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The growth of hair follicles and its relation to the adjacent dermal structures[1]

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Hair growth in the mouse is a cyclic phenomenon in that short periods of rapid hair production alternate with relatively long periods of quiescence (Chase & Eaton, 1959). After the first coat, waves of hair growth sweep from the ventrum dorsally, progressing anteriorly and then posteriorly (Butcher, 1934, 1951).

Changes in the follicle during the cycle of hair growth have been described for the mouse in great detail (David, 1934; Collins, 1918) when plucking of hairs from resting follicles was found to induce activity. Dry (1926) described the various types of hair of the mid-dorsal pelage of the mouse after removing them with warmed acetic acid; the undercoat contains a single type of hair and accounts for approximately 82 % of the pelage while three different types of guard hair can be distinguished.

Correlated with the development of the hair follicles (Chase, Montagna & Malone, 1953), the adjacent skin undergoes profound changes. The mechanism of the downward growth of the follicle from the level of the dermis during the quiescent phase through the panniculus adiposus to the panniculus carnosus during the period of growth and differentiation, and the relation of the downgrowth to changes in the skin have yet to be explained. Growth and upward movement of the hair involves the addition of new cells from the matrix of the follicle and an enlargement of each cell (Montagna, 1962). Chase *et al.* (1953) find that the increase in thickness of the skin during early follicular growth is not due to mitotic activity in the fat or corium. Increased storage of fat in the adipose cells is assumed to account for the increase in thickness of the adipose layer. Gibbs (1941) studied the combined thickness of the dermis-adipose layers in relation to post-natal development of the skin and hair of the mouse, and Chase *et al.* (1953) correlated the cyclic changes in the follicle with the thickness of the epidermis, the corium, and the adipose layers of the skin.

It has been suggested that the cyclic changes in the follicle are invoked by the dermal papilla (Butcher, 1965), because of the associated alterations of the latter during each follicular growth cycle, but there is no evidence of how the papilla is related to the growing hair follicle.

High resolution autoradiography combined with specific labelling of DNA (Hughes *et al.* 1958) provides a powerful tool for the study of DNA synthesis. In the mouse, Cattaneo, Quastler & Sherman (1961), using ^{3}H-thymidine, determined the proliferative cycle in the growing hair follicle 10 d after plucking. The rate of growth of the hair follicle of the mouse throughout the hair cycle has not been described, apart from

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the rate of growth of the free hair between the 11th and 18th d after plucking with ^{35}S-cystine being used as the label (Ashmore & Uttley, 1965).

In this investigation, the morphology of the skin and the growing hair follicle throughout its cycle has been studied by histological techniques applied to thin sections of skin (Expt. I). The length of the various hair follicles, and of the hairs, and the areas of the skin components between small follicles have been measured in sections of skin, and the results correlated with hair follicle growth, and hair growth induction (Expt. I). The rate of growth of the follicles has been determined for cell populations on a quantitative basis, by means of high resolution autoradiography with ^{3}H-thymidine (Expt. II). Autoradiographs of bulb squashes have been compared with sections of longitudinally cut hair follicles at various intervals during the hair cycle.

MATERIALS AND METHODS

Expt. I. Twenty-four male albino mice aged 3–4 months were used; they were given a standard laboratory diet *ab libitum*, and were of the BALB/c strain obtained from the Chester Beatty Institute. An area of approximately 2 cm^2 on the dorsum was plucked under ether anaesthesia at the beginning of the experiment and again after 30 d to ensure that the follicles were quiescent (Telogen). Animals were killed at times ranging from 1 to 21 d after plucking.

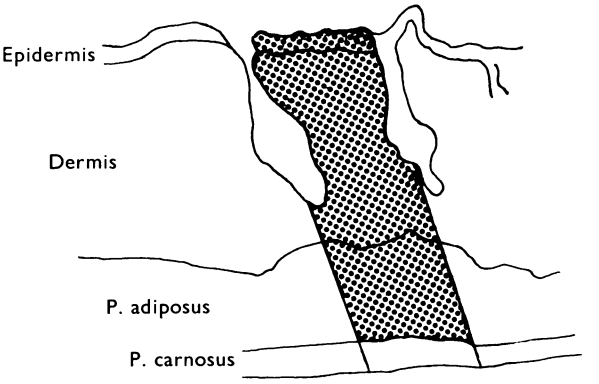


Fig. 1. 2 d follicle: camera lucida drawing of skin with two adjacent small hair follicles; the shaded area denotes the region measured in the various layers of the skin: epidermis, dermis, and fat. (× 176.)

For thick sections, the tissue was fixed in formol saline for 72 h, and then stained in a carmalum solution (5 ml carmalum, 50 ml water) for 24 h. The pieces of skin were trimmed and oriented to obtain the desired plane of section of the follicles. Then the tissue was cut at approximately 25 μm with a razor at an angle of 45° to the middle of the dorsum. The sections were then dehydrated and mounted in Canada Balsam.

