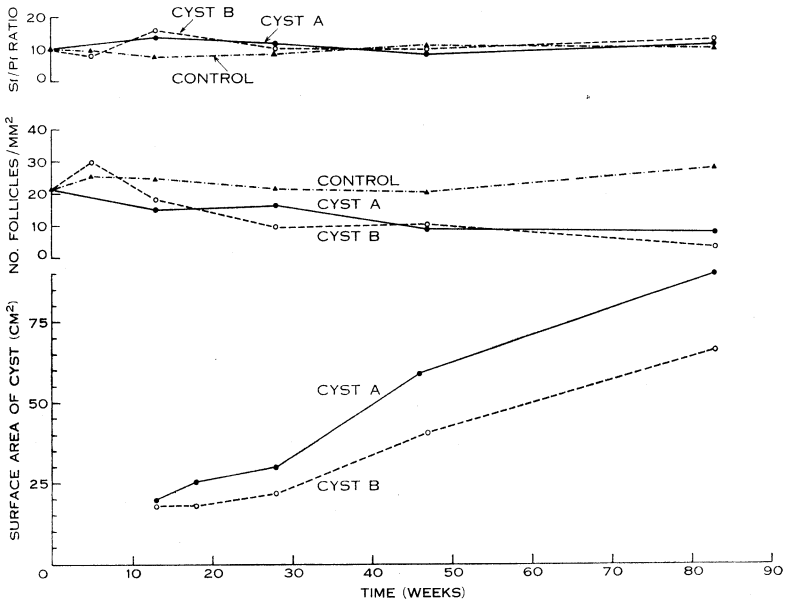

Fig. 1.—Relation between time after implantation, surface area of cysts, number of follicles per mm^2, and secondary/primary fibre (*Sf*/*Pf*) ratio in control and cyst samples in Merino B432.

Subsequent to the implantation and biopsy procedures it was expected that inflammatory changes would be present in the cyst walls. However, inflammatory reaction was slight and was restricted to a round cell infiltration immediately beneath the epidermis. This infiltration gradually disappeared as the time between biopsies was increased.

Up to 83 weeks after implantation no structural alteration in any of the epidermal derivatives of cyst skin had occurred as compared with surface skin. However, the arrangement of the follicle groups had altered considerably. For example, Plate 3, Figure 1, shows a transverse section of surface skin at the mid-sebaceous gland level in which the follicles are arranged in groups as described by Carter (1943). Plate 3, Figure 2, shows a similar section of cyst wall. Follicles are still grouped together but the groups are further apart as a result of an increase in the major trabeculae of connective tissue in which arrector pili muscles can be clearly seen. Within the group there is evidence that the primary follicles tend to become

isolated while the secondary follicles form subgroups as minor trabeculae of connective tissue increase in width. The outstanding features are the increase in connective tissue trabeculae and the absence of inflammation. To demonstrate this increase in connective tissue, diameters of follicle groups and distances between adjacent groups (measured from the centre of each follicle group) were recorded. For example, the average distance between 15 groups in a control sample of surface skin from animal B432 was 1030 μ and the average diameter was 850 μ. In cyst A

