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Effects of Grafting the Skin in Merino Sheep, with Special Reference to the Growth of Wool and Hair

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Abstract

Grafts of wool-growing trunk skin (wool grafts) and hair-growing inguinal skin (hair grafts) were studied in two black and two black and white adult Merino sheep. Skin growing black wool was transplanted to skin growing white wool and vice versa. Also, skin in both black and white regions was excised and returned to the same site. Inguinal skin was transplanted to black wool-growing skin.

The areas of the grafts, which initially ranged from about 1 to 25 cm^2, decreased to a minimum by 28 days after grafting. Most grafts then expanded slowly and returned to approximately their original size by the end of the experiment (118–194 days after grafting).

Grafting caused the temporary cessation of wool and hair growth. Old fibres were shed and new fibres, usually similar in colour, appeared above the skin surface about 28 days after grafting. Each animal was given a series of injections of L-[^{35}S]cystine and fibres were sampled from the grafts and control regions for length and diameter measurements. The fibres on most of the grafts showed marked increases in length growth rate and diameter, resulting in large increases in volume growth rates. However, the total amount of wool produced by the grafts was usually reduced because grafting caused a loss of follicles.

At the end of the experiment, skin samples were taken from the grafts and control regions for microscopic examination. The sebaceous glands were retained in the grafts except those associated with follicles that did not regenerate. The sweat glands and erector muscles were often destroyed by grafting, particularly in the wool grafts. Innervation was greatly reduced. Langerhans cells appeared to be as numerous in the grafts as in control samples. Melanocytes spread from black skin into wool and hair grafts of white skin and were most commonly seen in the epidermis, which increased in thickness. Clumps of pigment in the dermis appeared at the periphery of many of the grafts.

Introduction

Although skin grafts in sheep have been studied by various workers (Hardy *et al.* 1952; Priestley 1965; Rudall and Wickham 1965; Priestley *et al.* 1977; Ryder and Priestley 1977, 1979), very little information is available on the effects of grafting on the follicles and wool growth. The present investigation was designed primarily to examine this using the autoradiographic technique of Downes and Lyne (1959, 1961) and Downes *et al.* (1967). This method permits the growth in length of individual fibres to be measured with considerable accuracy, without having to remove the fibres from the sheep until the end of the experiment. Other effects of grafting, such as changes in the numbers of follicles and their associated sweat glands, sebaceous glands and erector muscles, the phenomenon of pigment spread, and innervation, are also described. Knowledge of such changes is of importance since it should throw some light on basic aspects of the skin and the factors responsible for controlling the mechanisms of wool and hair growth.

Materials and Methods

Thirty four full-thickness skin autografts (Table 1) were made in four adult Merino sheep [Nos 4940 (male) and 4952 (female), each black and white (piebald); Nos 9172 and 9173 (both castrated males, black)] using techniques similar to those described by Billingham (1961). The animals were kept indoors in metabolism cages during the experimental period (April–November 1971).

Halothane plus oxygen was used as a general anaesthetic. The recipient sites for the grafts, which were on the right and left sides of the trunk, were closely clipped and cleaned with Zephiran (Winthrop Laboratories, Ermington, N.S.W.). The grafts, which were from the wool-growing trunk region (wool grafts) and from the hair-growing inguinal region (hair grafts), ranged in area from 0·8 to 25 cm^2. The smaller grafts were cut with trephines 1, 2 or 3 cm in diameter, and the larger grafts (initially 12–25 cm^2 in area and rectangular) were cut with a scalpel. The skin was excised and either returned immediately to the same site (after being rotated 180°) or transplanted into another site. Because the recipient sites gaped and the donor skin contracted after excision, some of the grafts fitted well while others were initially separate from the host skin. To study the effects of freezing, six grafts were held in solid CO_2 or liquid nitrogen for 30 s before being returned to the same site. Grafts 12 cm^2 or greater in area were secured with sutures and Michel clips. The graft sites were then covered with Vaseline-impregnated gauze and sprayed with Nobecutane (Allen & Hanburys, Boronia, Vic.). The entire areas were then covered with gauze which was held in position with elastic bandages attached to the skin with Bostik cement (Bostik Australia Pty. Ltd.). The dressings were removed 12–28 days after grafting and the Michel clips and sutures were removed after 15 or 16 days. Subsequently, the grafts were examined, measured, and photographed at intervals up to 194 days.

After grafting, each animal was given a series of seven intravenous injections of tracer amounts of L-[^{35}S]cystine at 14-day intervals. At the time of the first injection (23 days after grafting for sheep 9172 and 9173; 59 and 99 days after grafting for sheep 4940 and 4952), the grafts and adjacent skin were closely clipped, and 5 days after the last injection hair and wool samples were plucked from the centres of some of the grafts, and control regions near the donor site of each graft. Length growth rate and diameter measurements of the wool and hair samples were made by means of autoradiography (Downes and Lyne 1959, 1961; Downes *et al.* 1967).

At the end of the experiment (118 days after grafting for 9172 and 9173; 154 and 194 days for 4940; 194 days for 4952), trephined samples of skin 0·5 cm in diameter were taken from the centres of some of the grafts and rectangular samples were taken from the graft-surrounding skin junctions. Two hair grafts (Table 1—4940, graft 8; 4952, graft 6), including a small region of the surrounding skin, were removed entire. Control samples of skin were taken with a 1·0 cm diameter trephine from sites adjacent to the origin of the grafts on the trunk and inguen.

Skin samples were studied using the histological and histochemical techniques described by Lyne and Hollis (1968*a*). Lipids were coloured with Oil Red O. The distribution of alkaline phosphatase (AP) and acetylcholinesterase (AChE) was investigated in frozen sections 50 μm thick. Paraffin sections, cut vertical to the skin surface and stained with haematoxylin, eosin, and picric acid (HEP), were used for the measurements of mean epidermal thickness by the method of Lyne and Hollis (1968*b*).

Follicles were counted in sections cut parallel to the skin surface at the sebaceous gland level on at least four fields 1 mm^2 in area (Table 2). The number of follicles per unit area was corrected for skin shrinkage as described by Carter and Dowling (1954). All follicles in the samples of inguinal skin (grafts and controls) were counted. The total numbers of follicles grafted were estimated from the areas of skin grafted and the numbers of follicles per 1 mm^2 in the control biopsies. It is known from other studies (Lyne 1961, 1964) that the number of follicles per unit area of undisturbed skin remains more or less constant in adult sheep.

Results

Macroscopic Observations

Thirty of the 34 grafts showed no evidence of rejection when they were first examined after removal of the dressings 12–28 days after grafting. Two of the four

rejected grafts had been held in liquid nitrogen (Table 1). Some of the grafts on one sheep, photographed 188 days after grafting, are shown in Fig. 1.

Table 1. Summary of grafts and changes in their areas in four Merino sheep

Sheep No.	Graft[A]	Origin of graft, graft site and treatment[B]	Area of recipient site at excision (cm^2)	Area of donor skin at excision (cm^2)	Area of graft after 28 days (cm^2)	Area of graft at last measurement (cm^2)	Days after grafting when last measured
4940	1	B—rotated	3·1	3·1	1·8	3·7	194
	2	W to B	7·0	3·1	1·3	3·1	194
	3	B to liquid N_2	3·1	3·1		Rejected	—
	4	W to B	3·1	7·0	2·0	6·8	194
	5	B to W	7·0	3·1	1·3	2·3	194
	6	B to W	3·1	7·0	2·5	5·7	194
	7	W—rotated	3·1	3·1	1·1	2·3	194
	8	Inguen to B	0·8	0·8	0·5	0·6	194
	A	Inguen to B	25·0	25·0	11·6	17·6	154
	C	B to W	25·0	25·0	9·8	21·2	154
	D	B—solid CO_2	3·1	3·1	—	3·6	154
	E	W to B	25·0	25·0	10·0	22·4	154
	F	W to B	6·2	6·2	—	7·0	154
	G	B to W	6·2	6·2	—	—	—
	H	W—solid CO_2	3·1	3·1	1·4	3·6	154
4952	1	W—rotated	3·1	3·1	1·5	3·1	194
	2	W to B	0·8	3·1	0·6	0·9	194
	3	B to W	3·1	7·0	2·4	6·4	194
	4	W to B	7·0	4×0·8[C]		(a) 0·8 (b) 0·9 (c) 1·1 (d) 1·1	194
	5	B to W	0·8	0·8		Rejected	—
	6	Inguen to B	0·8	3·1	0·6	0·6	194
	7	B—liquid N_2	3·1	3·1		Rejected	—
	8	B—rotated	3·1	3·1	1·1	2·5	194
	A	Inguen to B	16·0	16·0	8·3	7·9	59
	C	B to W	16·0	16·0	8·3	6·5	59
	D	B—solid CO_2	3·1	3·1	0·4	0·3	59
	E	W to B	16·0	16·0	6·1	5·4	59
	F	W to B	6·2	6·2	—	1·0	59
	G	B to W	6·2	6·2		Rejected	—
	H	W—solid CO_2	3·1	3·1	0·6	0·6	28
9172	1	B—rotated	12·0	12·0	8·6[D]	10·8	118
	2	Inguen to B	12·0	17·5	11·4[D]	17·5	118
9173	1	B—rotated	12·0	12·0	8·8[D]	8·8	118
	2	Inguen to B	12·0	17·5	11·3[D]	15·9	118

