FACTORS DETERMINING SWEATING COMPETENCE OF CATTLE SKIN

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Abstract

Significant differences in sweating rate between micro-areas of cattle skin have been demonstrated and the differences in morphology between these contrasting areas investigated.

Variation in sweating rate over the skin was most marked when the coat was longest and follicle activity and sweating rate least.

Among the characters most closely related to sweating performance were percentage of follicles in anagen and arterial supply to the sweat gland layer. Neither mean sweat gland volume nor follicle density had a direct effect on sweating rate.

The growth phase of the hair follicle has a strong influence on the capillary supply to the sweat gland and this appears to be a critical factor in sweat gland performance.

I. Introduction

Differences in sweat gland size have been demonstrated between different breeds of cattle and between different strains within breeds (Nay and Hayman 1956; Nay and Dowling 1957; Nay and Hayman 1963; Pan 1963). Breeds and strains which are better able to control body temperature have the largest glands. Zebu cattle and their crossbreeds have been shown to have higher follicle density than British-breed animals (Nay and Hayman 1956; Turner, Nay, and French 1962), although Pan (1963) found no significant difference between Jersey and Sahiwal cows.

Pan, Donegan, and Hayman (1969) showed there was a positive correlation between the density of a sweat gland population in a body region and its sweating rate. The correlation between mean volume of individual sweat glands in an area and sweating rate was negative, and the correlation between total sweat gland volume in an area and its sweating rate was not significantly different from zero. This agrees with Nay and Dowling (1957) who found no evidence of an association between sweat gland size and sweating rate.

Observations of sweating rate at different times of the year (Schleger and Turner 1965) have demonstrated the seasonal nature of sweating competence, maximum sweating rates being significantly lower in winter than summer. When coat score and sweating rate were determined at the one time, the type of coat was associated with capacity to sweat. Animals with sleek coats showed a higher sweating capacity than animals with rough coats, even though the sleek ones had cooler skins. The body regions shown by McLean (1963) and Pan, Donegan, and Hayman (1969) to have the highest sweating rates are those which have the sleekest coats. Since the sleekest animals are those whose follicles are most active, this observation suggests a relationship between sweat gland function and metabolic status of the hair follicle, a member of the same structural unit as the sweat gland.

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The purpose of this investigation is to locate sites of high and low sweating rates within limited areas of the same body region and to look for differences between these sites in morphological features which might contribute to sweating function.

II. Materials and Methods

(*a*) *Measurement of Sweating*

Sweating rate was measured by applying 21 uniformly distributed disks of cobalt chloride paper to a skin area of 12·9 cm^2 and noting the time taken for the colour of each disk to change to bright rose. The disks were prepared as follows.

Chromatography paper, grade No. 1, was immersed in 20% cobalt chloride, dried at room temperature on a sheet of glass, and then in an oven at 90°C. Disks of 3 mm diameter were punched out and redried before mounting on 2·5-cm wide adhesive cellulose tape with graph paper and a glass slide as backing. The disks were mounted in seven rows of three, with a spacing of 0·5 cm between them. After mounting, the slides were placed in an oven and the disks redried to a uniform blue colour. The strip of adhesive with attached disks was then fixed to a second slide and stored in a desiccator. Slides were prepared no longer than 24 hr before use. In the hot room the tape with attached disks was removed from its slide and applied to an area of skin previously clipped and dusted.

The amount of moisture required to change the colour of the disk was found experimentally to be 43 g/m^2, as compared with 22 g/m^2 to change a disk of 10% cobalt chloride paper (Schleger and Turner 1965). The time taken by each disk to change colour was measured in seconds. As the relative reaction time of each disk or site was the essential criterion, the rates were expressed in arbitrary units as 10^4 times the reciprocal of time in seconds.

Sweating measurements were made on seven animals, six of which were Hereford × Shorthorn crossbreds while one was a Brahman crossbred. A total of 19 trials were carried out throughout the year, each trial involving the one body region. As several areas were studied in some trials, data were obtained from 42 areas in all. At least two sweating determinations were made on each area; the total number of determinations or runs was 117. Most replicate determinations of sweating were made on the one day but two of the areas were studied over more than one day. In all trials the temperature of the hot room varied from 38·5 to 40·0°C with a relative humidity of approximately 50%.