For thin sections, the tissue was fixed for 24 h in formol saline, dehydrated through the alcohol series to cedar wood oil, and embedded in paraffin. Sections were cut at 5 μm and stained by Carmalum, Wilder's Silver Method, periodic-acid Schiff after diastase digestion (PAS), Carmalum–Orange G–Aniline Blue, or Ehrlich's haematoxylin and eosin, and mounted in Euparol.

The length of the hairs (Figs. 8, 9), the length of the corresponding follicles (Table 2, Fig. 9) and the thickness of the layers of the skin (Table 1) were measured with an ocular micrometer on thick sections at a magnification of 100. The length of the whole hair was measured from above the bulb of the follicle to the tip of the hair (Whole Hair). The length of the hair follicles below the surface of the skin, of the hairs above the skin (Free Hair) and the length of the dermal papilla were measured. The type of hair was related to the general size of the bulb, large or small.

We also determined the area of the various layers of the skin between small hair follicles in thin sections (5 μm); since all our sections were of the same thickness, the area of each layer was proportional to its volume. Camera lucida drawings were made and the surface areas of each layer between adjacent small hair follicles were determined with a planimeter (Fig. 1). The figures listed in Table 3 are direct planimeter readings, and 15 units on the planimeter equal 1 cm^2; the magnification of the camera lucida drawings was 176 and thus for the arbitrary units in Table 3, 1 unit equals 215 μm^2.

Expt. II. Thirty male mice, aged 3–4 months, of an inbred strain derived from the 'pathogen free' Walter Reed Hospital Swiss mice were used. Approximately half an hour before an area of approximately 4 cm^2 on the dorsum was plucked, the mice were anaesthetized with sodium pentobarbital solution (1·5 c.c. normal saline, 1·0 c.c. Nembutal); 30 d later, hair was plucked from the same area on the mid-dorsum to ensure that the follicles were in quiescence (Telogen). Each mouse received 100 μc ^{3}H-thymidine (sp.act. 3·6 c/mM) purchased from New England Nuclear Corporation, Boston, 45 min before they were killed at various intervals between the time of plucking and 21 d later. The integument was immediately fixed in cold acid alcohol (1 part glacial acetic acid to 3 parts 100 % ethanol) for 24 h, gradually hydrated, then hydrolysed in HCl at 60 °C for 10 min and stained by the Feulgen technique. For squashes, the skin was transferred to 45 % acetic acid in a depression slide. Under the dissecting microscope, the panniculus carnosus and adiposus were peeled off leaving the follicles protruding like hundreds of fingers projecting upwards in rows. The bulbs were dissected from the follicles with fine needles, transferred to a clean slide and squashed. The cells spread out, and separated well in most of the squashes (Fig. 2). For sections, the stained tissue was embedded in paraffin according to routine methods; longitudinal sections of hair follicles were cut at 5 μm.

Autoradiographic procedure

NTB liquid emulsion from Eastman Kodak was applied by the dipping technique (Messier & Leblond, 1957). For exposure, the slides were placed in light-proof boxes with the desiccant Dryerite, and kept for 5 d at −20 °C. After being developed in Kodak D19 solution, fixed in Kodak acid fixer, and washed in running tap water for a minimum of 1 hr, the preparations were permanently mounted in Euparol.

Quantitative data

For quantitative analysis in autoradiographs, squash preparations were used, and the total number of bulb cells, mitoses, and labelled cells was determined. Connective tissue cells were easily distinguished by their long spindle-shaped nuclei, and ex-

cluded from the count. Only nuclei with 5 or more grains were considered to be labelled. The mean labelling index (L.I.), the mean mitotic index (M.I.), and the mean number of cells was determined for each squash preparation for both the large and small bulbs:

$$\text{L.I.} = \frac{\text{Number of labelled cells}}{\text{Total number of cells}} \times 100.$$

$$\text{M.I.} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100.$$

RESULTS

A. *Morphology*

The plucking of hairs from quiescent follicles removes the inner sheath which is interlocked with the hair, and leaves a cavity surrounded by the partially collapsed wall of the outer sheath (Fig. 3). Pycnotic cells are observed within the cavity and in the pilary canal. Adhering to the basal border of the outer sheath is the basement membrane which separates the epithelial tissue of the skin and follicle from the connective tissue of the dermis, the dermal papilla, and the connective tissue sheath

Table 1. *Measurements (μm) of the mean thickness of the various layers of the skin during the hair cycle*