[A] 1–8, grafts on left side of trunk; A–H, grafts on right side of trunk.
[B] W, white wool-growing skin; B, black wool-growing skin.
[C] Four separate grafts transplanted to one site.
[D] Area of graft after 23 days.

The areas of the grafts contracted during the first 28 days after grafting (Table 1). Most of the grafts then slowly increased in area, and at the termination of the experiment several of them had returned to approximately the size that they were at the

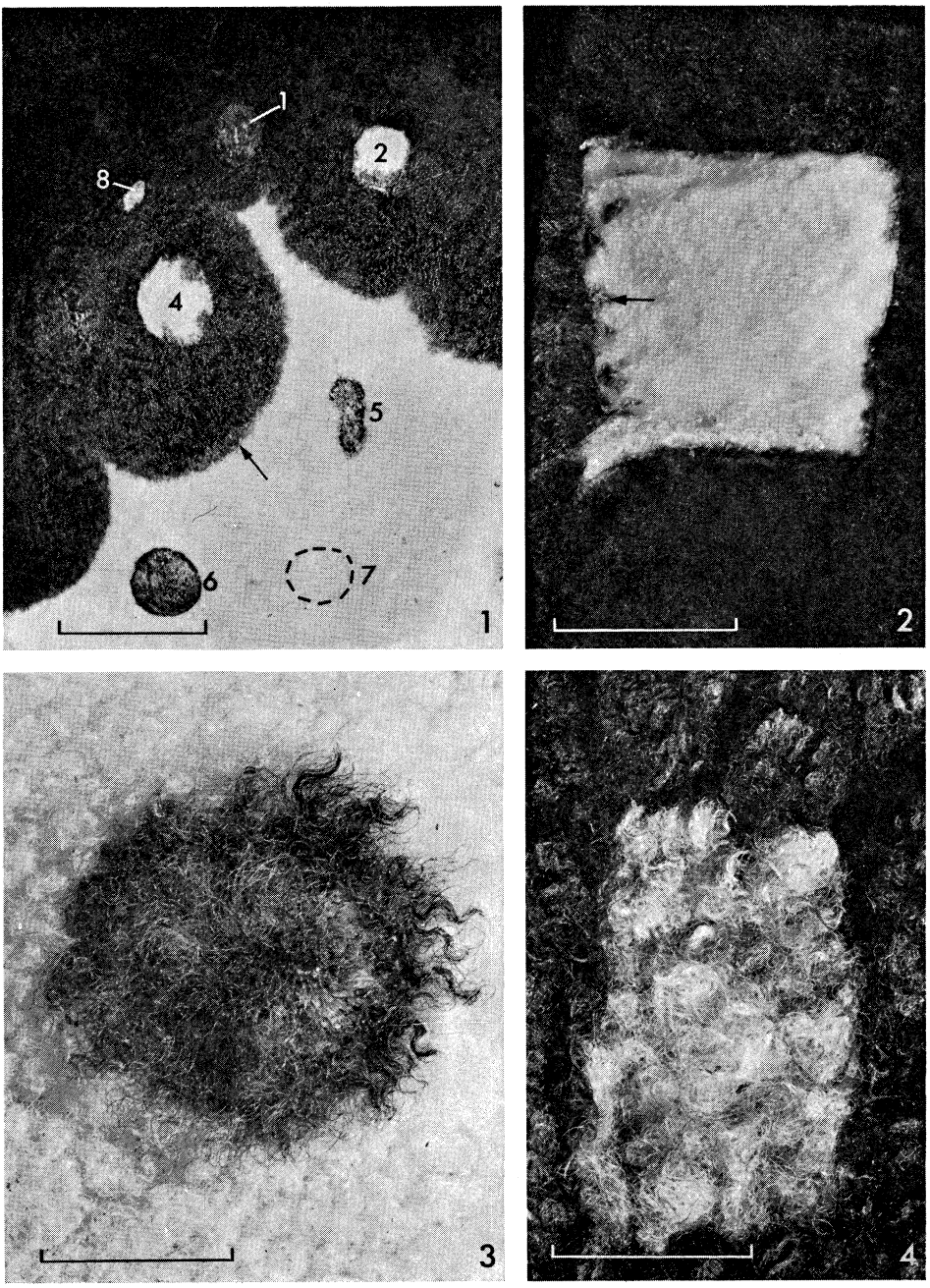


Fig. 1. Left side of sheep No. 4940 showing grafts 1, 2, 4–8 (see Table 1). Photograph taken 188 days after grafting and close clipping of the fibres. Arrow indicates irregular junction of black and white wool-growing regions of control skin. Scale line 5 cm.

Fig. 2. Hair graft A in sheep No. 4940 28 days after grafting. The old hairs have been shed and the new ones have not yet emerged. Note pigment spreading into graft (arrow). Scale line 2 cm.

time of excision. There were no obvious differences in the pattern of area change between grafts of different sizes or between hair and wool grafts.

Grafting caused the temporary cessation of wool and hair growth (Fig. 2). The fibres present in the follicles were shed from most of the grafts by 21 days. This fibre shedding was first observed at 15 days but in several grafts some old fibres were still present at 36 days.

New fibres were first observed above the skin surface at 28 days, usually appearing at the periphery before the centre of the graft. Usually, grafting did not change the pigmentation of the skin or fibres (Table 2). In several of the black wool grafts a considerable reduction of pigmentation was observed in the new fibres (Fig. 4). Fibre regrowth on white wool grafts was mostly white (Fig. 5) but one of the hair grafts (No. 4940, graft 8, Table 2) showed a marked increase in the percentage of pigmented hairs compared with the control.

Table 2. Effect of grafting on the number of follicles per unit area of skin and pigmentation of the fibres

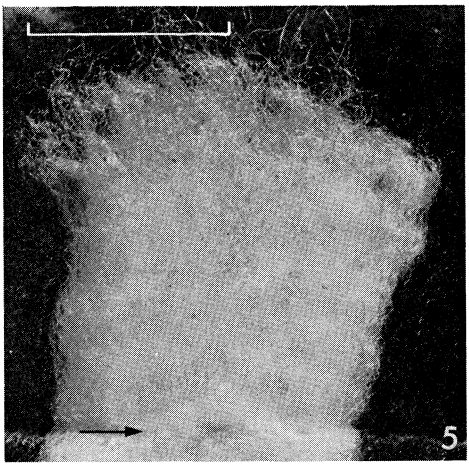
Sheep No.	Graft[A]	Origin of graft, graft site and treatment	Days from grafting to biopsy	Follicles per 1 mm² of skin[B]		Pigmented fibres (%)[C]	
				Control	Graft centre	Control	Graft centre
4940	4	W to B	194	66·7 (8)	23·6 (6)	0 (790)	0 (163)
	6	B to W	194	68·0 (8)	28·3 (6)	92 (731)	88 (215)
	C	B to W	154	68·0 (8)	21·2 (5)	96 (702)	93 (134)
	E	W to B	154	66·1 (8)	10·3 (6)	0 (721)	0 (80)
	8	Inguen to B	194	0·8 (86)	0·8 (78)	5 (73)	55 (58)
	A	Inguen to B	154		1·4 (20)		8 (27)
4952	4(a)	W to B	194	49·8 (7)	35·8 (4)	0 (694)	20 (5)
	4(b)	W to B	194		34·0 (5)		0 (207)
	6	Inguen to B	194	0·4 (156)	1·5 (60)	89 (56)	59 (88)
9172	1	B—rotated	118	33·2 (8)	28·4 (8)	92 (378)	2 (300)
	2	Inguen to B	118	0·3 (78)	0·1 (20)	100 (23)	50 (2)
9173	2	Inguen to B	118	0·9 (78)	0·3 (40)	100 (70)	100 (3)

[A] See Table 1 for details of grafts.
[B] No. of 1-mm² fields examined is given in parentheses.
[C] No. of fibres examined is given in parentheses.