(*b*) *Histology: Consideration of Techniques*

(i) *Capillary Density*

In this laboratory histochemical reactions involving alkaline phosphatase have proved to be the most satisfactory in demonstrating the capillary supply to the papillary layer of cattle skin. The silver technique as used by Diemer and Henn (1964) has been unsatisfactory due to the positive staining of elastic tissue generally. The method of Graham, Lundholm, and Karnovsky (1965), using 3-amino-9-ethylcarbazole and based on the peroxidase activity of the red blood cells, gives a better definition of the capillaries than the benzidine method (Pearse 1961), but exsanguinated areas may give a false pattern.

Montagna and Ellis (1957) claimed that the azo-dye technique for alkaline phosphatase has all the advantages and none of the disadvantages of injection techniques in demonstrating the endothelial cells of human capillaries. Hashimoto and Ogawa (1963) demonstrated the capillaries of rat skin with the alkaline phosphatase reaction while Johnson and Menne (1968) used it to show the capillaries of the skin in man, mouse, rat, and pig. On the other hand, Cormia and Etnyey (1961) and Cormia (1963) pointed out that not all capillaries react positively with the alkaline phosphatase technique, which therefore does not provide complete information on the vascular pattern.

The density of capillaries positive for alkaline phosphatase is particularly relevant to sweating function. The presence of alkaline phosphatase in minute blood vessels is associated with transmembranous solute transfer and may be indicative of the functional activity of the tissue

(Cormia 1963). Kormano (1967) has cited a number of reports relating alkaline phosphatase activity to transport across the capillary wall. Mikhail, Farris, and Gimbel (1965) suggest that the marked phosphatase reaction in the papillary loops may be related to some function, such as temperature regulation, subserved by this peripheral part of the circulatory system.

(ii) *Nerve Supply*

The cholinergic nerve supply to the upper dermis of cattle skin may be assumed to represent the total nerve supply to this region. Jenkinson, Sengupta, and Blackburn (1966), in their study of the innervation of cattle skin, concluded that the nerve distribution shown by the monoamine oxidase and specific cholinesterase techniques was the same as that observed after the treatment of the skin with methylene blue and with silver, but that nerves above the sebaceous gland contained only specific cholinesterase. In this laboratory, nerves reacting for monoamine oxidase have not been found above the sebaceous gland level but a limited number of nerves reacting for non-specific cholinesterase have been demonstrated.

(iii) *Procedure*

Fast and slow sweating sites were sampled for microscopic study in August and January. Skin biopsies were taken with a 1-cm diameter trephine in the early morning, generally 2 days after the hot room measurements. After fixing for 4 hr in chilled 10% neutral formalin, surface diameters of the skin biopsies were measured with a stereomicroscope at a magnification of $\times 10$. The biopsy was dissected in the follicle plane and sections of 100 μm thickness were cut with a freezing microtome. Sections of 32 μm thickness were used in the photomicrographs illustrating the capillary supply to the sweat glands. The biopsy for these sections was taken from the flank region of a Hereford $\times$ Shorthorn cow.

The capillary and nerve supply to the upper dermis was demonstrated by the reactions for non-specific alkaline phosphatase (Gomori 1952) and specific cholinesterase (Koelle and Friedenwald 1949). The capillary loops appeared to terminate at the epidermis, because the positive phosphatase reaction was confined to the arteriolar end of the capillary endothelial lining. They could thus be assessed in the same way as the terminating cholinergic nerves which were counted in each of three sections, each count being adjusted to a section length of 0·33 cm. If T is the total count of nerves or capillaries in a section of 1 cm length and 100 μm or 10^{-2} cm thickness, then 10^2T is the number per square centimetre. Assuming that when the skin biopsy contracts after fixation the circular surface area of 1 cm diameter contracts to an elliptical area with axes a_1 and a_2, therefore

$$\text{degree of contraction} = \text{contracted area/fresh biopsy area}$$

$$= 0{\cdot}7854\, a_1a_2/0{\cdot}7854 \times 1$$

$$= a_1a_2.$$

Since density of nerves or capillaries *in vivo* equals the degree of contraction $\times$ density in fixed biopsy, then

$$\text{density } \textit{in vivo} = a_1a_2 10^2T.$$

A number of sections were stained with haematoxylin and eosin for the determination of follicle phases, density of dermal arteries, sweat gland area, and follicle density. The proportion of hair follicles in stages between anagen III to anagen VI inclusive was used as percentage active follicles. The density of dermal arteries was estimated by counting the number of arterial cross-sections in the glandular layer of each section. Projected sweat gland area was obtained by measuring with a planimeter, the image drawn with the aid of a camera lucida attachment. Ten sweat gland areas were averaged for each sample. An index of follicle density was gained from vertical sections, transverse sections not being available. From the thickness and length of skin sections the density of follicles per 1 cm^2 could be calculated. This was adjusted for biopsy contraction before sectioning. The follicle density so determined was higher than that reported for transverse sections, probably due to the dissection of a proportion of follicles.

