

EFFECT OF SUBDERMAL PRESSURE ON THE SKIN AND ITS APPENDAGES IN THE SHEEP

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Summary

A method was devised by means of which pressure in an outward direction could be exerted on the undersurface of the skin. In each of two sheep (one black, one white) a Perspex disk was inserted subdermally and connected by means of a pin projecting through the skin to a spring supported on a light tower on the animal's back. The device was well tolerated and a pedicle of skin was formed during a period of some months. Macroscopically, there was no change in the skin or wool which showed normal growth, colour, and crimping. No obvious shedding of the fibres occurred.

Microscopically, there was a change in fibre crimp, particularly in the time taken to form each crimp. There was a decrease of approximately 50% in the density of the follicle population over the disks but no change in the ratio of secondary to primary follicles. There was no change in length growth rate of fibres over the disks but there was a small increase in the fibre diameters.

The epidermis over the disks increased in thickness and a distinct and continuous granular layer formed. Pigmentation of the epidermis was unchanged except near the pin in the black animal, where both melanocytes and melanin were increased. Within the dermis of the black animal, there was an obvious increase in the concentration of melanocytes and melanin adjacent to the pin, both of which were probably derived from degeneration of follicles. Elsewhere over the disk, the follicle outer root sheaths showed exaggerated tongue-like projections in the mid-region below the level of the sebaceous glands. These projections contained melanocytes and melanin-containing cells, some of which appeared to be separating and migrating from the outer root sheath.

The arrangement of the follicles over the disks appeared normal, as did the sebaceous glands. However, both dermal and follicle nerve networks were reduced. In both animals, sweat gland ducts appeared normal but, in the white animal, the secretory parts of the glands were reduced in size and number.

Small, localized areas of chronic inflammation were seen consistently around the upper part of the primary follicles and their sweat gland ducts above the level of the sebaceous glands.

I. INTRODUCTION

Little is known of the effects of subdermal pressure on the skin. This investigation was designed to observe any changes that might be produced in the skin and its appendages in the sheep by subdermal pressure. It was hoped also to determine whether outward pressure on the wool follicles would influence the crimping of the wool fibres, since Chapman (1965) suggested that movement of the follicles was

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necessary for the production of crimped wool. It could be reasoned that subdermal pressure on the follicles might interfere with any such movement.

The possibility of producing a skin pedicle by using a subdermal device to elevate an area of skin was also explored. A method of producing additional skin in a particular area in the form of a pedicle could be of value for experimental purposes. Such a method could also prove useful in human reconstructive surgery.

II. MATERIAL AND METHODS

A device was made to exert outward pressure beneath a small area of skin on a sheep's back. It consisted of two components: the first comprised a Perspex disk in the form of a shallow cone 29 mm in diameter (Fig. 1) to be inserted subdermally and a stainless steel pin that would be inserted through the overlying skin and screwed into the disk; the second component comprised a stainless steel wire tower with a central spring suspended from the top which could be attached to the pin projecting through the skin (Fig. 2). The adjustable screw in the centre of the tower made it possible to vary the tension exerted by the spring on the subdermal disk. In order to increase the versatility of the device, several springs were made of different lengths and different strengths.

Part of the sheep's back was closely clipped and the skin prepared for surgery. Under local anaesthesia, an anteroposterior incision was made through the skin just behind the midback position and approximately 10 cm lateral to the midline. Through this incision, the skin was separated by blunt dissection from the underlying muscle layer and the disk was inserted to a mid-dorsal position. The incision was closed and a small hole was made through the skin over the centre of the disk and the stainless steel pin inserted and screwed into place.

The wire tower was centred over the pin and held in position with sutures. After 7 days, when the incision had healed, the adjustable central spring was attached to the projecting pin (Fig. 2) and the device adjusted to give slight tension on the underlying disk. The area was observed every few days and, as the disk and overlying skin became elevated above the surrounding skin level, the central spring was taken up to maintain the subdermal pressure and thus promote the formation of a pedicle. With different springs and adjustments, it was found that the pull exerted on the disk ranged approximately from 170 to 400 g (i.e. equivalent to a pull of 26–61 g/cm² on the disk). In order to determine the height of the pedicle throughout the experimental period, measurements were made from a known point on the tower to the top of the pin projecting through the skin.

In the first experiment, an adult white Merino ewe was used; the entire procedure was repeated in a second experiment with minor variations using an adult "black" Merino ewe. In the latter, the spring was attached on the twelfth day instead of the seventh day and the pedicle was not raised as high as in the first experiment for reasons which are given in Section III. On completion of the second experiment, the disk and pin were placed in a new midback position in the black animal in order to determine what effects the disk and pin alone had on the overlying skin and its appendages, and thus serve as a control.

At intervals in each experiment, wool samples were plucked from different small areas of the skin over the disk and from nearby normal areas outside the base of the tower. These samples were used for measurement of fibre length growth rate and fibre diameter. During the period in which the control disk was in the black animal, intravenous injections of tracer amounts of L-[³⁵S]cystine at 4-day intervals were administered to check by autoradiography (Downes, Clarke, and Dagg 1967) the length growth rates and fibre diameters of wool samples taken from over the control disk and from normal areas. In all experiments, tracings were made of wool fibres at a magnification of $\times 10$ for evaluation of crimp form. At the termination of each experiment, the entire pedicle and adjacent skin were removed for microscopic examination.

For general histological studies, samples of skin from the pedicle and adjacent areas were fixed in 10% neutral buffered formalin. Serial sections (8 μ thick) were cut both perpendicular to the skin surface (parallel to the long axes of the follicles) and parallel to the skin surface (transverse sections). The sections were stained with light haematoxylin (H), or haematoxylin, eosin, and picric acid (HEP). The innervation of the skin was demonstrated using the silver impregnation method of Winkelmann and Schmit (1957).