The skin in the white wool-growing regions of two sheep (Nos 4940 and 4952) was almost free of pigment, whereas the black wool-growing skin of all sheep was pigmented. The skin in the inguinal regions of only one sheep (No. 4940) was non-pigmented. However, this animal had a few pigmented fibres (Table 2). Several of the white to black wool and hair transplants showed evidence of pigment spreading

Fig. 3. Wool graft 6 in sheep No. 4940 at 55 days after grafting. The new fibres on the graft are already longer than those on the surrounding skin. Both regions were closely clipped at the time of grafting. Scale line 1 cm.

Fig. 4. Regrowth of mostly non-pigmented fibres on skin which was growing mostly pigmented fibres before grafting. Sheep No. 9172, graft 1. Photograph taken 112 days after grafting. Fibres on the graft and surrounding skin were closely clipped 89 days previously. Scale line 2 cm.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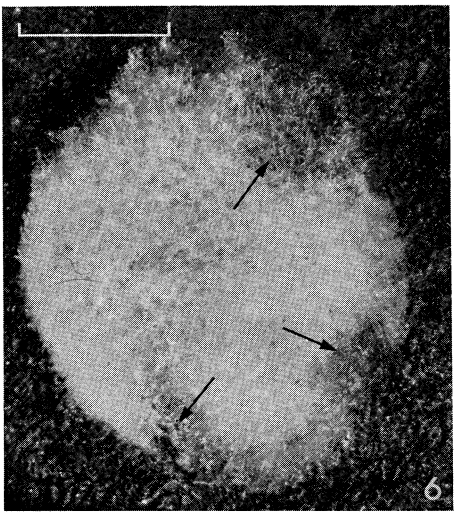
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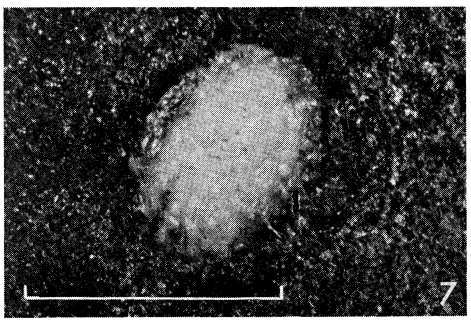
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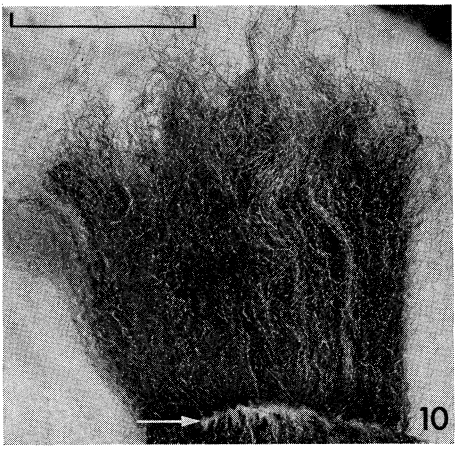
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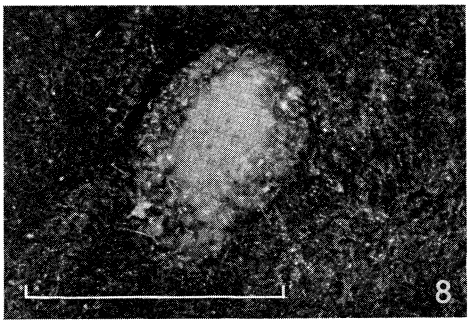
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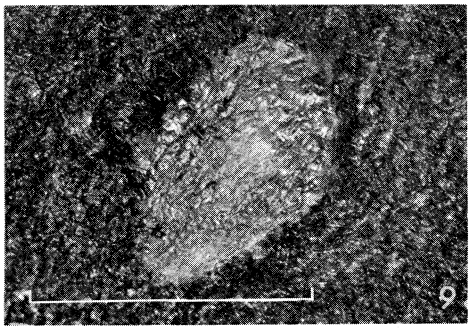
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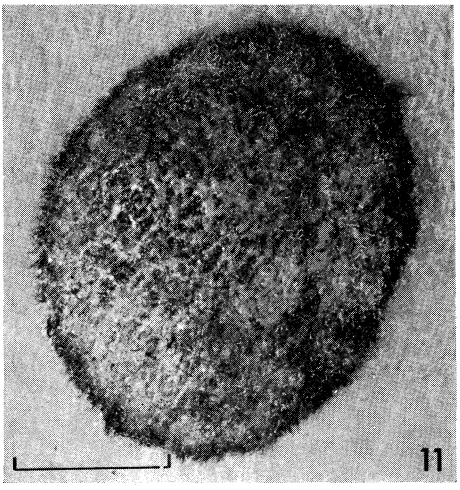
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from the black to the white skin (Figs 6–9). Usually, the spread of pigment was uneven and ceased before it reached the centre of the graft. Pigment spread from black grafts into surrounding white skin (Fig. 11) was not observed. Skin pigmentation and regrowth of wool on the grafts exposed to solid CO_2 did not differ from that found on the grafts that were not frozen.

The staple length of the wool on some of the grafts differed markedly from that on the surrounding skin (Figs 3, 10). Another feature of some of the grafts was the presence of small areas with few or no fibres.

Comparison of Wool Growth on Grafts with Control Regions

Results of the autoradiographic measurements of fibres plucked from the grafts and control regions are shown in Table 3 and Fig. 12. Most of the wool grafts, including those that were merely rotated, showed increases both in length growth rate and diameter of the fibres compared with the control. The increases in the mean length growth rate usually ranged from 10 to 25% and the increases in mean diameter from 15 to 35%. These changes resulted in increases up to 131% in the volume growth rates of the fibres from the grafts (Table 3). The extent of the changes was apparently not related to the treatments.

Examination of fibres (wool and hair) from the grafts of two sheep (Nos 9172 and 9173) revealed that fibre growth had not commenced by 23 days after grafting, the time when these animals were given the first injection of labelled cystine. Because of this, fibre growth for these animals was determined for only five 14-day intervals (Table 3 and Fig. 12). The increases in length growth rate and diameter for most wool grafts were still apparent during the last 14-day growth period measured (Fig. 12). The length growth rates and diameters of the fibres of pigmented and non-pigmented wool grafts and wool grafts of different sizes were similar.

Growth of hair on grafts from the inguen (Table 3 and Fig. 12) could not be compared with hair growth in their controls because most of the follicles in the latter were in a non-growing (telogen) phase of the hair cycle when the cystine injections were given. However, the length growth rates of the hairs produced by the hair

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Fig. 5. Wool parted to show regrowth of non-pigmented fibres similar to those growing on the skin at the time of grafting. Sheep No. 4940, graft 4. Photograph taken 188 days after grafting. The wool on the graft and surrounding skin was closely clipped 89 days previously. Arrow indicates skin surface. Scale line 2 cm.

Fig. 6. Same graft shown in Fig. 5 after close clipping at 188 days after grafting. Arrows indicate regions where pigment has spread into the graft from the surrounding pigmented skin. Scale line 1 cm.