III. Results

(*a*) *Sweating Pattern*

A typical pattern of sweating rate is shown in the following tabulation, which lists the "sweating times" in 21 sites distributed over a 12·9 cm² area of skin (one of the four areas in trial 4, Table 1):

52	72	111	125	92	103	117
64	69	130	147	158	159	118
56	102	128	146	151	108	108

The mean of three estimates of the time taken for the cobalt chloride disks to change colour varied from 52 to 159 sec. That is, the rate of the fastest site was three times that of the slowest one. The distribution of rates was not random. Adjacent sites were often found to have comparable sweating rates. For example, the three sites in the first column of the tabulation have a relatively high average rate, while three of the sites in the fifth and sixth columns are comparably slow.

Table 1

DIFFERENCE BETWEEN SITES WITHIN AREAS AND THE HOMOGENEITY OF SITES BETWEEN RUNS

Trials carried out on seven animals from August 1969 to July 1970

Trial	Highest: Lowest Sweating Rate†	Sites		Sites × Runs		Intra-class Correlation	Variance Ratio
		D.F.	M.S.	D.F.	M.S.		
1	3·11	20	11·429	80	1·412	0·587	8·097**
2	3·46	20	19·814	80	1·829	0·660	10·835**
3	2·28	40	5·812	80	1·107	0·586	5·249**
4	2·04	80	22·136	300	2·165	0·648	10·224**
5	1·59	60	7·059	200	3·472	0·205	2·033**
6	1·42	100	0·851	300	0·508	0·144	1·675**
7	2·31	60	1·768	120	0·606	0·390	2·917**
8	2·60	80	2·312	240	0·386	0·555	5·983**
9	2·27	80	4·493	160	0·794	0·608	5·659**
10	1·84	80	0·701	160	0·179	0·493	3·914**
11	1·56	20	3·836	80	1·404	0·257	2·731**
12	1·72	20	3·053	60	1·077	0·314	2·835**
13	1·50	20	0·127	60	0·033	0·420	3·894**
14	1·43	60	2·015	100	2·148	−0·021	0·938
15	1·21	20	1·835	80	2·348	−0·046	0·781
16	1·36	20	4·435	60	4·902	−0·024	0·905
17	1·58	20	1·187	60	1·443	−0·046	0·823
18	1·38	20	2·090	60	2·986	−0·081	0·700
19	1·37	20	0·258	60	0·246	0·013	1·051

** $P < 0·01$.

† Ratio of highest to lowest rate in 21 sites within an area. Where the number of areas in each trial (sites d.f. multiple of 20) is > 1, the ratio is the mean of the ratios for the several areas.

If the sweating pattern departs significantly from a random one there will be a high ratio of slowest to fastest site times in the one area and a high repeatability in the ranking of sites between runs. Table 1 summarizes the results for the 19 sweating

trials. In addition to the ratio of slowest to fastest times, the table shows the intraclass correlation coefficient, an index of the homogeneity of the same sites in different runs. The significance of the intraclass correlation in each trial is indicated by the variance ratio, which tests the effect of sites against the sites × runs interaction.

In 13 of the 19 trials the variation between sites was highly significant. Of the remaining six trials, four were on a short-haired Brahman crossbred, and five of them took place in summer months. On the Brahman crossbred animal, the only trial showing a significant difference between sites was that carried out on the flank region in midwinter. On Shorthorn × Herefords, the only two trials that showed no difference between sites were those on the shoulder in January.

(*b*) *Morphology of the Skin from Fast and Slow Sites Sampled in August*

Various structures of the papillary layer were compared in skin taken from fast and slow sites of the shoulder region of animal 6-178 and the flank region of animal 7-314. The mean and standard error of sweating rate and of six skin characters from fast and slow sites are shown in Table 2. The significance of the difference between sites is indicated by the value for *t*.