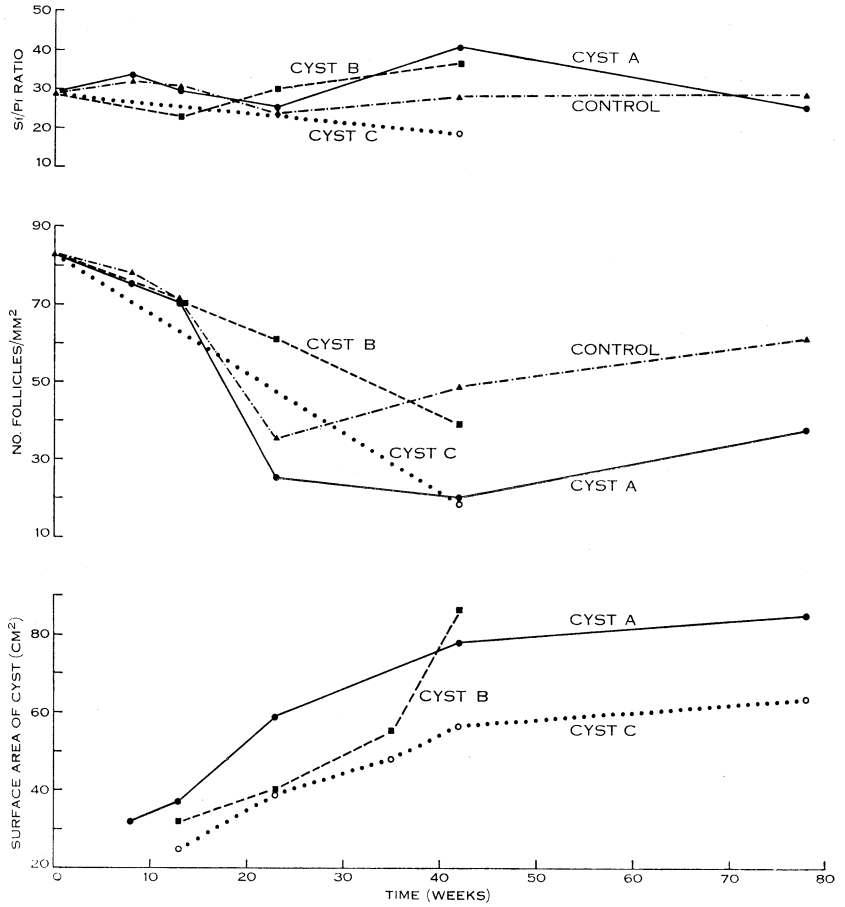

Fig. 2.—Relation between time after implantation, surface area of cysts, number of follicles per mm², secondary/primary fibre (*Sf*/*Pf*) ratio in control and cyst samples in Merino B195.

of the same sheep 83 weeks after implantation, the average distance between four groups was 1480 μ and the average diameter was 1090 μ. Only a few satisfactory measurements could be made on the cyst samples because of their small diameter (0·5 cm), the wide separation of their groups, and the difficulty of unequivocally defining the outlines of follicle groups.

Examination of the cyst samples below the sebaceous gland level showed the presence of bud-like structures originating from the outer root sheath. They occurred

at a level just above the dermal papilla of the follicle bulb and multiple structures were seen to originate from a single follicle (Plate 2, Fig. 2). The larger structures resembled small cysts attached by a stalk to the outer root sheath and having a lumen lined by tissue similar to the cornified layer of epidermis. Similar structures have been described by Burns and Clarkson (1949), Auber and Ryder (1955), and Epstein and Kligman (1956). Examination of biopsy samples showed that these structures were present in all the control samples of all the sheep and that their occurrence was apparently more frequent in the cyst wall. This apparent increase in frequency may depend upon the large number of follicles in the cyst samples which have been cut obliquely or longitudinally, thus enabling the structures to be more readily seen.

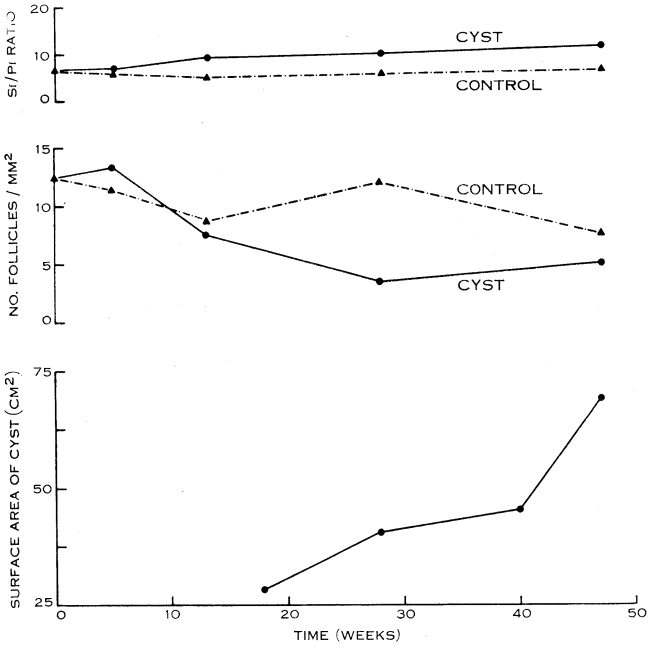

Fig. 3.—Relation between time after implantation, surface area of cyst, number of follicles per mm², and secondary/primary fibre (*Sf*/*Pf*) ratio in control and cyst samples in Corriedale A459.

The number of *P* plus *S* follicles per mm² and *S*/*P* fibre ratios (*Sf*/*Pf*) for each skin sample, together with the changes in surface area of the cysts, are shown in Figures 1–4.