Figs 7–9. Hair graft 8 in sheep No. 4940 showing spread of pigment into graft from the surrounding skin. *7*, 28 days after grafting. *8*, 36 days after grafting. *9*, 68 days after grafting. The fibres on the graft and surrounding skin have been closely clipped. Scale lines 1 cm.

Fig. 10. Wool parted to show regrowth of pigmented fibres similar to those growing on the skin before grafting. Sheep No. 4940, graft 6. Photograph taken 188 days after grafting. The wool on the graft and surrounding skin was closely clipped 89 days previously. Arrow indicates skin surface. Scale line 2 cm.

Fig. 11. Same graft shown in Fig. 10 after close clipping of the fibres at 188 days after grafting. Note that pigment has not spread from the graft into the surrounding non-pigmented skin. Scale line 1 cm.

grafts were much less than those of the wool grafts or the control regions adjacent to the wool grafts.

Numbers of Follicles and Relation to Wool Growth on Grafts and Control Regions

The numbers of follicles per unit area in skin biopsies from some of the grafts and control regions are shown in Table 2. Separate counts of the primary (P) and secondary (S) follicles could not be made for most of the grafts since many of the P follicles had lost their sweat glands and erector muscles, accessory structures which permit their identification. When the P and S follicle types could be distinguished, the S/P follicle ratios in the controls and grafts were similar, suggesting that the S and P follicles were affected to the same extent by grafting. There was no evidence of follicle neogenesis in any of the skin samples examined but it could have occurred earlier during the time of follicle regeneration.

Table 3. Length growth rates, diameters, and percentage increases in volume growth rates of fibres from 22 grafts compared with control fibres

Each wool sample measured contained approximately 30 fibres and each hair sample contained from 7–28 fibres. Values are means ± s.e.m.

Sheep No.	Growth period (days after grafting)	Graft[A]	Origin of graft, graft site and treatment	Length growth rate of fibres (μm/day) Control	Graft	Diam. of fibres (μm) Control	Graft	Increase in volume growth rate (%)
4940	99–183	1	B—rotated	339±2·7	388± 4·3	18·6±0·1	19·5±0·3	25
		2	W to B	359±5·7	399± 2·5	18·4±0·2	21·2±0·3	47
		4	W to B	359±5·7	421± 4·0	18·4±0·2	22·9±0·3	82
		5	B to W	339±2·7	379± 3·7	18·6±0·1	22·6±0·3	61
		6	B to W	339±2·7	425± 8·2	18·6±0·1	22·7±0·4	87
		7	W—rotated	359±5·7	406± 2·5	18·4±0·2	21·9±0·4	60
	59–143	A	Inguen to B	—	210± 7·5	—	27·3±1·3	—
		C	B to W	338±2·3	414±11·2	18·4±0·2	25·0±0·4	131
		D	B—solid CO_2	338±2·3	424± 3·2	18·4±0·2	22·3±0·2	86
		E	W to B	361±3·9	451± 7·9	19·1±0·1	24·1±0·3	100
		F	W to B	361±3·9	429± 2·3	19·1±0·1	23·1±0·4	74
		H	W—solid CO_2	361±3·9	407± 2·6	19·1±0·1	20·0±0·4	22
4952	99–183	2	W to B	339±1·7	352± 4·5	20·0±0·2	19·9±0·2	4
		3	B to W	339±1·7	360± 3·4	20·0±0·2	23·0±0·2	42
		4(b)	W to B	297±9·8	321± 1·7	16·7±0·3	22·1±0·4	88
		8	B—rotated	297±9·8	334± 2·8	16·7±0·3	22·0±0·1	98
	59–143	A	Inguen to B	—	194± 2·3	—	23·5±0·6	—
		C	B to W	319±6·3	321± 1·3	20·2±0·2	22·8±0·2	27
		E	W to B	319±6·3	317± 6·4	20·2±0·2	20·3±0·2	1
9172	37–107	1	B—rotated	337±6·3	339±35·3	19·5±0·4	22·8±1·4	48
		2	Inguen to B	—	189± 7·2	—	22·4±1·4	—
9173	37–107	2	Inguen to B	—	256± 8·1	—	26·8±1·0	—

[A] See Table 1 for details of grafts.

All wool grafts examined showed a reduction in the numbers of follicles per 1 mm^2 (Table 2). The total numbers of follicles in four of the six grafts examined in detail at the end of the experiment (Table 4) were less than the estimated total numbers of follicles in these grafts at the time of grafting. Although there was a reduction in the total numbers of follicles, there was an increase in the volume growth rate of the wool produced by the remaining follicles. In these four grafts the increase in wool

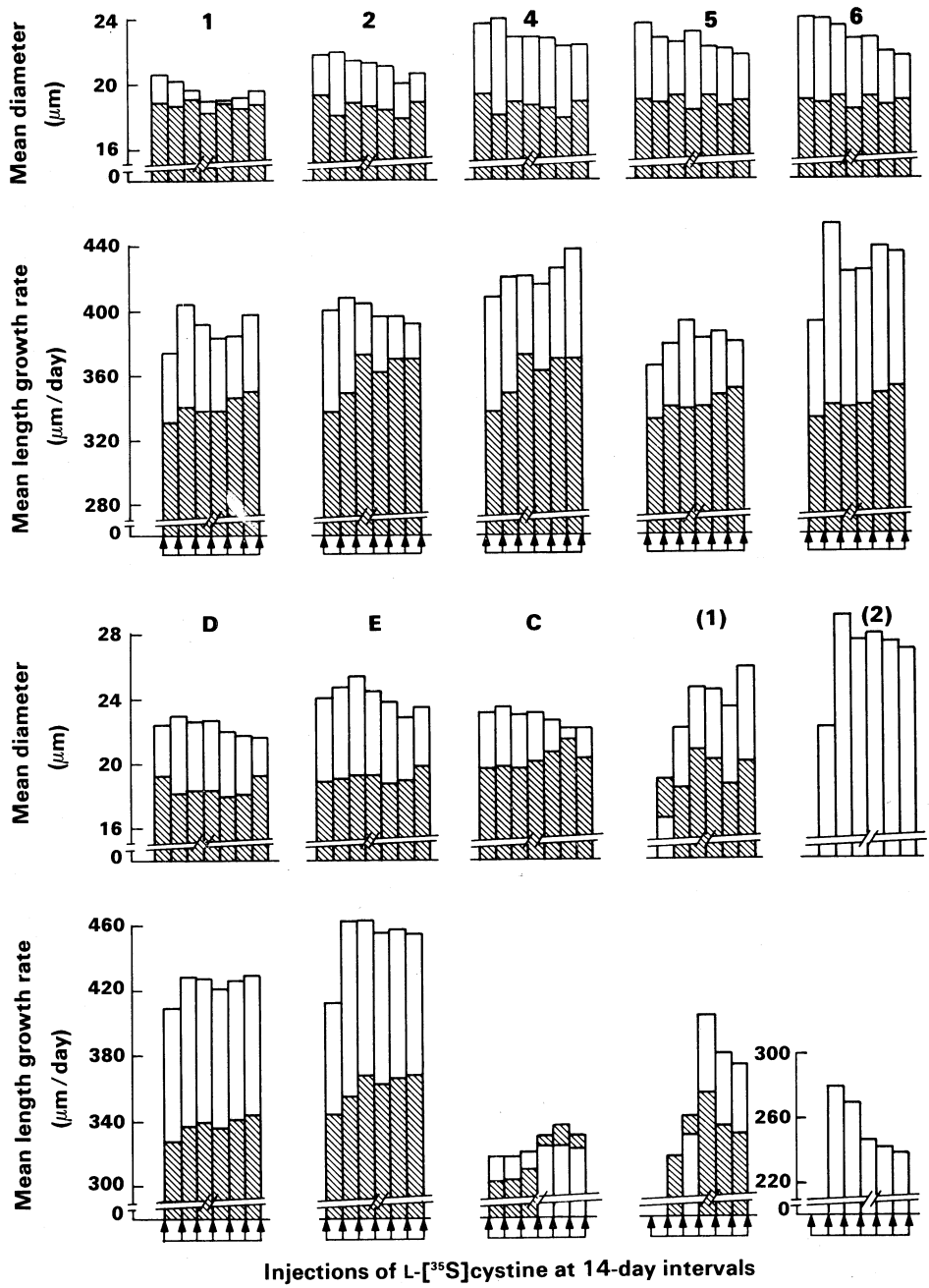


Fig. 12. Comparison of mean length growth rates and mean diameters of fibres from 10 grafts (open histograms) and control regions (shaded histograms) during each 14-day interval of the growth periods measured (see Table 3). Grafts of different sizes and pigmentation were selected to show their response to different treatments. Sheep No. 4940, wool grafts 1, 2, 4, 5, 6, D, E; sheep No. 4952, wool graft C; sheep No. 9172, wool graft (1); sheep No. 9173, hair graft (2). The mean length growth rate of the fibres from the wool graft of sheep No. 9172 between injections 2 and 3 (205 μm/day) is not shown. The growth rate of the hairs in the control region for the hair graft in sheep No. 9173 was not obtained (see text).

production of the remaining follicles (Table 3) did not compensate for the loss of follicles. However, in the other two grafts the net effect was an increase in wool production.