Time after Plucking (d)	Epidermis	Dermis	Adipose	Epidermis to p. carnosus
2	28	290	120	438
7	23	290	270	583
11	17	350	460	827
15	17	280	440	737

of the hair follicle. After plucking, a ball of compact papilla cells is separated by a gap from the cells of the germinal bulb (Fig. 4). A cone of reticulin fibres (PAS+) and spindle-shaped fibrocytes extends to the panniculus adiposus from the papilla of each follicle (Fig. 5). The thickness of each layer of the skin is greatly reduced (Chase *et al.* 1953). The fat cells are oriented parallel to the epidermis (Fig. 5). Within 24 h, the outer sheath collapses, forming a stalk of cells between the sebaceous glands and dermal papilla which replaces the cavity formed by the excision of the hair and its inner sheath (Fig. 4). By 37 h after plucking, the compact ball of papilla cells begins to change into an inverted triangle with its base slightly curved upward for maximum contact with the germinal bulb cells. The round papilla cells move apart and begin to become spindle-shaped. The cells of the adipose layer are parallel to the epidermis.

Two days after plucking, the dermis is 2–3 times as thick as the adipose layer (Table 1). The hair follicles are within the dermis, and the longer, thicker bulbs of the guard hairs can be easily distinguished from the much shorter, thinner bulbs of the undercoat. All follicles are growing and expand downward into the cone of reticulin fibres; by the 3rd d the dermal papilla is completely enclosed by the bulb of

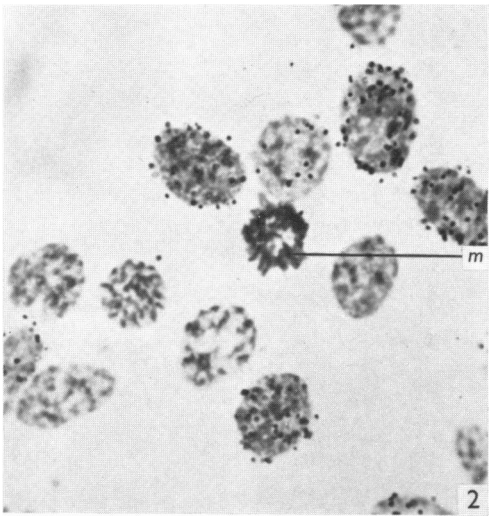
the large hair follicle which is beginning to form the hair, but in the small hair the papilla is not fully enveloped by the follicle, nor has hair formation begun (Fig. 8). The fat cells still form a layer parallel to the epidermis. By the 4th d, all the large follicles have grown down to and a few into the adipose layer itself, while the small follicles are still within the dermis. The basement membrane around the growing follicles is intact. In the large follicles the hair has grown up to the base of the sebaceous glands while in the small follicles it has begun to develop. By the 5th d, the larger follicles are penetrating the adipose layer within the reticulin cone, while the fat cells become oblong and begin to align themselves parallel to the length of the hair follicles. Hairs from large bulbs reach the epidermis and some even protrude above the skin for approximately 30 μm (Fig. 8), but in the small follicles they have only reached the sebaceous gland. The large follicles in the p. adiposus begin to curve or bend as they approach the p. carnosus on the 6th d.

The large follicles have penetrated the bottom of the adipose layer by the 7th d but the small ones are still in the dermis with their bulbs just touching the adipose layer. During the first week after plucking, the rate of growth of the small follicle is 57 % that of the large one (Fig. 9, Table 2). The hair in the large follicles protrudes well beyond the surface of the skin and that of the small follicles has just reached the surface of the skin with only a few hairs piercing the surface. The large papillae are completely encased by the bulb, and a cap of connective tissue consisting of extruded dermal papilla cells forms at the junction of the papilla with the dermis. In the small follicles also the bulb encloses the dermal papilla completely, but the cap has not appeared. The fat cells are all oblong and aligned parallel to the length of the follicles; the dermis equals the adipose layer in thickness (Table 1).

Table 2. *Measurements (μm) of the mean length of the hair follicles and dermal papilla during the hair cycle*

Time after plucking (d)	Hair follicles		Dermal papilla	
	Large	Small	Large	Small
1	256	205	50	30
2	298	235	—	—
5	590	381	—	—
7	951	585	110	70
11	1200	1020	130	70
13	1480	1280	220	90
15	1400	1270	150	90
17	1340	1260	120	90