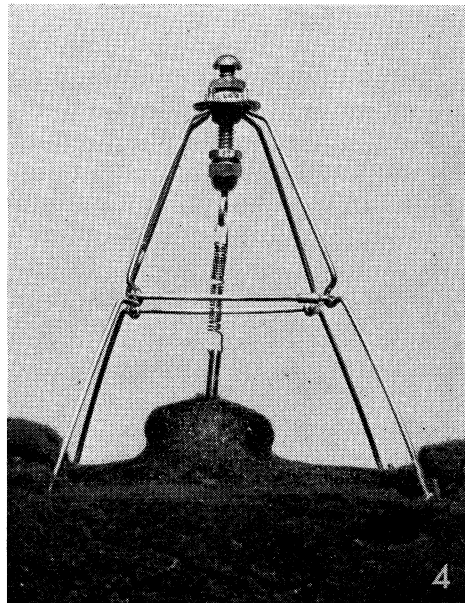
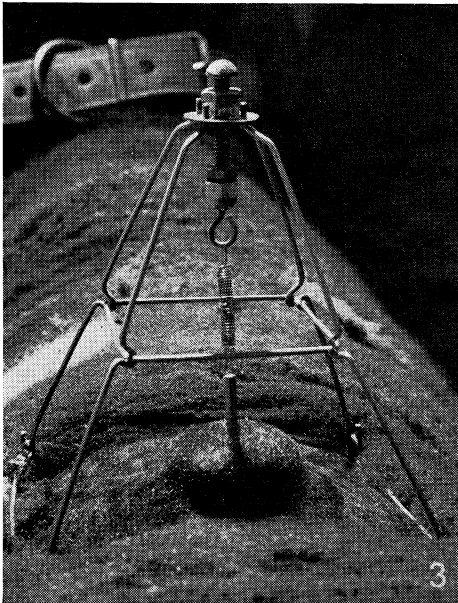
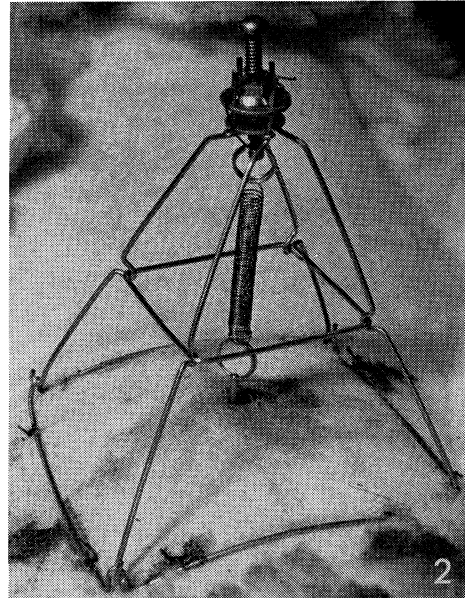
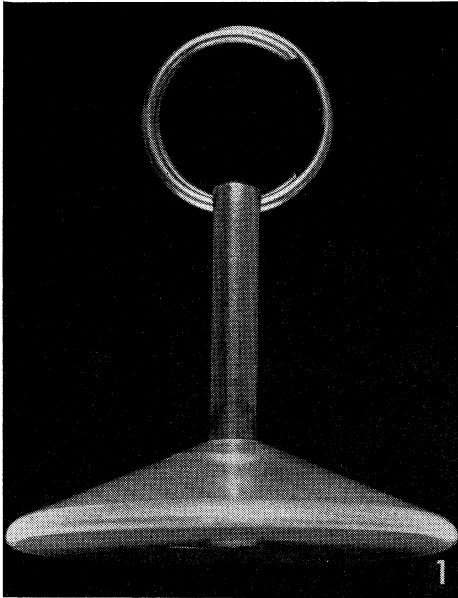


Fig. 1.—Perspex disk in the form of a shallow cone (diameter 29 mm). A stainless steel pin with ring is screwed into the centre of the disk.

Fig. 2.—Midback of white sheep showing the wire tower with adjustable central spring, immediately after its connection to the pin which in turn is attached to the disk (Fig. 1) beneath the skin.

Fig. 3.—Posteroanterior view of midback of black sheep showing the pedicle of skin formed, 35 days after connection of the spring.

Fig. 4.—Lateral view of skin pedicle illustrated in Figure 3 showing the "waisting" on the anterior and posterior sides.

The distributions of alkaline phosphatase and acetylcholinesterase were investigated in frozen sections ($50\ \mu$ thick) of other samples from the pedicle and adjacent skin fixed in 10% neutral formalin using, respectively, the methods of Gomori (1952) and Montagna and Ellis (1957). Lipids were coloured with Oil Red O.

The follicles were counted (using a microprojector at magnification $\times 215$) in transverse sections at the mid-sebaceous-gland level of the primary follicles. Most of the counts were made on at least six fields of $1\ \text{mm}^2$ on HEP-stained sections. The follicle density counts were corrected for skin shrinkage as described by Carter and Clarke (1957).

The follicle terminology is that used by Hardy and Lyne (1956). There are two main types of follicles called primary and secondary. Primary follicles are identified by having an apocrine sweat gland, an arrector muscle, and a sebaceous gland, while secondary follicles have only sebaceous glands.

III. RESULTS

(a) *Development of the Skin Pedicle*

In the early stages, the subdermal pressure produced a hump in the skin. As the hump increased in height, a pedicle was formed which took on a characteristic shape: the top reflected the shape of the disk, while the lateral sides sloped steeply down to the surrounding skin (Fig. 3). The anterior and posterior sides of the pedicle developed a waisting or hour-glass appearance with the narrowest part of the pedicle at approximately the centre of its height (Fig. 4).

The implanted disk and projecting pin were well tolerated and the skin appeared to remain normal over the disk and up to the pin. In the first experiment, the skin began to ulcerate over the anterior margin of the disk after approximately 100 days at which stage the experiment was terminated. In the second experiment, the rate of raising the pedicle was reduced in an attempt to avoid this complication. Under the conditions of the experiments, the rate of production of the skin pedicles was slow, and heights of approximately 60 and 30 mm respectively were achieved in the two experiments in about 100 days. Little progress was made in the second experiment in increasing the height after approximately 100 days (Fig. 5).