Table 4. Effect of grafting on the total numbers of follicles and wool production

Sheep No.	Graft[A]	Origin of graft, graft site and treatment	Total No. of follicles grafted	Volume growth rate of wool at grafting (mm^3/day)[B]	Total No. of follicles in graft at biopsy	Volume growth rate of wool of graft (mm^3/day)[C]	Change in wool produced by graft (%)
4940	4	W to B	46 700	4·42	16 000	2·76	−38
	6	B to W	47 600	4·40	16 100	2·79	−37
	C	B to W	170 000	15·11	44 900	9·21	−39
	E	W to B	165 000	17·10	23 100	4·77	−72
4952	4(b)	W to B	3980	0·26	3100	0·37	+42
9172	1	B—rotated	39 800	3·99	30 700	4·55	+14

[A] See Table 1 for details of grafts.
[B] Estimated from volume growth rate on control skin.
[C] See Table 3 for growth periods.

Changes in Skin Structure

(i) *Epidermis*

Measurements of epidermal thickness in biopsies taken 118–194 days after grafting are compared with control measurements in Table 5. In all animals, the thickness of

Table 5. Effect of grafting on epidermal thickness

Sheep No.	Graft[A]	Origin of graft, graft site and treatment	Days from grafting to biopsy	Epidermal thickness (μm) Control	Graft	Increase in epidermal thickness on graft (%)
4940	4	W to B	194	13	22	69
	6	B to W	194	15	31	107
	C	B to W	154	15	22	47
	E	W to B	154	13	19	46
	A	Inguen to B	154	19	38	100
4952	4(a)	W to B	194	14	23	64
	4(b)	W to B	194	14	27	93
9172	1	B—rotated	118	15	31	107
	2	Inguen to B	118	18	30	67
9173	2	Inguen to B	118	28	35	25

[A] See Table 1 for details of grafts.

the epidermis was increased by grafting and the percentage increases for wool and hair grafts were similar (Table 5). The Langerhans cells (Figs 13, 14), which are

AChE-positive, in the graft epidermis appeared to be as numerous as they were in the control epidermis. In addition to the Langerhans cells, AChE-positive cells, which appeared to be mainly non-dendritic (Figs 13, 14), were present in the basal layer of the epidermis and in the upper part of the dermis.

(ii) *Follicles*

The grouping and orientation of the follicles were irregular in the grafts when compared with controls (Figs 15, 16). These changes were greatest at the graft edges and in the adjacent skin, where some of the follicles were orientated parallel to the skin surface.

Most of the follicles in the wool grafts were active at the time of biopsy. However, in one of the wool grafts [No. 4952, graft 4a] nearly all the follicles were regressed or partly regressed at the time of biopsy (Figs 17, 18). Macroscopic observations of this graft revealed that it remained almost naked after shedding the old fibres following grafting. The hair canals of the follicles were cyst-like and contained a large amount of keratinized material (Fig. 17). Below some of these enlarged canals, the follicles had transformed into short strands of cells (Fig. 18), many of which did not extend below the level of the few remaining sebaceous glands. Several of the hair grafts contained similar cyst-like hair canals (Fig. 19). Many of the follicles in the hair grafts contained club hairs and were in the telogen phase of the growth cycle, having completed at least one cycle.

The follicles in most of the grafts appeared to be similar in size to those in control biopsies. AP was present in the dermal papillae and the number of follicles showing asymmetric distribution of AP in the follicle matrix region, as described by Lyne and Hollis (1967), was similar in graft and control samples of wool and hair follicles. AP activity in the connective tissue sheath was present in some of the graft follicles but not in the control follicles.

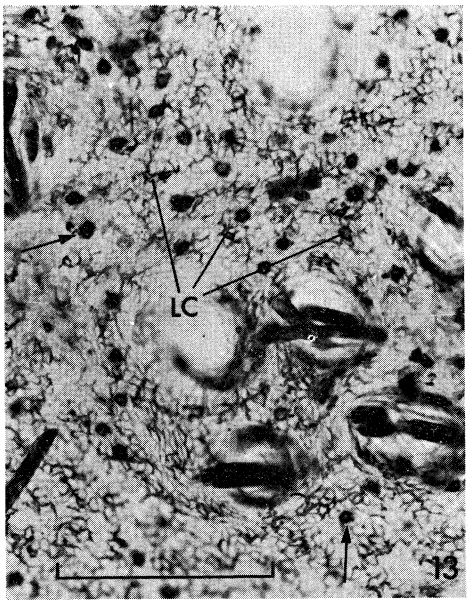
(iii) *Glands*

The regenerated follicles in the grafts retained their sebaceous glands (Fig. 16) with no obvious change in size. All the sebaceous glands in the grafts and control samples were associated with follicles and were strongly AChE-positive (Fig. 22). Some AP activity was present in the mature sebaceous cells of control samples but none was seen in the grafts.

The sweat glands associated with all the P follicles in the control biopsies were less numerous but much larger in the inguen than in the wool-growing trunk skin. Usually, the sweat glands were lost from the wool grafts but retained by the hair grafts (Fig. 20). The retention of sweat glands without associated follicles was rare. AP activity in the myoepithelium of the sweat glands was greater in the inguen than in the wool-growing skin and was unchanged by grafting (Fig. 20). AChE activity, usually confined to the ducts of the sweat glands in the grafts and controls, was more often observed in the hair-growing inguinal skin than in the wool-growing trunk skin.