On the 11th d both types of follicle extend down to the p. carnosus but the large, thick follicles are bent to allow for additional growth parallel to the epidermis. Cap formation now begins on the small bulbs. The oblong adipose cells are still parallel to the length of the follicles (Figs. 6, 7). By the 13th d both types of follicles have grown 6× longer than on the 1st d after plucking (Table 2); the dermal papilla of the large bulbs is 2× as long as that of the small bulbs (Table 2). The adipose cells are still oblong, and parallel to the length of the follicles, and the basement membrane around the follicles remains intact.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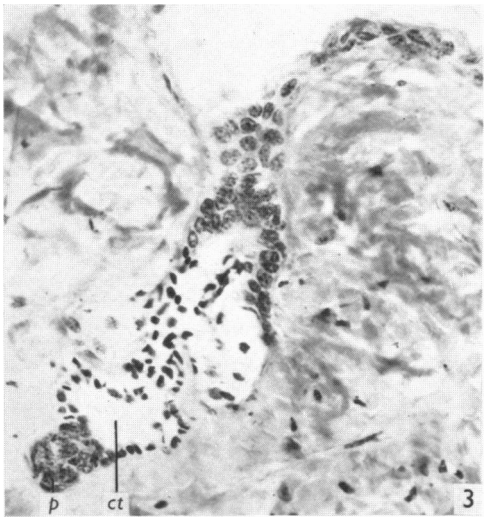
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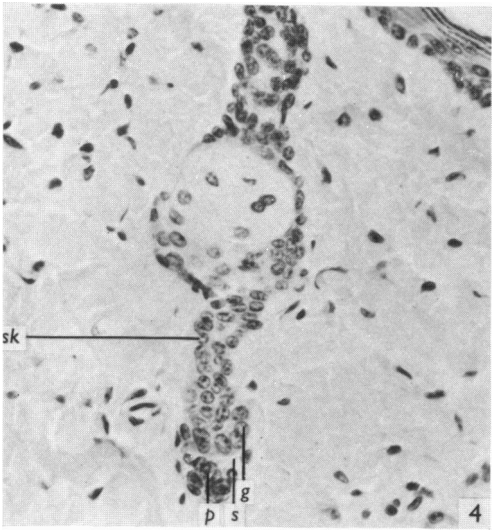
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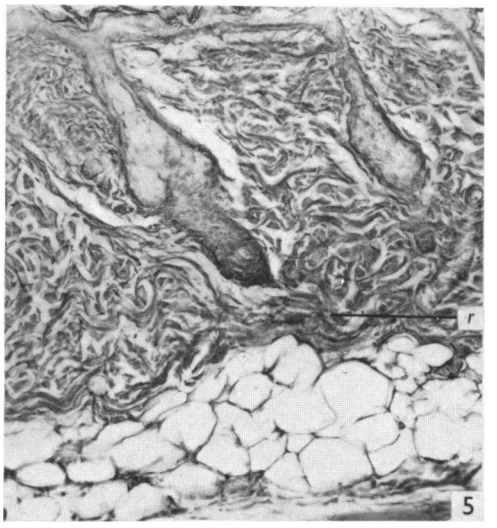
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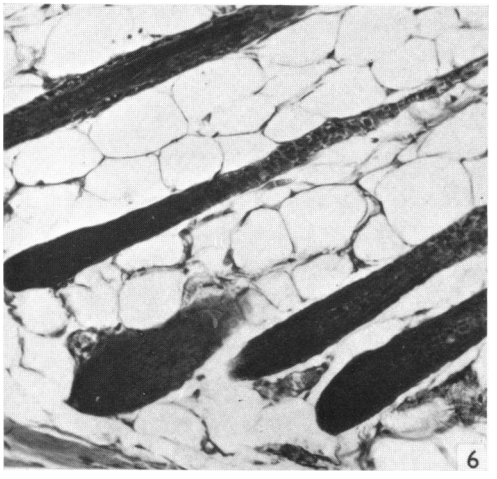
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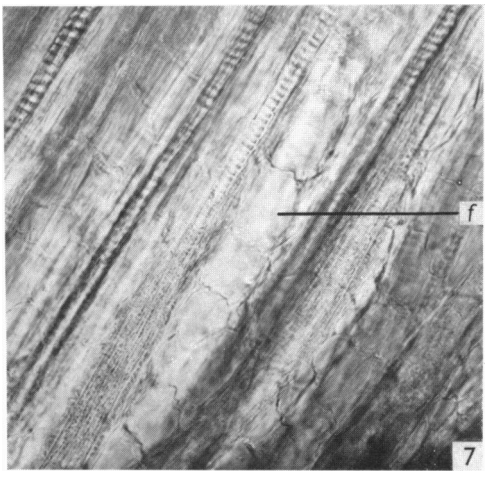
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B. *Kinetics*

To determine whether the increase in the thickness of the adipose and the fibrous dermal layers during the hair growth cycle was due to a redistribution and reorientation of tissue, or to an increase in the amount of each, the area (volume) of the various skin layers and the rate of growth of the large and small follicles and their hair, were measured.