TABLE 2

COMPARISON OF SWEATING RATE AND SKIN STRUCTURE OF FAST AND SLOW SITES FROM TWO BODY REGIONS

Trials carried out in August 1969. Values are means ± standard errors

Parameter	Animal No. 6-178, Shoulder		*t*	Animal No. 7-314, Flank		*t*
	Fast	Slow		Fast	Slow	
Sweating rate $\times 10^4$ (sec^{-1})	18·0±1·0	5·3±0·4	11·78**	16·7±0·7	3·7±0·4	16·04**
Active follicles† (%)	20	15		34	23	
No. of dermal arteries per cm^2	22	10		10	6	
No. of upper dermal nerves per mm^2	17±0·6	13±0·5	1·94	24±0·7	18±0·3	2·97*
No. of upper dermal capillaries per mm^2	6±0·1	19±0·4	12·03**	4±0·4	9±0·7	2·08
Sweat gland area $\times 10^{-4}$ (cm^2)	183·0±13·0	212·4±13·7	2·36*	204·6±13·0	204·8±11·4	0·02
Follicle density per mm^2	15±0·2	20±0·2	6·25**	21±0·4	19±0·6	0·99

* $P < 0·05$. ** $P < 0·01$. † Anagen III–Anagen VI.

The fast sites have a greater density of dermal arteries, as indicated by the density of transverse arterial sections. The proportion of follicles that are active is higher in the fast sites, being one-third and one-half greater than in the slow sites of the shoulder and flank regions respectively. As shown by Jenkinson, Sengupta, and Blackburn (1966), the cholinergic nerves and arteries of the skin are closely associated throughout the dermis. The larger nerve trunks of the papillary layer give off finer

branches which terminate at the epidermis (Fig. 1). In view of the higher density of dermal arteries in the fast sites, it is to be expected that the density of upper dermal nerves will be higher also. This is so in the shoulder region and in the flank region; the difference between sites is significant in the latter. The upper dermal capillaries, however, are significantly denser in the slow sites.

There is no difference between sites of the flank region of animal 7-314 in mean sweat gland size or follicle density. In the shoulder region of animal 6-178, the sweat gland area and follicle density are significantly less in the fast sites.

TABLE 3

COMPARISON OF NERVE AND CAPILLARY SUPPLY TO THE UPPER DERMIS OF FAST AND SLOW SWEATING SITES FROM THE FLANK REGION OF THREE ANIMALS

Trials carried out in January 1970. Values are means ± standard error

Parameter	Animal No. 8-1		Animal No. 8-254		Animal No. 8-314	
	Fast	Slow	Fast	Slow	Fast	Slow
Sweating rate × 10^4 (sec^{-1})	12·6±1·1	9·8±0·6	12·8±0·8	6·8±0·3	12·8±1·0	10·5±0·7
No. of epidermal nerves per mm^2	54±3	30±1	47±4	27±1	39±4	24±2
No. of epidermal capillaries per mm^2	25±3	30±4	19±1	28±4	7±1	11±1

Analysis of Variance

Source of Variation	D.F.	Epidermal Nerves		Epidermal Capillaries	
		M.S.	*F*	M.S.	*F*
Fast *v.* slow sites	1	1701	85·1**	150	6·5*
Animal × sites	2	40	2·0	12	0·5
Error	12	20		23	

* $P < 0·05$. ** $P < 0·01$.