The surface area of all cysts in all animals increased with time. In animals B195 and B432 multiple implantations were made and the growth of the resulting cysts observed for 78 and 83 weeks respectively. Although individual cysts in the same animal did not attain the same size, their growth curves indicated a similar growth pattern (Figs. 1 and 2). The least growth occurred in the Merino mutant without sweat glands. In general, the rate of growth tended to decrease with time.

The density of the follicles in the cyst samples decreased as the age of the cyst increased. The low incidence of shed or abnormal follicles in the cyst skin when compared with control skin indicated that the decrease in density was the result of an increase in surface area, which was dependent upon an increase in connective tissue between follicle groups and an increase in the epidermis. The smallest decrease in follicle density occurred in the Merino mutant without sweat glands. In some cases there was an initial rise in follicle density which was considered to be the result of skin contraction.

Because of the low incidence of shed or abnormal follicles the S/P fibre ratios are equivalent to the S/P follicle ratios. The constancy of the S/P ratios in the cyst and control samples supports the conclusion that the decrease in follicle density

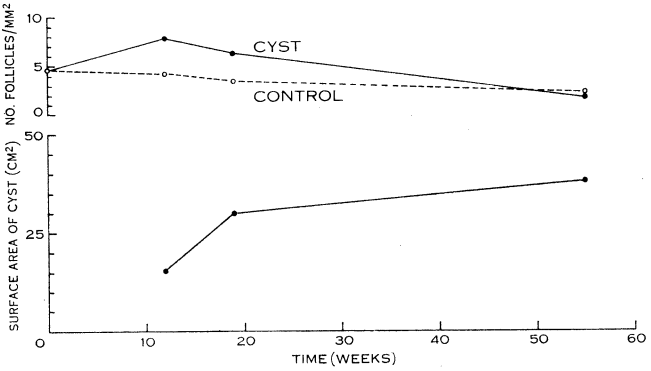

Fig. 4.—Relation between time after implantation, surface area of cyst, and number of follicles per mm² in control and cyst samples of Merino mutant without sweat glands, A445.

was mainly the result of the separation of follicle groups. The apparent increase in the S/P ratio in the Corriedale cyst samples was thought to be due to an inadequate number of follicle groups in such small samples in an animal with such a low follicle density. Because of the absence of sweat glands in animal A445 no attempt was made to identify the primary follicles. Thus no S/P data appear in Figure 4.

IV. Discussion

The sheep has proved to be an ideal experimental animal for the study of cyst growth. Knowledge of the biology of sheepskin has enabled the wool follicles to be used as growth markers to reflect the influences of the environment.

To study growth in an artificially induced biological system, such as an experimental dermal cyst, the system must be reproducible if data are to be interpretable in physiological terms. Although the sheep chosen provided a wide range of skin characteristics, the similar behaviour of all cysts indicates that the implantation technique can be used successfully to form standard epithelium-lined cysts which will follow a predictable growth pattern; this is taken as evidence of the stability of the cyst system. Results showed that increase in surface area of cysts was related to their age, and that the growth rate gradually decreased with time.

As expected, the growth of cysts was accompanied by a decrease in wool follicle density per unit area. This decrease in density is apparently not the result of loss of follicles but rather a separation of follicle groups by growth of interstitial connective tissue. This view is supported by the apparent constancy of the S/P ratio and particularly by the absence of shed or abnormal follicles in the cyst wall as compared to the control skin samples

It was found in the cysts on the sheep without sweat glands that after 55 weeks only slight growth had occurred and that the follicle density had fallen only slightly below that of the control skin. It would thus seem that factors contributing to cyst contents, such as secreting epithelium or inflammatory exudate, are probably important in determining the rate of growth and eventual size of such cysts.

The absence of abnormalities in either wool follicles or their accessory structures, the normal growth rate of intracystic wool, and the similar distribution of sulphydryl and disulphide bonds in the cyst wall to that in surface skin, all indicate that the cyst environment causes no detectable change in the metabolism of the implanted skin. Although the apparent absence of structural or metabolic change suggests that the cyst wall is not subjected to pressure, the forcing back of the syringe plunger when aspirating fluid cyst contents and the spherical shape assumed by cysts indicate the presence of positive intracystic hydrostatic pressures. It is probable that the intracystic pressure is balanced by the active growth of connective tissue and an increase in the local blood pressure within the cyst wall.

The selection of animals of different breeds and with specific genetic characteristics can extend the scope of these artificially induced cysts as a biological tool. For example, the potassium content of cyst fluid from an animal with sweat glands was 121 m-equiv/l. This value agrees with the potassium concentration of sweat collected by other methods and the potassium content of cyst fluid from an animal without sweat glands was 9·2 m-equiv/l, which is similar to the potassium value for extracellular body fluids (Short, Stacy, and Brook, personal communication).