(b) *Fibres and Follicles*

Macroscopically, there appeared to be no difference in the rate of wool growth over the disks compared with the surrounding areas; nor was there any apparent change in the colour or crimping of the wool that grew over the disks in the experimental periods. No evidence of fibre shedding was observed. Comparison of tracings of fibres from over the control disk with those of fibres from nearby normal areas in the black sheep showed no change in the crimping of the wool. However, tracings of fibres from over the experimental disks in both animals showed a marked decrease in the "frequency" of crimp formation (Fig. 6), that is, an increase in the time taken to form each crimp. In the black animal only, the decrease in frequency was accompanied by an apparent increase in amplitude.

Table 1 shows the mean length growth rate and mean diameter of the wool fibres and the follicle population in experimental and normal areas for various periods after insertion of the disks. The heights of the pedicles at the end of each period are also shown.

The length growth rates of the fibres over the disks were not significantly different from those in adjacent areas. It is interesting to note that the subdermal pressure did not *decrease* the length growth rates of the fibres. However, after prolonged subdermal

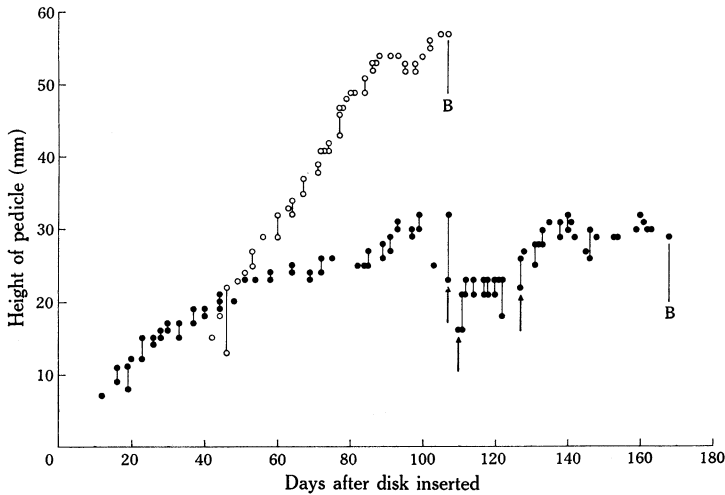


Fig. 5.—Graph showing the rate of formation of the skin pedicles (height plotted against time) in the white (○) and black (●) sheep. In many instances, two heights are shown for the one day; the lower point indicates the height as first measured and the upper point the height after upward adjustment of the spring. Where it was obvious that a spring had weakened, it was replaced by a new spring (indicated by arrows). B, biopsies taken and disk removed.

pressure, fibre diameters in general showed a tendency to increase over the disks. This was most definite in the last period of the first experiment and in the last three periods of the second experiment. In the first period of each experiment, there was little change

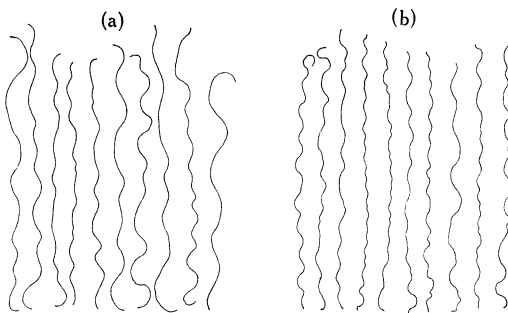


Fig. 6.—Tracings of 10 randomly selected fibres which had grown over the experimental disk in the black animal during the period of subdermal pressure (a), compared with tracings of 10 randomly selected fibres grown during the same period in a nearby normal area (b).

in fibre diameter. Since length growth rate was not significantly altered while diameters increased, the volume of wool produced per follicle was increased by up to 60% (Table 1).

TABLE 1
EFFECT OF SUBDERMAL PRESSURE ON THE MEAN LENGTH GROWTH RATE AND MEAN DIAMETER OF THE FIBRES, AND NUMBER OF FOLLICLES PER SQUARE MILLIMETRE OF SKIN
Number of fibres measured in each case are given in parenthesis

Sheep	Period (days after disk inserted)	Approximate Height (mm) of Disk Skin at End of Period*	Length Growth Rate (mm/day)		Diameter (μ)		Change in Mean Fibre Volume per Day (%)	No. of Follicles per mm ² (6 mm ² measured)†	
			Normal	Over Disk†	Normal	Over Disk†		Normal	Over Disk
White	0-60	30	0.38 (108)	0.33 (92)	24.5 (100)	23.9 (100)	-17.3	—	—
	60-107	57	0.32 (295)	0.33 (115)	21.4 (295)	26.7 (115)	+60.9	41.6 [19.6]	23.2 [18.8]
Black	0-47	21	0.33 (108)	0.36 (88)	21.5 (108)	23.1 (83)	+18.3	—	—
	47-72	25	0.42 (108)	0.44 (75)	22.1 (100)	24.8 (75)	+31.1	—	—
	72-121	23	0.47 (200)	0.53 (185)	21.6 (200)	24.2 (185)	+44.2	—	—
	121-168	27	0.35 (201)	0.32 (82)	21.5 (200)	26.2 (73)	+19.7	46.0 [14.6]	20.1 [15.0]
Black	0-53	Disk not raised	0.34 (208)	0.34 (198)	21.7 (200)	22.9 (198)	+10.3	—	—
	91-111 §	Disk not raised	0.32 (78)	0.30 (43)	22.0 (78)	22.3 (43)	+1.6	—	—
	53-155	Disk not raised	0.32 (216)	0.27 (160)	21.6 (228)	22.3 (154)	-1.7	41.6 [11.5]	41.4 [14.1]
				0.33 (207)	21.9 (208)				

* Above control skin.

† Two values represent samples from opposite sides of pin.

‡ Values in square brackets are the ratio of secondary to primary follicles.