By the 11th d after plucking, the area of fat between two small follicles increases

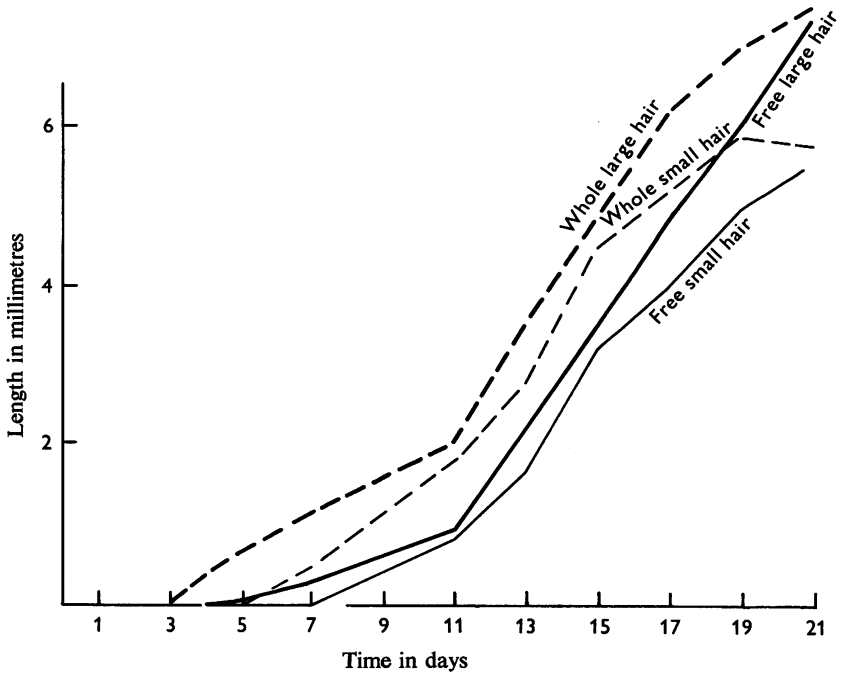

Fig. 8. Graph showing the growth in length of the large (Guard) and small (Undercoat) hairs after plucking.

Fig. 2. Autoradiograph of squashed bulb, 11 d: note the metaphase chromosomes (*m*) in the centre surrounded by labelled and unlabelled proliferative cells of the bulb matrix. Feulgen. (× 2000.)

Fig. 3. 0-d follicle: note the cavity (*ct*) in the follicle below the sebaceous gland where the plucked hair was formerly located, and the dermal papilla (*p*). Haematoxylin and eosin. (× 375.)

Fig. 4. 1-d follicle: note the compact ball of dermal papilla cells (*p*) at the bottom of the follicle separated by a space (*s*) from the germinal bulb (*g*) of the follicle below the stalk (*sk*) of cells formed by the collapsed outer sheath. Van Giesen. (× 375.)

Fig. 5. 2-d follicle: note the rounded fat cells in the adipose layer and the cone of reticulin fibres (*r*) extending down to the p. adiposus. Haematoxylin and eosin. (× 175).

Fig. 6. 11-d follicle (thin section): note fully developed, elongated fat cells parallel to the length of the follicles. PAS+. (× 175.)

Fig. 7. 11-d follicle (thick section): a section of the same tissue in Fig. 6, but to retain the maximum amount of lipids, the tissue has not been processed through the alcohols or xylene. Note ovoid fat cells (*f*). Carmalum. (× 175.)

sharply by a factor of 8, while the area of the dermis increases gradually by a factor of 2 (Table 3). The thickness of the adipose layer quadruples by the 11th d (Table 1), whereas the thickness of the dermis remains unchanged during the first week, and then reaches its maximum by the 11th d after plucking. The thickness of the epidermis is maximal on the 2nd d after plucking, and steadily decreases until the 11th d. The planimeter and micrometer measurements of the adipose layer indicate that the increase in area of the fat is double the increase in thickness of the adipose layer between the 2nd and 11th d.

Table 3. *Planimeter measurements of the areas of the dermis and fat between small hair follicles*

(1 unit = 215 μm^2.)

Time after plucking (d)	Dermis	Fat
2	170	100
7	272	407
11	379	803

The length of the dermal papilla of the small follicles doubles during the first week, and has trebled by the 13th d after plucking (Table 2).

The large follicles initially grow faster than the small follicles (Fig. 9, Table 2). However, for a period during the second week, the small follicles grow at a somewhat faster rate than the large follicles; between the 7th and 11th d after plucking, the growth of the large follicles begins to taper off while that of the small follicles increases.

The downward growth of the hair follicle within the cone of reticulin fibres extending down to the p. adiposus is facilitated by the change in orientation of the fat cells, and the increase in volume of the dermis and especially of the p. adiposus. The follicle grows as a unit without piercing the basement membrane and simultaneously increases in width and differentiates through the production of keratin. As the follicles thicken and lengthen, the spherical fat cells change from an orientation parallel to the epidermis to one parallel to the growing follicle (Figs. 6, 7). The follicles become thickest in the adipose layer where the actively growing part lies, and this leads to the continued diminution of the space between adjacent follicles in mid-Anagen. The increase in volume and thickness of the dermis and adipose layers precedes the growth of the follicles, allowing them to change their angle to the epidermis from almost 90° during Telogen to approximately 45°. The increase in volume of the fat provides the follicles with more space for 3-dimensional growth and offers less resistance to expansion than the fibrous dermis. The change in angle of the follicle, the increase in thickness and volume of the p. adiposus, and the bending of the follicle bulbs on reaching the p. carnosus allow the follicle to grow much longer than the maximum thickness of the skin between the epidermis and p. carnosus (Tables 1, 3).