Because cyst skin is similar in structure and metabolism to surface sheepskin and because it would be possible to maintain a high antibiotic concentration in the protected cyst environment over an extended period, these cysts could be used as a source of sterile adult skin and sterile wool. The use of experimental dermal cysts to produce artificially the environment necessary for the development of "pink rot" has already been discussed (Molyneux 1959).

The establishment of these cysts as a reliable biological system suggests their use in studies of skin metabolism and also will enable the study of cyst growth to be continued.

V. Acknowledgments

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Explanation of Plates 1–3

Plate 1

Fig. 1.—Experimental cyst removed from Merino sheep without sweat glands 55 weeks after implantation (×3).

Fig. 2.—Experimental cyst removed from Merino sheep with sweat glands 83 weeks after implantation (×3).

Plate 2

Fig. 1.—Photomicrograph of cyst wall from Merino sheep showing sweat glands with active epithelium 83 weeks after implantation (×160).

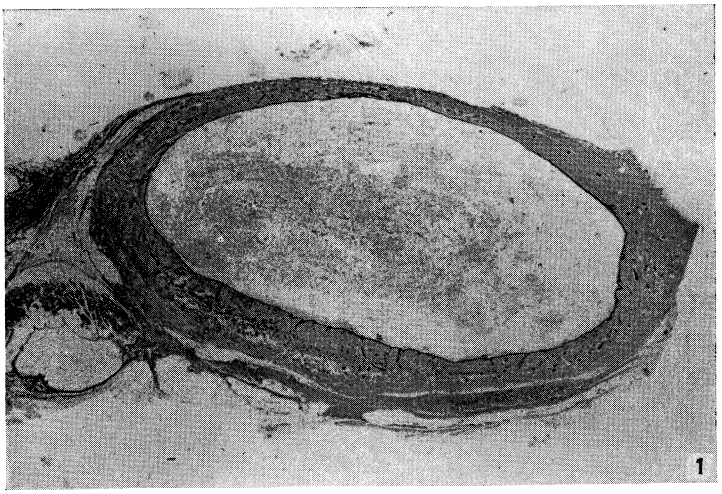
Fig. 2.—Photomicrograph of wool follicle in cyst wall 83 weeks after implantation showing two cyst-like structures arising from the outer root sheath of a single follicle (×180).

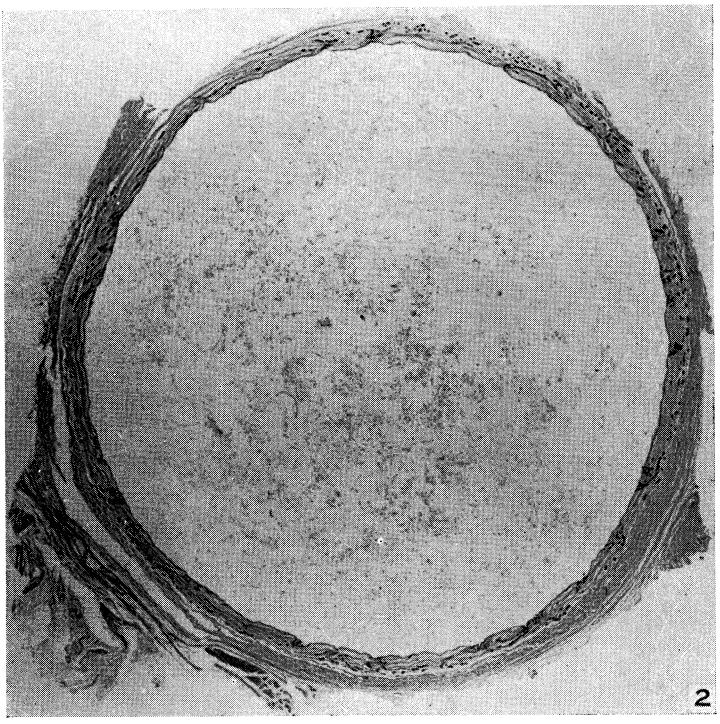
Plate 3

Fig. 1.—Section showing group arrangement of follicles in control skin sample of Merino B432 (×20 approx.).