§ 20-day period when sheep received injections of L-[³⁵S]cystine at 4-day intervals.

|| Means of measurements of cystine-labelled parts of fibres.

As skin samples could only be taken once in each experiment, follicle population counts were only made at the end of the final period in each experiment. At the end of the two subdermal pressure experiments, the number of follicles per square millimetre over the disks was approximately half the number in the normal samples and in the skin over the disk when subdermal pressure was not applied. However, the ratios of secondary to primary follicles in samples taken from over the two experimental disks and the control disk were similar to the corresponding ratios in normal samples taken nearby.

The group arrangement of follicles over the experimental disks was essentially normal except that the amount of connective tissue increased between groups and, to a lesser extent, between the follicles. Similar changes were observed by Molyneux and Lyne (1961) in the walls of experimental cysts produced by the subcutaneous implantation of full-thickness skin in sheep.

The dermis overlying the experimental disks was compressed by the upward pressure and was considerably thinner than normal and the follicles, in general, were inclined more than normal from vertical. Some were seen to lie almost horizontally in the dermis.

In the greater part of the skin over the disks the follicles showed no evidence of destruction or degeneration. This was borne out by the relatively normal wool growth over the disks. However, the depth of the follicle layer over the disks was slightly reduced compared with that in normal skin. In the immediate vicinity of the pin there was evidence of both partial and complete degeneration of some follicles.

(c) *Epidermis*

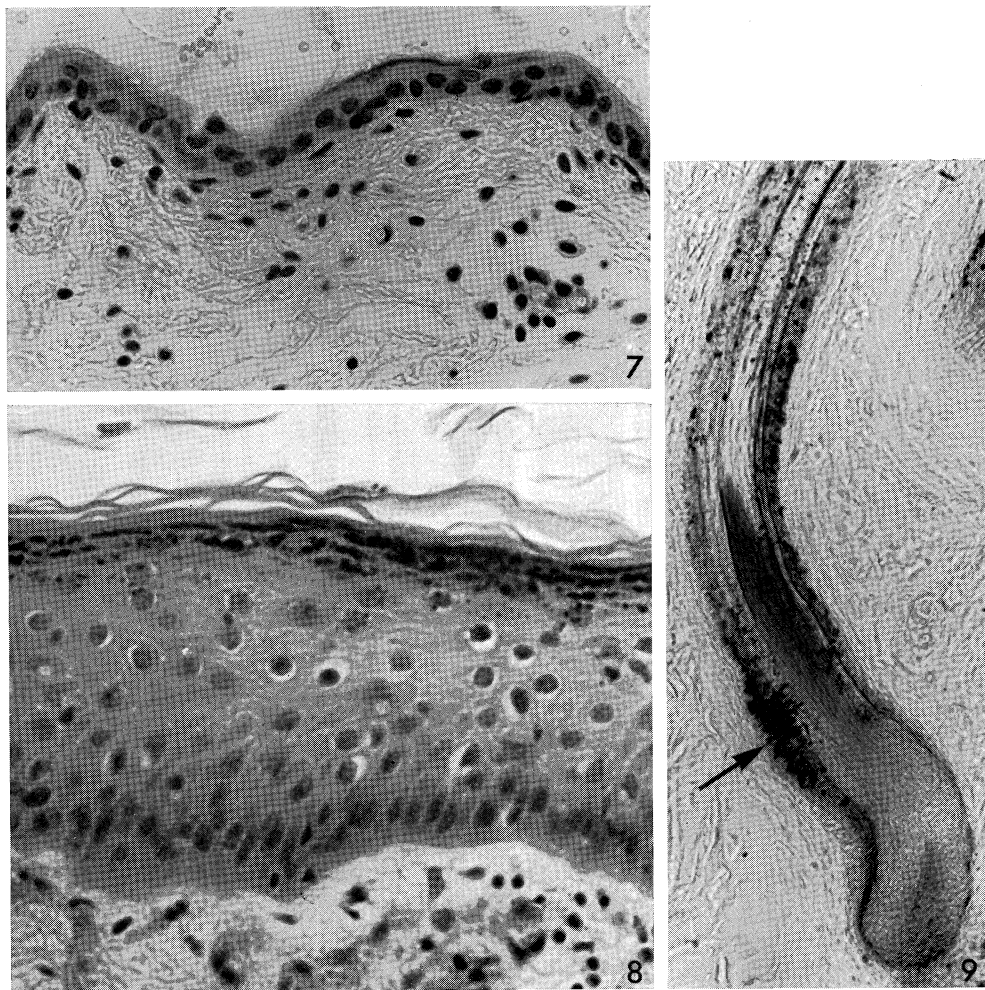
Normal midback epidermis in these animals was approximately $20\ \mu$ thick and had no granular layer except around the follicle openings. Two outstanding changes occurred in the epidermis over both the experimental and the control disks: an increase in its thickness and the development of a marked and continuous granular layer (Figs. 7 and 8). The increase in the thickness of the epidermis over each experimental disk was greatest near the pin (up to six-fold, $128\ \mu$), less (two- to threefold, $40\text{--}60\ \mu$) over the rest of the disk, and tapered off to a very slightly increased thickness down the side of the pedicle. The epidermis over the control disk increased greatly in thickness near the pin but the increase over the rest of the disk was not as marked as that over the experimental disks. Part of the increase in epidermal thickness over both the experimental and control disks was due to an increase in the cornified layer.

The granular layer over all disks had altered in several respects. It had become continuous instead of being confined to the region of the follicle openings; it was much thicker and some of the granules were deep in what would generally be regarded as the spinous layer; and finally, the granules were larger.

Acetylcholinesterase-positive branched cells (Lyne and Chase 1966) were present in normal numbers along the basal layer throughout the epidermis over the disks. However, in the immediate vicinity of the pin fewer were present and they were more superficially located and extended up to the granular layer (Fig. 16).