The large follicles grow faster than the small follicles during the first week but by the 11th d, both grow linearly at the same rate (Table 2), and reach their maximum length by the 13th d which is maintained until the 17th d (Table 2, Fig. 9). The

dermal papilla of both types of follicles reaches its maximum length at the same time as the follicles, i.e. on the 13th d, (Table 2). On the 21st d the Whole Hair in the large follicles is 5× and in the small follicles 4× as long as the follicle on the 13th d after plucking (Fig. 9); thus a follicle produces a hair 4–5× its own maximum length. At the end of the hair growth cycle (Catagen), the follicles become shorter and narrower, thus decreasing the Whole Hair; the length of the Free Hair continues to lengthen because of continued keratinization of the hair shaft (Fig. 8). The growth of the Free Hair of both the small and large follicles is approximately linear between the 11th and 21st d after plucking.

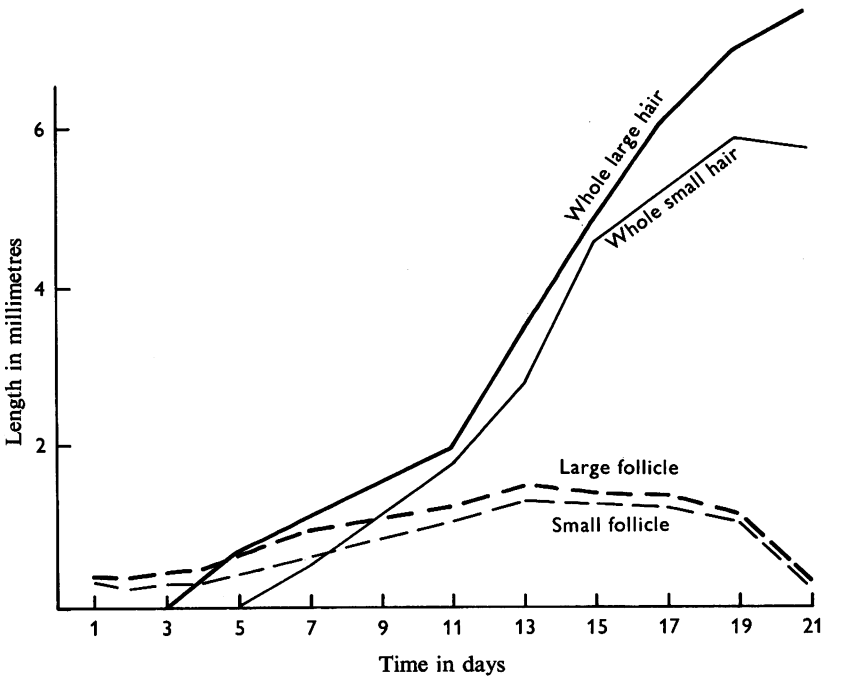

Fig. 9. Graph showing the growth in length of follicles and whole hair following the plucking of hairs from large and small hair follicles.

C. *Cytology*

The number of epithelial cells in the bulb of the large hair follicles is greater than that of the small follicles (Fig. 10). The long, overcoat hairs have 1·5–3× as many medullary cells as the short, undercoat hair with its single layer of medullary cells. During mid-Anagen, i.e. day 11, the bulb of the large follicles has a mean number of 3499 epithelial cells compared with 1246 in the small follicle (Fig. 10). The total number of epithelial cells in the large bulbs rises sharply between the 1st and 11th d after plucking, whereas in the small bulbs it increases very gradually. Between the 11th and 18th d after plucking, however, the cell population of the large bulbs declines much more rapidly than that of the small bulbs (Fig. 10).

The growth rate of the bulbs has been analysed in terms of the labelling index (L.I.) and the mitotic index (M.I.) in autoradiographs of squashes made after injection of

^{3}H-thymidine. One day after plucking, the mean L.I. in the large bulbs is almost double that of the small bulbs (Fig. 10), but by the 11th d the L.I. in the small bulbs is greater than that in the large ones. Subsequently, the L.I. of the small bulbs decreases sharply while in the large bulbs it remains stationary at first and then falls rapidly on the 17th d; in both, the L.I. reaches the same low level by the 18th d (Fig. 10). Mitosis is absent for 24 h after plucking in both types of bulb. By the 11th d, the M.I. is at its peak in both large and small bulbs with the large bulbs having a lower M.I. than the small (Fig. 11). By the 19th d, mitosis ceased (Fig. 11) in both types of bulbs. The L.I. and M.I. of the dermal papilla is nil at all times.