Fig. 2.—Section showing group arrangement of follicles in cyst skin from Merino B432, 83 weeks after implantation (×20 approx.). *M*, major connective tissue trabecula separating follicle groups; *m*, minor connective tissue trabecula dividing follicle group into subgroups; *P*, primary follicles isolated from follicle group; *A*, arrector pili muscle in major trabecula.

DERMAL CYSTS IN SHEEP





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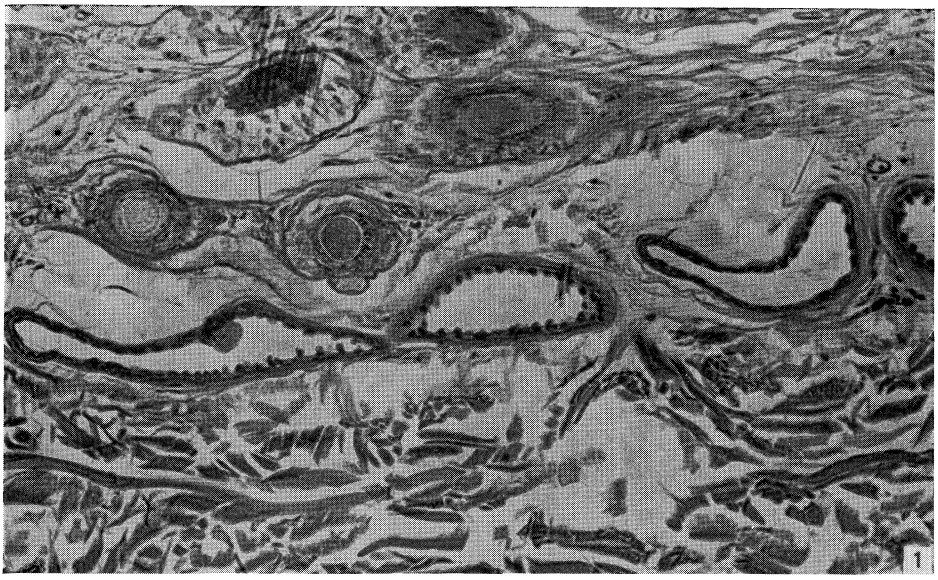

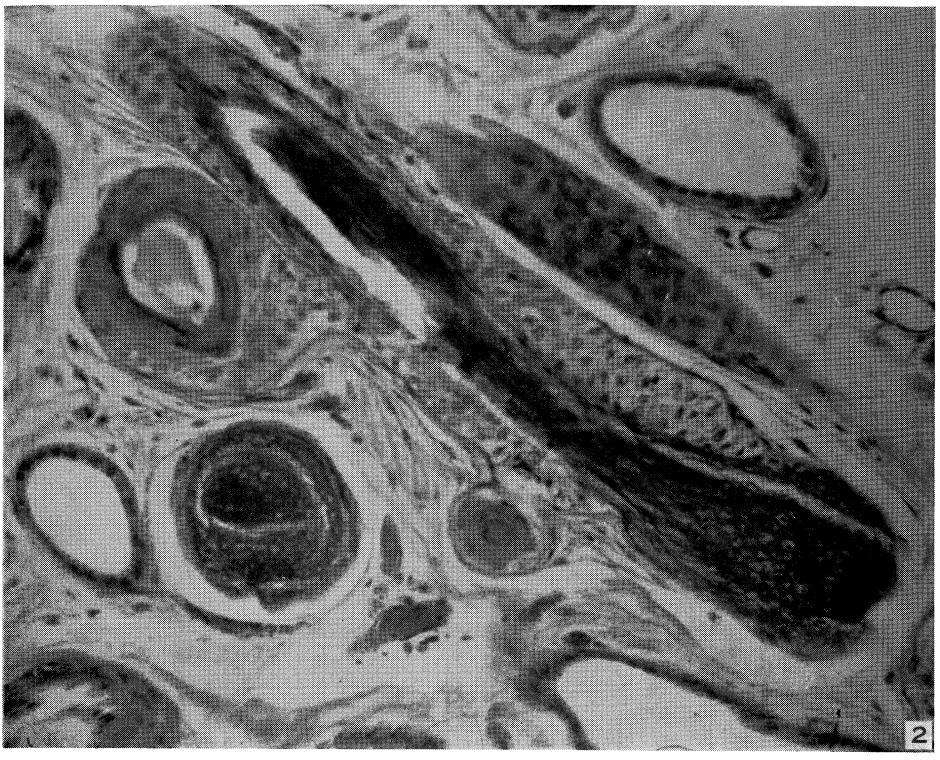

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