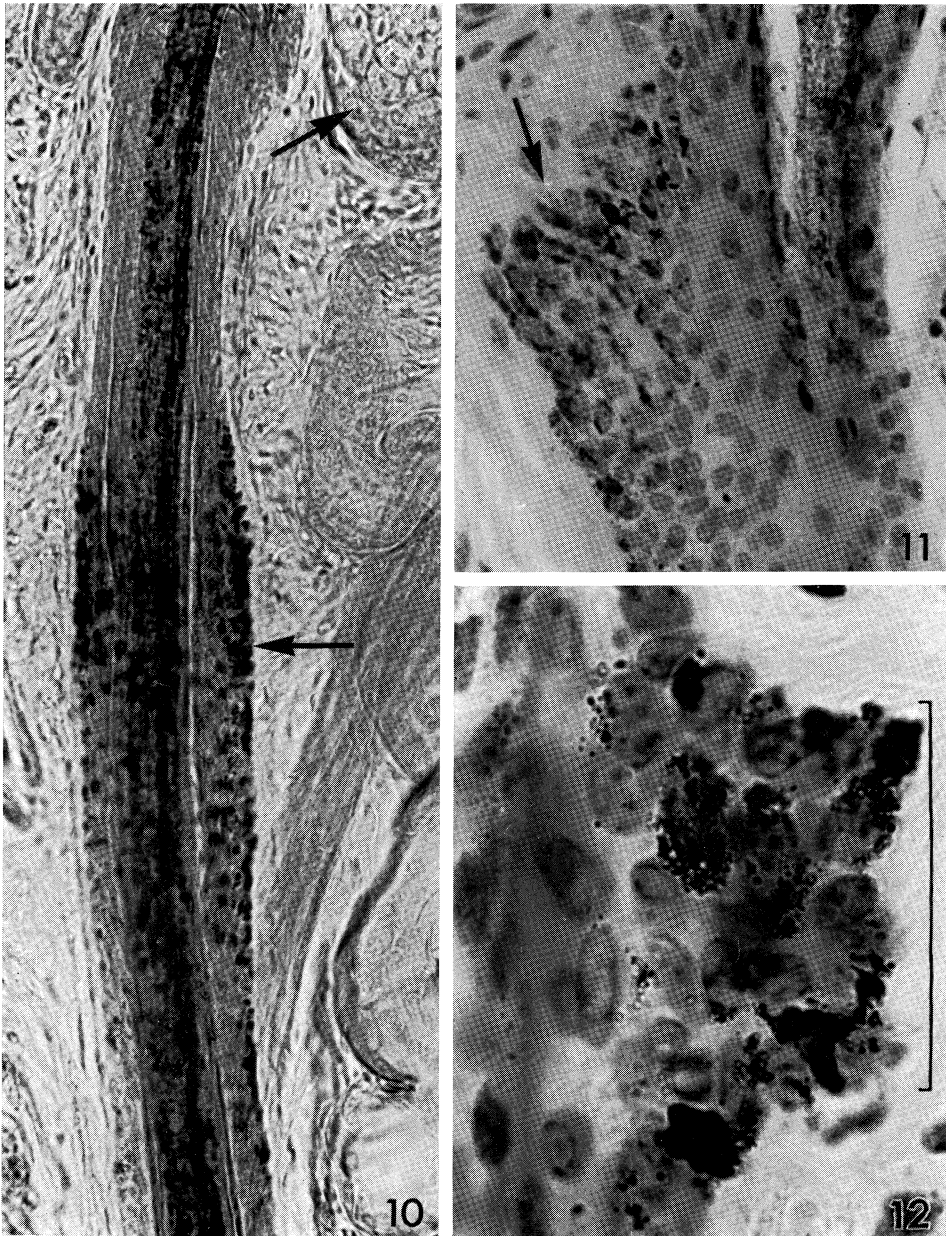
(d) Pigmentation

Lyne and Hollis (1968*a*, 1968*b*) showed the melanocytes in the follicle outer root sheath of sheep as occurring close to the top of the follicle bulb and well below the level of the sebaceous glands (Fig. 9). Cells containing melanin move upwards along the outer root sheath and finally pass into the follicle lumen somewhere below the openings of the sebaceous gland ducts.