(iv) *Erector muscles*

Erector muscles, which were strongly AChE-positive (Fig. 21), were lost from nearly all the grafts (Fig. 22). However, these muscles were present at the periphery of some grafts but the original graft-surrounding skin junctions could not be accurately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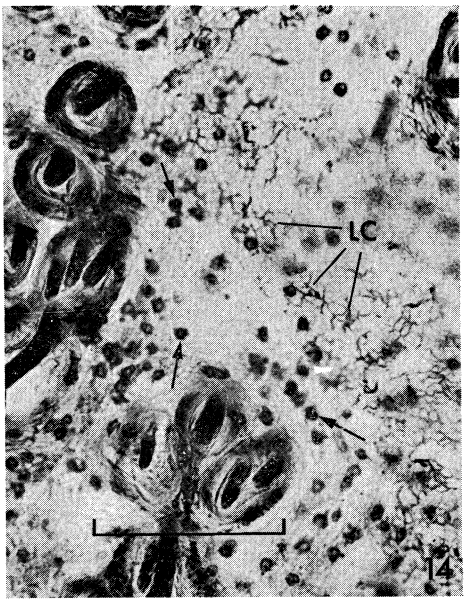
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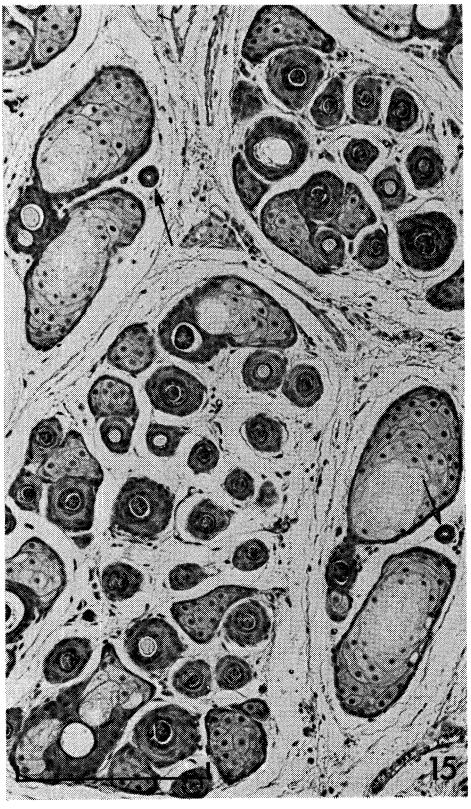
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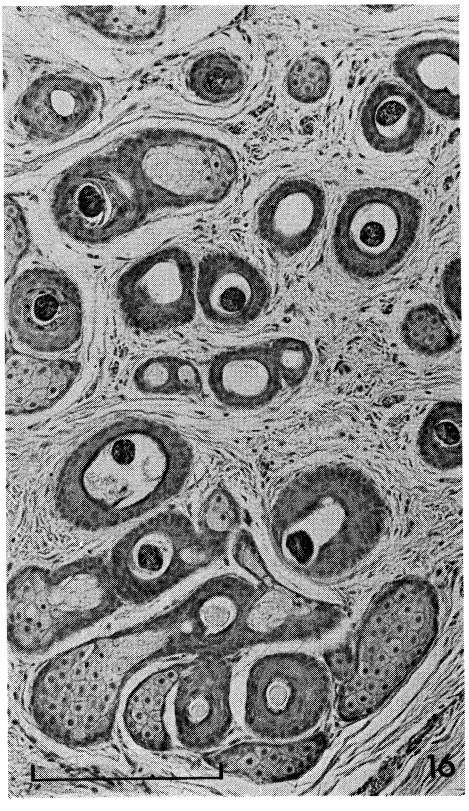
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determined at the time of the biopsies. These indeterminate regions were usually less than 1 mm wide.

(v) *Blood vessels and nerves*

The blood vessels, which were AP-positive, were similar in the grafts and control biopsies. These vessels were most commonly seen close to the follicle bulbs and within the dermal papillae.

In general, there was a marked reduction in the innervation of the grafts compared with the controls, as revealed with AChE. This was particularly evident in the wool grafts. Many of the hair grafts were sparsely innervated (Fig. 22), although a few of the follicles near the periphery possessed nerve networks (Fig. 23) similar to those in control biopsies (Fig. 21). AP-positive nerve networks were only observed around follicles in control biopsies from the inguen.

Networks of AChE-positive nerves were associated with the erector muscles in the control biopsies from the inguen but were not revealed in the hair grafts. Tactile discs and encapsulated sensory end-organs, similar to those described in other sheep (Lyne and Hollis 1968*a*), were extremely sparse in the control biopsies and were not observed in any of the grafts.

(vi) *Pigmentation of epidermis and dermis*

Melanocytes were present in the epidermis of all controls and grafts growing black wool or hair. Some of the white to black wool grafts were completely free of epidermal melanocytes. However, grafts from white wool- and hair-growing regions in one sheep (No. 4940, grafts 2, 4, 8, A), which were almost non-pigmented at the time of grafting into black wool-growing skin, contained epidermal melanocytes when biopsied (Fig. 24). These were more commonly located at the periphery of the grafts and were unevenly distributed. Examination of biopsies taken from the white wool graft shown in Fig. 6 indicated that the dark patches at the periphery contained melanocytes. The centre of the graft was free of epidermal melanocytes.

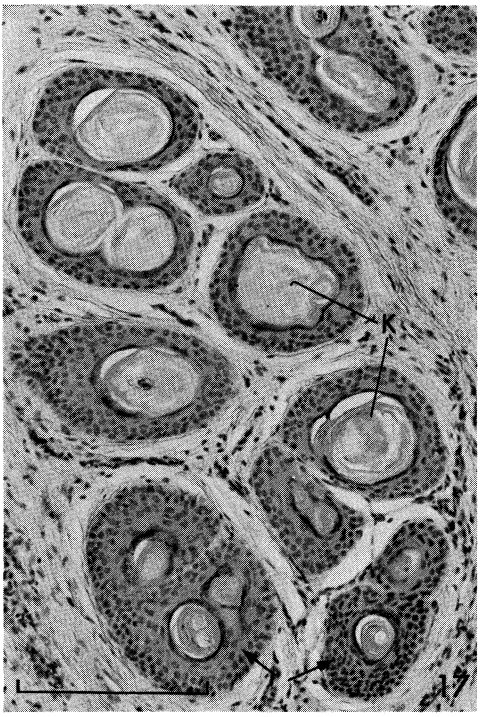
The dermis at the periphery of some of the black to white wool grafts and one of the white hair grafts (No. 4940, graft 8) was pigmented. This pigment was mainly located in the upper part of the dermis and usually in small clumps which were unevenly distributed. Distinct dermal melanocytes were not seen. Some clumps of pigment were also seen in the adjacent surrounding skin. The two hair grafts in sheep 4940 (8 and A) differed in that pigment was present in the dermis of graft 8 but absent in graft A. In the control samples of inguinal skin from two sheep (Nos 4952, 9172) there were no dermal melanocytes whereas grafts from this region contained these cells at the periphery.

Fig. 13. Tangential section of control epidermis in wool-growing region of sheep No. 4940 showing the dendritic AChE-positive Langerhans cells (*LC*). Arrows indicate non-dendritic AChE-positive cells. Scale line 200 μm.

Fig. 14. Tangential section close to that shown in Fig. 13 showing AChE-positive non-dendritic cells (arrows) in upper dermis and Langerhans cells (*LC*) in epidermis. Scale line 200 μm.

Fig. 15. Arrangement of follicles seen in transverse section of control skin growing mostly black fibres. Sheep No. 4940. Arrows indicate sweat gland ducts. HEP. Scale line 200 μm.

Fig. 16. Arrangement of follicles seen in transverse section of skin of graft shown in Figs 10 and 11 194 days after grafting. Sweat gland ducts are absent. HEP. Scale line 200 μm.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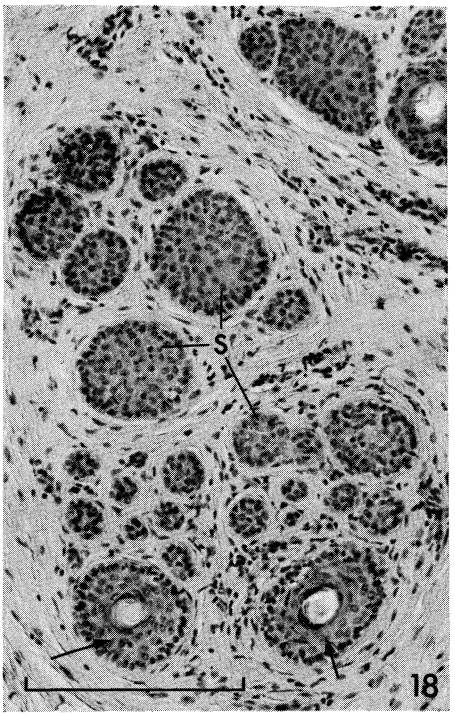
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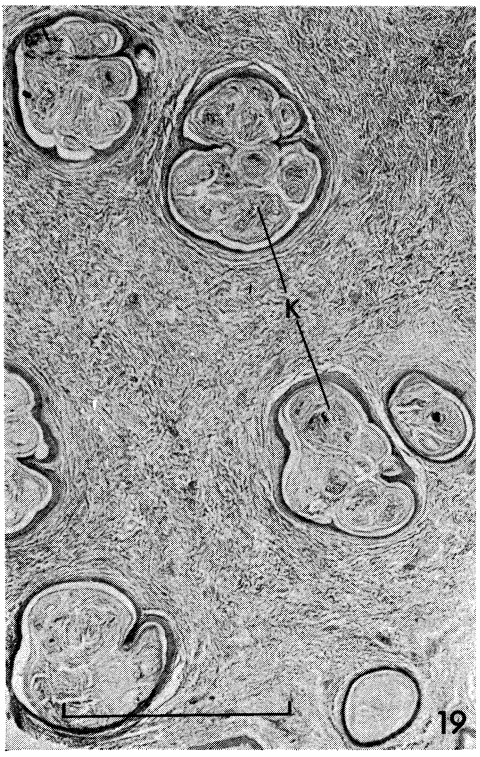
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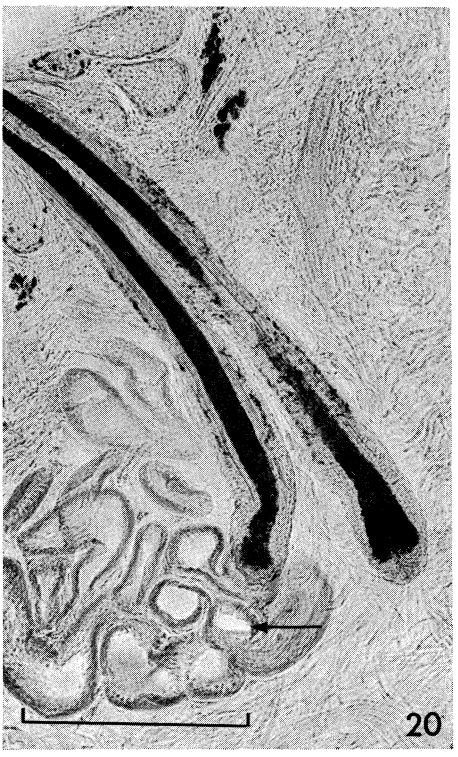
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(vii) *Pigmentation of follicles and glands*