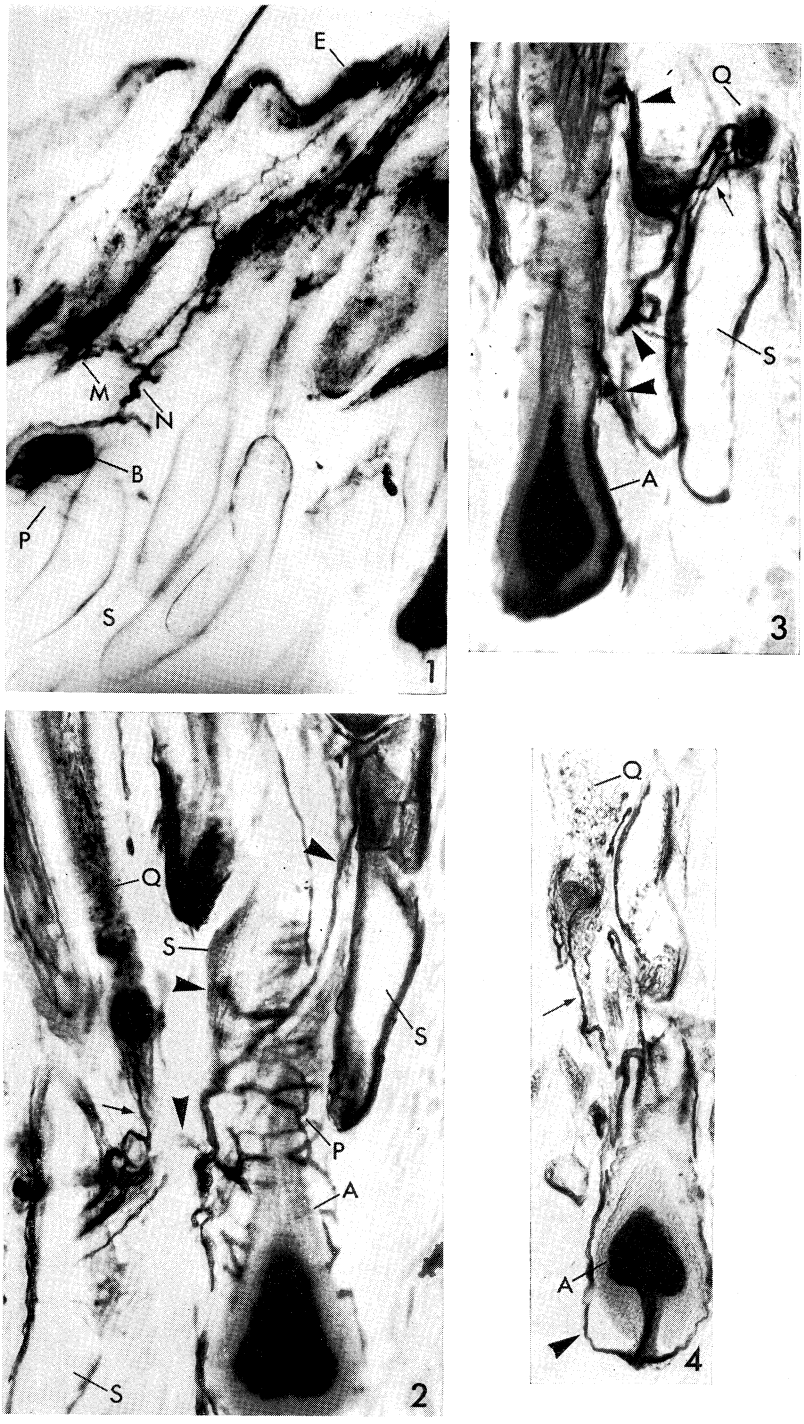
(c) *Morphology of Skin from Fast and Slow Sites Sampled in January*

Fast and slow sites were selected from within the experimental areas of the shoulder and flank regions of three animals, and the same characters were studied as in August. There was no consistent difference between the fast and slow sites of the

Fig. 2.—Demonstrating the difference between an active and a quiescent follicle in the capillary supply to nearby sweat glands. Arrowheads indicate the larger capillaries from the active follicle (*A*), while the small arrow indicates the fine capillary linking the quiescent follicle (*Q*) with the nearby sweat gland (*S*). *P*, capillary plexus of the keratogenous zone of the active follicle. Alkaline phosphatase. ×95.

Fig. 3.—Demonstrating large capillaries (arrowheads) leaving the keratogenous zone of an active follicle (*A*) at different levels. The dermal papilla of the quiescent follicle (*Q*) has only a fine capillary connection (small arrow) with its adjacent sweat gland (*S*). Alkaline phosphatase. ×95.

Fig. 4.—Contrasting the capillaries (arrowhead) from the dermal papilla of an active follicle (*A*) with the fine capillary (small arrow) associated with the dermal papilla of a quiescent follicle (*Q*). Alkaline phosphatase. ×95.



Figs. 1–4.—Photomicrographs of cattle skin.