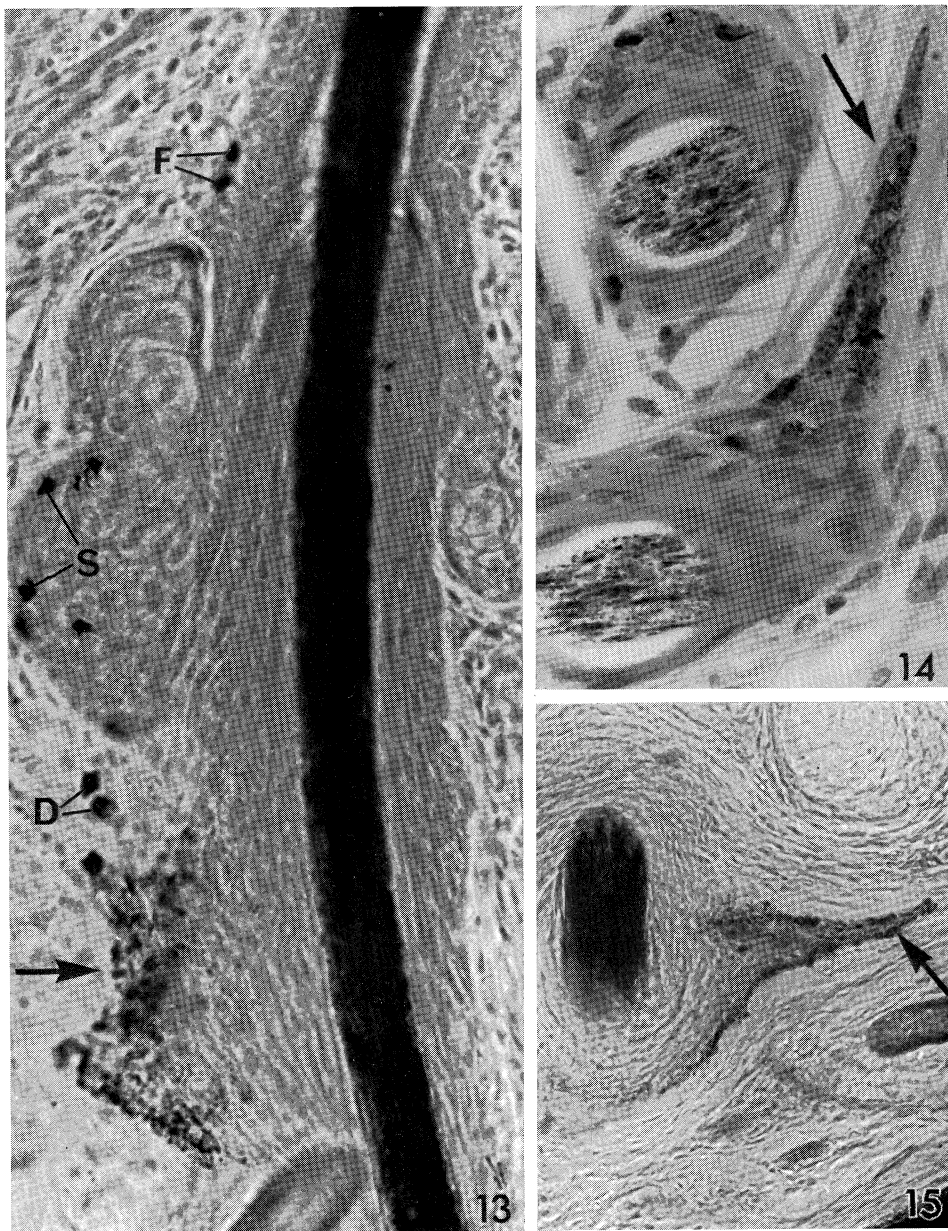


Figs. 7 and 8.—Vertical sections of epidermis at the same magnification ($\times 383$) from the white sheep. HEP. 7, Normal midback position away from disk. The epidermis is characteristically thin and lacks a granular layer except around follicle openings (not shown). There is a thin but continuous cornified layer. 8, In contrast, samples from over the disk showed a greatly thickened epidermis with a conspicuous and continuous granular layer. The cornified layer is much thicker than normal.

Fig. 9.—Longitudinal section of lower part of a follicle in a pigmented sheep showing melanocytes (arrow) in outer root sheath. $\times 112$.



Figs. 10-12.—Longitudinal sections of parts of three follicles from normal midback position of black sheep. **10**, Melanocytes and melanin-containing cells (lower arrow) are located approximately at the middle of the region of the outer root sheath between bulb and sebaceous gland (upper arrow). Alkaline phosphatase. $\times 265$. **11**, Outgrowth of pigmented outer root sheath cells (arrow). HEP. $\times 366$. **12**, Outgrowth of pigmented outer root sheath cells (bracket). HEP. $\times 1146$.



Figs. 13-15.—Parts of three follicles from over experimental disk in black sheep, showing outgrowths (arrows) of outer root sheath cells containing melanin and melanocytes. **13**, Longitudinal section of follicle; some melanin-containing cells, probably derived from the outer root sheath, are seen in the dermis (*D*), on the surface of the sebaceous gland (*S*), and on the wall of the follicle (*F*) above the sebaceous gland. Alkaline phosphatase. $\times 336$. **14**, Transverse section (slightly oblique) of follicle. HEP. $\times 484$. **15**, Transverse section (slightly oblique) of follicle. Alkaline phosphatase. $\times 229$.

in black sheep showing melanocyte (middle arrow) in basal layer, and melanin-containing cells in both epidermis (upper arrow) and dermis (lower arrow). H. $\times 1296$.