A change in the percentage of pigmented fibres in the grafts compared with the controls (Table 2) was related to the number of follicle bulbs containing melanocytes. These follicles were present at the periphery of some of the white wool grafts within black wool-growing skin. In one graft (No. 4940, graft 4), follicles growing pigmented fibres were up to 5 mm from the edge. In black wool grafts within white wool-growing skin, the surrounding skin remained non-pigmented.

Melanocytes were present in the outer root sheath of all follicles growing pigmented fibres. At the periphery of the black wool grafts there appeared to be a small increase in the number of these melanocytes. An increase in the number of melanocytes within the upper part of the follicles was also observed at the periphery of several of the black wool grafts. Some of the white wool grafts within black skin also contained melanocytes in the upper part of the follicles at the time of biopsy.

Melanocytes were present in the sebaceous glands in all the control samples from regions growing black wool and absent from the sebaceous glands in regions growing white wool. The distribution of these melanocytes remained unchanged in the grafts except in sheep 9172 (graft 1), in which they were lost. Melanocytes were also lost from the sebaceous glands in hair grafts in three out of the four sheep. In the remaining sheep (No. 4940) melanocytes were absent from the sebaceous glands in the control sample from the inguen but present in one of the grafts (graft 8).

Melanocytes associated with the sweat glands were present in the control sample of inguinal skin from only one sheep (No. 9173) and they were retained in the graft (graft 2). In the black wool-growing skin of two sheep (Nos 4940 and 9172), melanocytes were present in the wall of the upper part of the sweat gland ducts in control samples but absent in the grafts.

Discussion

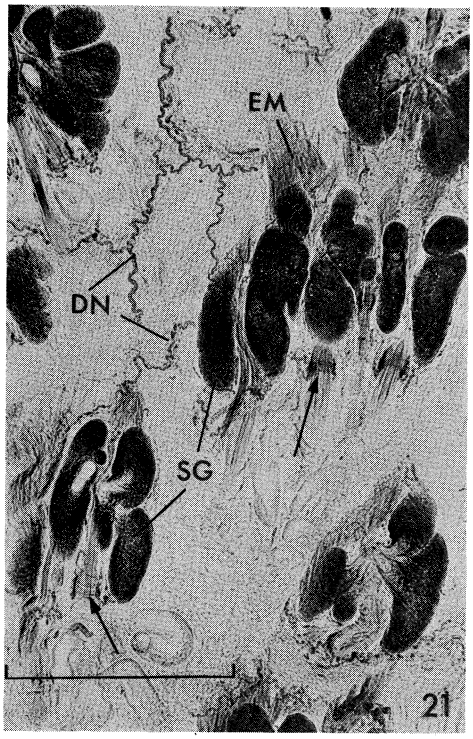
The present study has demonstrated that hair- and wool-growing skin up to 25 cm^2 in area can be successfully autografted in Merino sheep. Other studies on skin grafting in sheep appear to have been confined to smaller areas of skin. Initially, all the grafts contracted and then most of them expanded slowly. The skin grafts of adult Wiltshire and Soay sheep studied by Ryder and Priestley (1977), which ranged in area from 4 to 7·5 cm^2, only contracted to different extents in different individuals, whereas their skin grafts in Soay lambs (8·25 cm^2 in area) increased in area as the animals grew. Similarly, Rudall and Wickham (1965) found that skin removed from Romney and Kerry sheep foetuses (69 to 109 days after conception) and returned to the same animals after birth, expanded rapidly.

Fig. 17. Transverse section of the upper parts of two follicles with fibres (arrows) and a number of follicles without fibres in wool graft (graft 4a) in sheep No. 4952. A large amount of keratinized material (*K*) is present in the hair canals of the follicles without fibres. HEP. Scale line 200 μm.

Fig. 18. Transverse section of the same group of follicles shown in Fig. 17 at a lower level. Arrows indicate the two follicles with fibres. The other follicles appear as cross sections of cords of epithelial cells (*S*) without associated dermal papilla cells. HEP. Scale line 200 μm.

Fig. 19. Transverse section of greatly regressed follicles in hair graft (graft 2) in sheep No. 9173 118 days after grafting. *K*, keratinized material in hair canal. HEP. Scale line 1 mm.