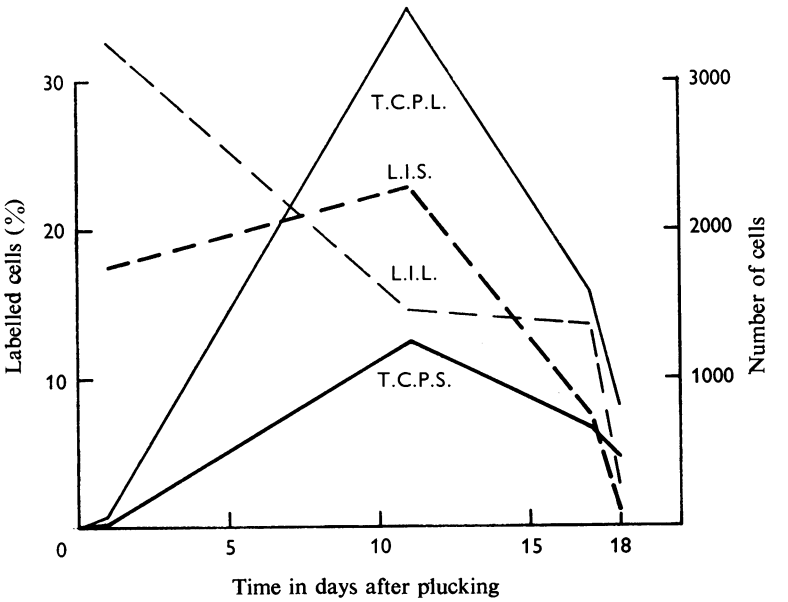

Fig. 10. Graph of the total cell population and labelling index of large and small hair follicles. T.C.P.L. = total cell population of large hair follicle bulbs; T.C.P.S. = total cell population of small follicle bulbs; L.I.L. = labelling index of large follicle bulbs; L.I.S. = labelling index of small follicle bulbs.

Up to the 11th day the large follicles and hairs generally grow faster than the small ones, but subsequently the growth rates, M.I. and L.I. of large and small follicles and hairs are about the same. Between the 11th and 21st d the rate of growth of the hair itself rises sharply at a time when the growth of the follicles has ceased. Minor variations in L.I. and M.I. (Figs. 10, 11) do not affect the approximately equal rate of lengthening of small and large hairs which is due to the increase in the volume as well as the number of keratinizing cells. The contribution of the enlargement of the keratinizing hair cells to the growth in length is illustrated by the fact that small and large hairs continue to grow through the 21st d when the L.I. and M.I. are at their lowest level. The hair of the large follicles grows initially faster than those of the small ones, but later the rate of growth of small and large hairs is the same. For both, the rate increases on or about the 11th d and remains constant for the next 10 d.

DISCUSSION

Mice have relatively short- and short-lived hairs as compared with the scalp hair of man (Ebling, 1964) and of some sheep (Ryder, 1964). The length and girth of the overcoat and undercoat hair of mice is closely correlated with that of the follicles; this correlation is important for the formation and growth of hair and for its mechanical anchoring within the skin. There is obviously no such close correlation between length of the hair of sheep and men and the length of the follicles. The remarks that follow apply only to the mouse.

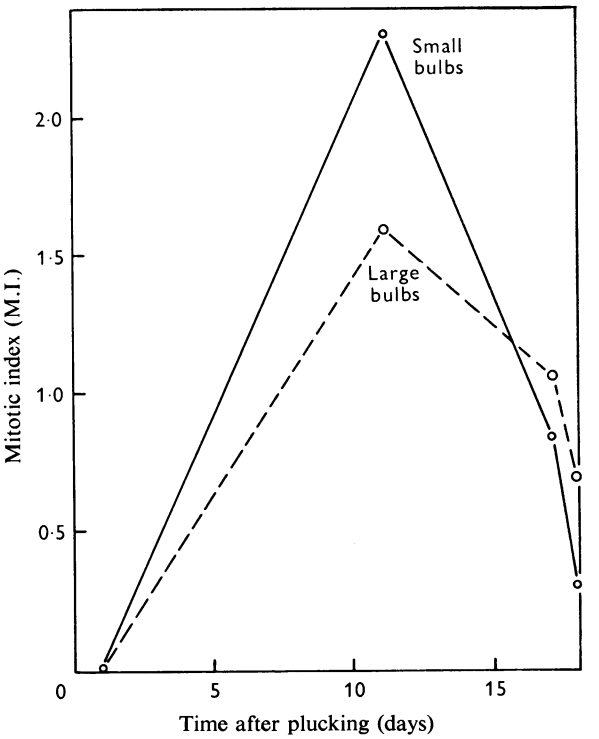

Fig. 11. Graph of the mitotic index of squashed large and small hair follicle bulbs.