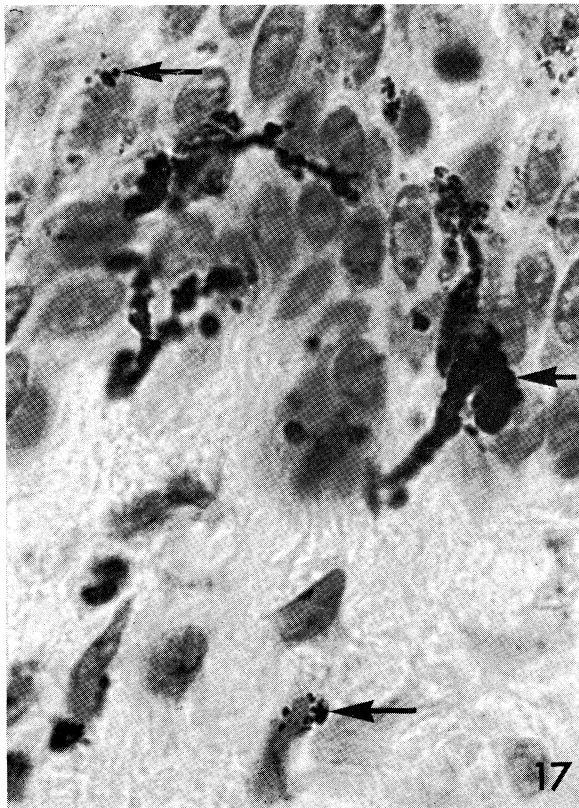
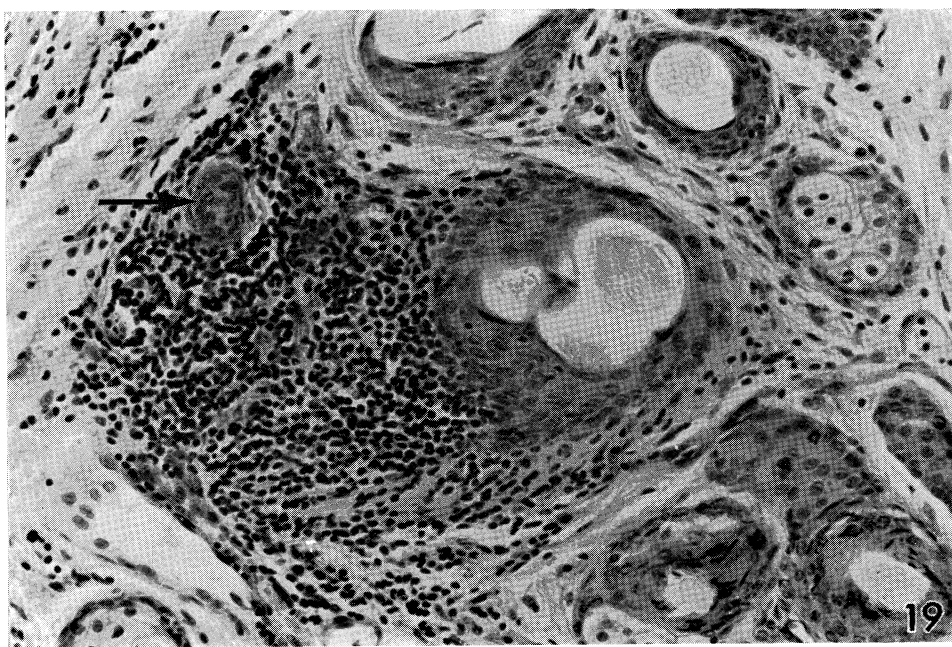
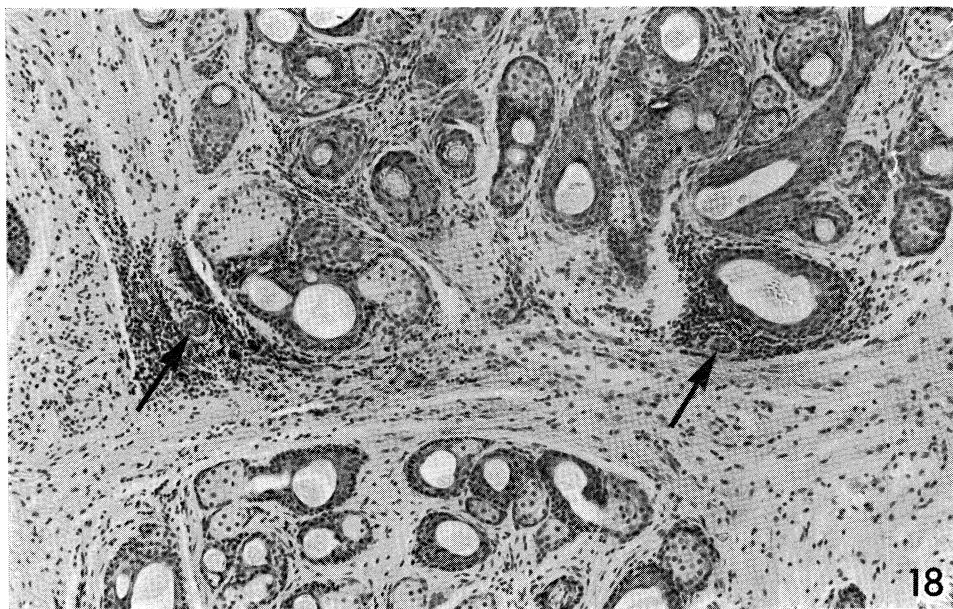


Fig. 16.—Vertical section of skin adjacent to the pin of experimental disk in black sheep showing concentration of melanin-containing cells (including some melanocytes) in deep layers of the epidermis and adjacent dermis. Arrows indicate more superficially placed acetylcholinesterase-positive branched cells. $\times 101$.

Fig. 17.—Vertical section of dermoepidermal junction in skin adjacent to the pin of control disk



Figs. 18 and 19.—Sections parallel to skin surface at level of upper part of sebaceous glands, taken from over the disk in the white sheep. Small localized patches of inflammatory cells are seen around the sweat gland ducts which are indicated with arrows. HEP. 18, $\times 92$. 19, $\times 215$.

In the black animal used in the present experiment two features were seen in the normal skin which may be peculiar to this sheep. First, the melanocytes and other melanin-containing cells of the outer root sheath were located at a high level (approximately at the middle of the region between bulb and sebaceous glands) (Fig. 10). Second, it was noted that a few follicles exhibited bulging or even small outgrowths of outer root sheath cells containing melanin and occasionally melanocytes (Figs. 11 and 12).

Over the experimental disk, pigmented outgrowths of the outer root sheath (Figs. 13–15) were also present, but were larger and more numerous than in normal skin. Some outgrowths were extreme and melanocytes and melanin-laden outer root sheath cells had separated from the follicles and migrated into nearby regions, a feature not observed in the normal skin. Some of these cells appeared to have lodged on the sebaceous glands and a few were seen in other regions usually free of melanocytes, for example on the outer surface of the follicle just above the sebaceous glands and at the base of the follicle bulb. The pigmented cells were a feature of the dermis in the experimental sections, but were extremely uncommon in the sections of normal skin. All of these changes in the distribution and concentration of pigment were most obvious near the pin.

The density and distribution of the melanocytes in the epidermis over both experimental and control disks appeared unchanged in both the black and the white animal, except for a small region in the black animal where the epithelium had proliferated downward adjacent to the pin. In this region there was a heavy population of melanin-containing cells including some recognizable melanocytes (Figs. 16 and 17). These cells were limited mainly to the deeper layers of the epidermis and the mid-region of the dermis. The level of the abnormal pigmentation in the dermis corresponded with the level of greatest concentration of melanin-laden cells in follicles in normal skin. This suggested that the abnormal pigmentation had been derived from degenerating follicles. Further, the number of melanin-laden cells linking the heavy concentration of similar cells in the epidermis with those in the dermis suggested a common origin, possibly from the degeneration of follicles. The changes in pigmentation in the sections taken from over the control disk were less than in the experimental sections.