Fig. 20. Longitudinal section of two active hair follicles and large sweat gland (arrow) in hair graft shown in Fig. 19. AP. Scale line 400 μm.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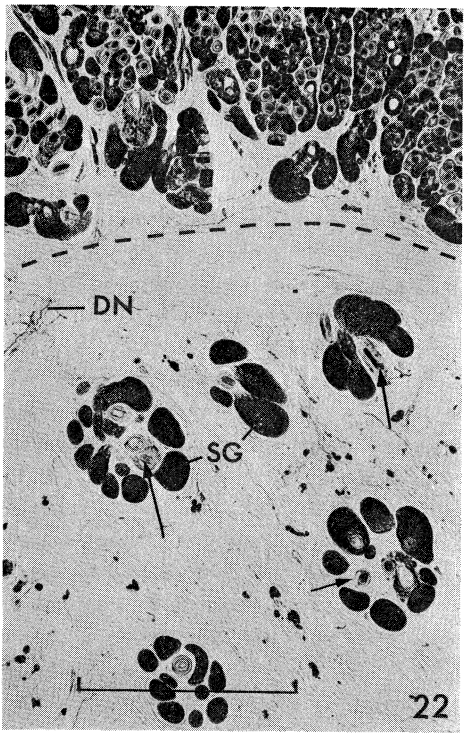
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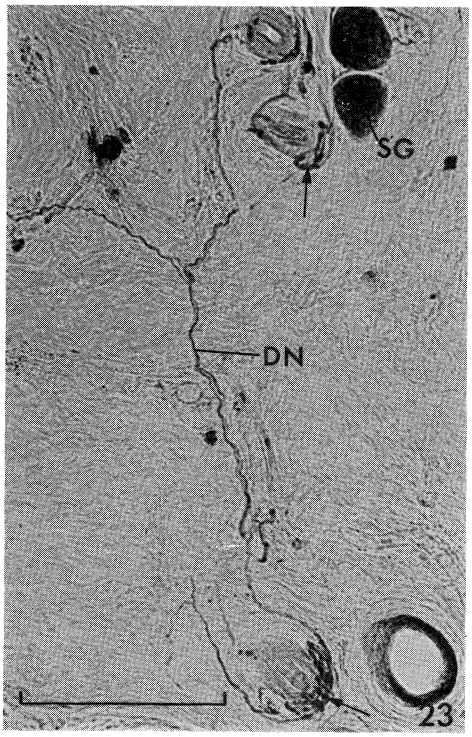
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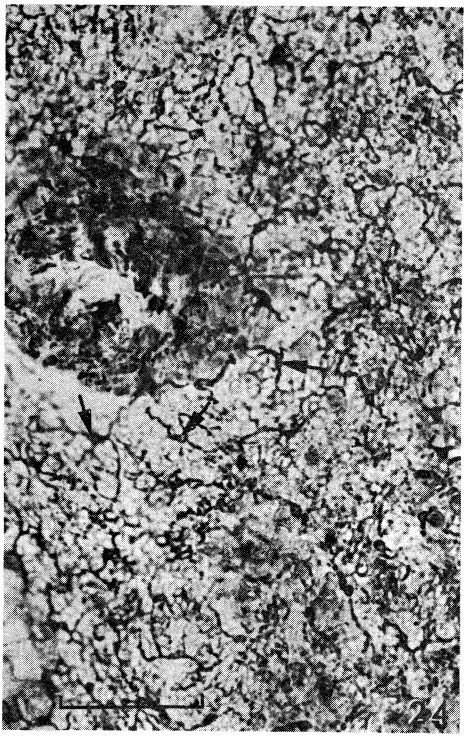
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Most of the follicles in the grafts of wool-growing skin were in the anagen (active) phase at the time of grafting whereas many of the follicles in the grafts of hair-growing skin were in the telogen (inactive) phase. The original fibres in the grafts were shed whether or not the follicles were in anagen or telogen at the time of grafting. Sanford *et al.* (1965), in their study of the effects of grafting during various stages of the hair growth cycle in mice, found that the later in anagen the grafting was done, the less likely the follicles were to return to hair production later. In the present study, there was a reduction in the number of follicles in all the wool grafts whereas only two out of five hair grafts examined showed evidence of a considerable loss of follicles. The reduction in the number of follicles in the grafts probably occurred at the time of follicle regeneration. Priestley (1965) studied the effect of interchanging grafts of wool-bearing skin from the midside with grafts of hair-growing skin from the nose, ear, axilla and breech in crossbred and Kerry Hill sheep and said that the survival of the follicles was good. In the grafting experiments of Ryder and Priestley (1977) about 25% of the secondary follicles were lost. They also said that some loss of primary follicles may have occurred but counts of the number of follicles per unit area were not included. Schinckel and Ferguson (1953) claimed that all the follicles already present in the skin of Merino and Border Leicester–Merino sheep foetuses (80–117 days of gestation) at the time of grafting regressed, and that a new population was formed. This is in contrast to the conclusion of Rudall and Wickham (1965) that some of the follicles, present in an immature state at the time of grafting foetal skin on to the same animals after birth, did not degenerate. No evidence of follicle neogenesis, similar to that described by Ryder and Priestley (1977) and Priestley *et al.* (1977), was found in the present study.

Although the use of autoradiography enables the length growth rates and diameters of fibres to be measured with considerable accuracy (Downes and Lyne 1961; Lyne *et al.* 1974), it does not appear to have been used previously in grafting studies. In the present study, the fibres in many of the wool grafts showed a marked increase in length growth rate and diameter from 37 to 183 days after grafting. This resulted in a considerable increase in the fibre volume growth rate per follicle. In four out of six grafts (Table 4) the increase in volume growth rate was offset by loss of follicles so that total wool production decreased. In the other two grafts, a net increase in wool production was observed. Priestley (1965) found no evidence of any increase

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Fig. 21. Slightly oblique section of a number of hair follicles in control skin sample from the inguen of sheep No. 4940. Arrows indicate follicle nerve networks. *DN*, dermal nerves; *EM*, erector muscle; *SG*, sebaceous glands; AChE. Scale line 1 mm.

Fig. 22. Transverse section of follicles (arrows) near edge of hair graft shown in Figs 7–9 at 194 days after grafting. The wool follicles which surround the graft are shown above the broken line. Note the absence of erector muscles in the graft. The sebaceous glands (*SG*) are strongly reactive for AChE. *DN*, nerves in dermis. Scale line 1 mm.

Fig. 23. Transverse section of follicles near edge of hair graft shown in Figs 7–9 at 194 days after grafting. Arrows indicate follicle nerve networks. *DN*, dermal nerve. *SG*, sebaceous gland. AChE. Scale line 300 μm.

Fig. 24. Tangential section of the epidermis of the hair graft shown in Figs 7–9 at 194 days after grafting. Dendritic melanocytes (arrows) are common in the basal layer of the epidermis. Langerhans cells are present but not clearly revealed. AChE. Scale line 100 μm.

in the length growth rate of the fibres on the grafts but there was an increase in diameter on wool grafts in hair-growing regions. Unfortunately, Priestley does not indicate the period after grafting when these measurements were made. Ryder and Priestley (1977) measured the growth of wool on their skin grafts but these measurements are not comparable to those presented here since their sheep had seasonal cycles of wool growth.

The blood supply to the grafts was not measured but the vascular network, as revealed by the AP technique, appeared to be similar in the grafts and control regions. In all grafts there was a great reduction in their innervation, which may have allowed a greater blood flow. Perhaps the increased wool production per follicle was partly due to the greater availability of nutrients following the destruction of many of the cutaneous nerves. Ferguson (1949) obtained a mean increase of 36% in wool growth rate for 10 weeks following unilateral sympathectomy and suggested that it was due to vasodilation of the denervated vessels. Slen (1958) suggested that mechanical trauma (such as that caused when a sheep is bumped), with its concomitant increase in blood supply to the site of injury, stimulated the growth of wool. Nerve networks associated with primary follicles in the control samples were rare in the grafts. Further studies are needed in order to determine whether the few nerve networks observed in grafts were those associated with the follicles at the time of grafting or new networks formed after grafting.

The colour of the new wool or hair which grew on the grafts was usually the same as that which grew prior to grafting. There were, however, some exceptions. For example, one of the large wool grafts changed from growing mostly black fibres before grafting to mostly white fibres after grafting. Butcher (1945) found that when skin producing black hair was interchanged with skin producing white hair in piebald rats, white hair frequently grew on the skin which formerly had black hair. He also found that if skin producing black hair was excised and sutured back to the same site it often produced white hair. Ryder and Priestley (1979) described the loss of pigment and repigmentation of the epidermis and fibres after freezing grafts of Soay sheep skin for 30 min. Similar loss of pigment and repigmentation of the epidermis, but not the fibres, was described by Lyne and Hollis (1968*b*) following freezing the pinnae of Suffolk sheep for 4 min. Repigmentation of the few grafts that lost pigment was not observed in the present experiments. However, melanocytes spread from pigmented surrounding skin into non-pigmented graft skin but not from pigmented grafts to non-pigmented surrounding skin, as described by Hardy *et al.* (1952) in a Merino lamb. The spread of pigment was mainly confined to the epidermis, and usually ceased before covering the entire graft. Pigment appearing in the dermis of grafts which were originally non-pigmented was mainly peripheral, suggesting that it originated from the surrounding follicles that were damaged at the time of grafting. The growth of pigmented fibres in place of non-pigmented fibres growing in the skin before grafting was uncommon. Ryder and Priestley (1979) found that the spread of pigment into the wool seldom appeared until over a year after grafting. Further studies are needed to determine the origin of the melanocytes that were responsible for this pigmentation change.

The increase in epidermal thickness following grafting was similar to that described by Ryder and Priestley (1977) in sheep at 2 and 4 months after grafting. However, they reported that the epidermis was of normal thickness 1 year after grafting. The

increases in epidermal thickness reported here were similar to those obtained following freezing the skin of sheep (Lyne and Hollis 1968*b*).

The sebaceous glands were retained but the sweat glands and erector muscles were often lost from the grafts as they were following freezing (Lyne and Hollis 1968*b*). Priestley (1965) and Ryder and Priestley (1977) also reported that sweat glands and erector muscles were often lost from grafts of wool- and hair-growing skin in sheep.

Further experiments are needed in order to determine the long-term effects of grafting on the structure and function of the skin and its appendages.

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