Fig. 1.—Showing the course of a nerve, positive for specific cholinesterase, as it passes from the sweat gland layer to the upper layer of the dermis. *S*, sweat gland; *P*, nerve plexus associated with blood vessel *B*; *N*, cholinergic nerve; *M*, arrector pili muscle. ×60.

shoulder region in any of the skin characters studied. In the flank region, the only significant difference between sites was in the density of nerves and capillaries to the upper dermis. This is illustrated in Table 3, where it can be seen that in the fast sites the nerve density is higher but the capillary density lower. The statistical significance of these differences is demonstrated in the analysis of variance (Table 3).

(*d*) *Follicle Activity and Capillary Supply to the Sweat Gland*

A comparison between an active and a resting follicle in capillary association with nearby sweat glands is seen in Figure 2. From the dense capillary network of the keratogenous zone (Montagna and Ellis 1958), relatively large capillaries can be traced from the active follicle to three distinct sweat glands. Only one fine capillary connects the dermal papilla of the resting follicle and the adjacent sweat gland. Large capillaries leave the keratogenous zone at three distinct levels in Figure 3 and vascularize the adjacent sweat glands. The papilla of the quiescent follicle again has a fine connection with the nearby sweat gland. The only capillary supply from quiescent follicles to the sweat glands originates from the dermal papilla and, in this regard, it is very inferior to that from the dermal papilla of active follicles (Fig. 4). As pointed out by Ryder (1956) for the sheep, the amount of vascular tissue in a dermal papilla is dependent on its size.

IV. Discussion

From the present study it can be seen that sweating performance may vary considerably within the same body region. Within skin areas of approximately 10 cm^2, smaller subareas can be defined which may differ in sweating rate by as much as 300%. This difference is as great as or greater than that reported between different body regions (McLean 1963; Pan, Donegan, and Hayman 1969) and between different seasons (Schleger and Turner 1965). The sweating pattern is most marked when the coat is longest and follicle activity and sweating rate least. That is, it is more evident in the flank region than in the shoulder, in British-breed animals than in Brahman crossbreeds, and in winter than in summer.

The two skin characters most closely related to sweating performance are density of dermal arteries and percentage of active follicles. From a comparison of fast and slow sites it can be seen that these two characters are closely associated. The growth phase of the hair follicle appears to have a critical effect on the capillary supply to the sweat gland. The capillary supply to sweat glands is largely derived from the capillary plexus of the keratogenous zone of active follicles. The quiescent follicles, which have regressed to a position above the sweat glands, make no such contribution. The only vascular connection between a quiescent follicle and its adjacent sweat gland is a fine and often single capillary from the dermal papilla. The dermal papillae of active follicles, as in man, contain large tufts of capillaries which extend to the follicle walls.

In his study of the vasculature of the normal scalp, Cormia (1963) showed that while the large, actively growing (anagen) follicle has a rich blood supply the alkaline phosphatase activity and calibre of these vessels decreases in catagen and telogen. A similar effect is seen in cattle. There is a direct relationship between transport through the capillary wall and alkaline phosphatase activity (Pearse 1958; Samorajski and

McCloud 1961; Maekawa *et al.* 1965), and so the greater phosphatase reaction product of capillaries derived from anagen follicles is an illustration of their greater solute transfer.

The poor relationship between sweat gland size and sweating rate must surely indicate that other factors have an important effect on sweat gland performance. The state of activity of the hair follicles would appear to be of prime importance. Exhaustive studies have shown that sweat gland size varies with each phase of the hair growth cycle (Schleger, unpublished data), although at no times does it approach zero as previously reported (Schleger 1966). The effect of hair growth phase on the capillary supply to the sweat gland is far more critical than its effect on sweat gland size and appears to be a limiting factor in sweat gland performance.

When the percentages of active follicles in August and January are compared, it can be seen that in summer there are three times as many active follicles in the shoulder region and twice as many in the flank region. These differences are comparable to more extensive data obtained from seasonal studies on the one group of animals. It would appear that when the level of follicle activity is low, the majority of growing hairs and the more efficient sweat glands are confined to the more highly vascularized areas of the skin. When follicle activity is high, growing hairs are more widely distributed throughout the skin so that capillary supply to the sweat glands and their efficiency of performance is more uniform.

The close relationship between blood supply and follicle activity in the rat and rabbit has been described by Durward and Rudall (1958), while its relevance to hypotrichosis in calves has been pointed out by Schleger, Thompson, and Hewetson (1967). The reduced physiological activity of sweat glands with old age in man is thought to be dependent on a decrease in blood supply and degeneration of the nervous control mechanism (Mackinnon 1954).

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