(e) *Inflammation*

Macroscopically, the tissue overlying the disk did not appear to be inflamed. However, on microscopic examination, localized patches of inflammatory cells (mainly lymphocytes) were seen. These patches were almost invariably associated with the upper part of the sweat gland ducts (Figs. 18 and 19), that is adjacent to the primary follicles above the level of sebaceous glands. Adjacent small blood vessels were engorged and showed a high content of inflammatory cells. Gram-stained sections revealed no evidence of infection as the cause of this inflammation.

A few of the areas of inflammation were close to the overlying epidermis which was also invaded by inflammatory cells throughout its full thickness. In these areas the most superficial blood vessels were engorged and intercellular oedema gave the epidermis a vesicular appearance.

(f) *Innervation*

In general, nerve fibres were less numerous than usual throughout the dermis over the experimental disks. Even more outstanding was the reduction in the follicle nerve networks which were found only occasionally.

(g) *Sweat Glands and Sebaceous Glands*

The sweat glands in the normal skin of the black animal were located more superficially than usual, while those in the white animal were at a normal level. Over both the experimental and control disks in the black animal, sweat glands were found in normal number, size, and distribution (for that animal). In the white animal, there was an obvious reduction in the number and size of sweat glands, in spite of the fact that sweat gland ducts were still present in normal number and relation to primary follicles above the level of the sebaceous glands.

Immediately adjacent to the pin, sebaceous glands were absent as were their associated follicles. Elsewhere, neither the morphology nor the function of these glands seemed to be altered. Oil Red O-stained sections showed the glands to be fully charged with secretion and there was an abundance of sebum in the superficial layers of the epidermis.

IV. DISCUSSION

It has been shown that a pedicle of skin can be produced by continuous subdermal pressure. In the field of animal research, such a technique might be useful for the production of folds or tubes of skin for *in vivo* observations, and to facilitate use of the transparent-window technique. Any practical application of this technique in the field of human surgery would be partly dependent upon the elaboration of a suitable device to produce subdermal pressure and also on the time required to produce a pedicle.

The fact that mean length growth rate of the fibres over the disk remained unchanged while the mean fibre diameter in most instances increased, showed that the actual amount of wool produced per follicle was increased by up to 60%. Three possible reasons can be given for the increase in wool production. The reduction in follicle population density may result in each follicle having (1) more nutrients available; (2) the reduction in innervation of skin over the experimental disks could have resulted in loss of nervous control over blood vessels, and an increase in blood flow with consequent changes in nutrition of the follicles; (3) in addition, it is possible that the inflammatory reaction adjacent to the upper parts of the primary follicles exerted a wider influence than was apparent and produced an appreciable increase in blood flow.

According to Chapman's (1965) dynamic hypothesis of crimp formation, follicle movement (producing cyclic change in the flexure of the follicle bulb) determines the various features of fibre crimp. It is reasonable to suggest that pressure on the under-surface of the dermis, by compressing the cells, could only *reduce* follicle movement and, since crimp formation was impaired in the present experiment, this may be taken as evidence for Chapman's hypothesis.

The tracings of fibres taken from skin subjected to subdermal pressure in the black animal indicated both a decrease in frequency and an increase in amplitude. It may be speculated that if movement of the follicle is simply slowed down and its

range of movement not reduced, the crimp of the resultant fibre will exhibit both decrease in frequency and increase in amplitude.

It had been anticipated that the production of a pedicle by subdermal pressure would cause stretching and thinning of the epidermis. However, marked and consistent thickening was observed over the surface of the disk. Perhaps this occurred as a biological response to a physical stimulus—the increase in tension in the epidermis. Some light may be shed on this problem by the experiments of Lyne and Hollis (1968*b*) who showed that both reduction and complete destruction of hair and wool follicles resulted in an increase in thickness of the epidermis. These results agree with those of Spearman (1964), who stated that, in many species, epidermal thickness varies inversely with the density of the hair covering. The increase in thickness over the control disk as well, where subdermal pressure would be minimal, is difficult to explain. However, a change in the balance between the normal rate of cell proliferation in the basal layer and cell loss at the skin surface would produce a change in epidermal thickness. Thus, an increase in cell production without a corresponding decrease in shedding, or a decrease in shedding alone, or a combination of the two, would result in increased epidermal thickness. Decrease in rate of shedding may in turn be due to an initial increase in the cohesiveness of the newly formed cells which may only be reduced to the shedding point after they have been retained for a longer period in the skin and have moved a greater distance than normal from the dermis—their source of nutrients. In some unknown way the disk has been responsible for some such change.

Upward and inward migration of outer root sheath cells into the lumen of the follicle has been described in several species including the sheep (Lyne 1965; Lyne and Hollis 1968*a*; Straile 1965). Biopsies from over the experimental disk in the black sheep showed marked tongue-like projections of outer root sheath cells containing melanin and some melanocytes. Some of the pigmented cells appeared to have separated from these projections and were free in the adjacent dermis. Thus, for some reason, these cells were apparently unable to migrate normally into the lumen, and their upward movement required an alternative outlet from the outer root sheath. Melanin-containing cells seen on the surface of sebaceous glands were presumed to have migrated from the outer root sheath and, perhaps because of their affinity for epithelial tissue, had attached themselves to these glands.

Pigmentation of both epidermis and dermis was an outstanding feature immediately adjacent to the pin in the black animal (Fig. 16). This same region was the only area with an almost complete absence of hair follicles, while those present were in various stages of degeneration. From this, it may be inferred that the dermal and most of the epidermal pigmentation was derived from the disintegration of follicles and the migration of outer root sheath cells from other follicles.

The almost total restriction of inflammatory reaction to the upper part of primary follicles and adjacent sweat gland ducts was interesting and may have been caused, in part, by a reduction in normal sweat gland activity and therefore a change in normal skin function. This is deduced from the observation that the number of sweat glands was greatly reduced in one animal.

V. ACKNOWLEDGMENTS

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