The regression of the follicle in Catagen and its subsequent expansion in Anagen are associated with cyclical changes in the thickness of the fibrous dermis and the dermal fat (Chase *et al.* 1953). Since in Anagen the follicles increase in length and thickness while the total length of the mouse and of its skin remain constant, space for the expansion of the follicles can be obtained only by the thickening of the adjacent structures. This is achieved partly by a redistribution and partly by an increase in volume, particularly of the dermal fat. The fat is redistributed from an orientation parallel to the skin to a vertical arrangement parallel to the hair follicles. Planimetric measurements show a genuine increase in volume of fat and this along with the rearrangement of the fat increases the space in which the growing follicle can expand.

Additional space is gained through a shift in the angle of the follicle with the dermis and fat from about 90° to less than 45°, and by the bending of the end of the follicle parallel to the panniculus carnosus. Changes in dermal thickness during the hair cycle are less than those of the fat. This is evident from Tables 1 and 3 and is also indicated in the diagram of Chase *et al.* (1953), which shows between Telogen and Anagen VI a two- to threefold increase in the thickness of the fat layer and of only one-half of the dermis in the same period. As already pointed out, the greatest expansion of the follicle in length and girth occurs in the adipose and not in the fibrous layer of the dermis.

The increase in thickness of the fat layer occurs simultaneously with the growth of the follicle and may actually precede it. A direct comparison of the rate of expansion of fat and follicle (Tables 1 and 2) is made difficult by the change in the angle of the hair follicle. The slow rate of the change in angle of the follicle and of its growth may obscure any evidence of mechanical pressure of the growing follicle on the surrounding fat even if such an effect exists. The rapid metabolic activity of fat (Chalmers, 1964) with deposition in primitive fat cells in Anagen and resorption in Catagen and Telogen may help to facilitate the growth of the follicle. Whether these changes in turn are secondary to alterations in the amount of dermal collagen (Ebling, 1964) remains to be established. On the other hand, Carruthers, Davis & Quevedo (1960) find no evidence for a change in the percentage composition of the lipids, phospholipids, cholesterol esters, triglycerides, cholesterol, or water in the mouse epidermis and dermis (including the p. adiposus and p. carnosus) during Anagen VI and Telogen which represents the times of maximum morphological differences in skin thickness. As noted by Carruthers *et al.*, these results are in apparent disagreement with those of Chase *et al.* (1953), Gibbs (1941), and Andreasen (1953), who also found an increase in thickness of the fat layer during Anagen.

The dermal papilla is enclosed by the hair bulb only after the latter has started to grow, and is thus unlikely to induce this proliferation. As suggested by Van Scott & Ekel (1958), the papilla may have a function during Anagen, i.e. when hair formation begins. The dermal papilla and the follicle stop growing at about the same time, while the growth of the hair continues. The growth of the bulb before it makes closer contact with the apparently quiescent papilla, and the neogenesis of hairs (Breedis, 1954) make it unlikely that the papilla evokes the growth of the bulb. Montagna points out that the cells of the papilla do not seem to divide. This observation is confirmed by our data and extended by the fact that the papilla cells do not take up tritiated thymidine. Thus it appears that once formed the papilla cells persist throughout the life span of the follicle. The main function of the papilla may simply be the anchorage and positioning of the follicle within the integument and help in the formation of hair throughout some phases of the hair cycle. Without a papilla, the hair will still grow but the follicles lack definitive form (Butcher, 1965).

The epidermal thickness (Table 1) of our mice appears to decrease after Telogen, whereas in the diagram of Chase it increases until Anagen II. Our results agree with those of Chase, however, since our epidermal measurements were begun two days after plucking (Anagen I) when the epidermis would have reached almost its maximum thickness (Chase *et al.* 1953).

The hair follicle grows as a whole within its intact vitreous membrane and in

association with the surrounding structures. The overcoat follicles lead in the growth as shown by the labelling index, and measurements of length. Though later small and large hairs grow at the same rate, the large follicles start earlier and have a higher labelling index than the small ones (Fig. 10). On the other hand, their growth begins to decline earlier than that of the small follicles. It is difficult to account for the differences in the beginning and rate of growth of small and large follicles by changes in the environment rather than by the differences between the follicles.

SUMMARY

1. The developing hair follicles grow downward through the p. adiposus within a cone of reticulin fibres and with an intact basement membrane. As the growing hair follicle lengthens and thickens, the fat cells in the p. adiposus change from an orientation parallel to the epidermis to one parallel to the growing follicle.

2. An increase in the volume of the dermal-adipose layers precedes the downward growth of all types of hair follicles.

3. The cells of the dermal papilla do not take up any tritiated thymidine during the hair cycle and do not divide. In the early stages after plucking of hairs, the papilla cells are clumped and separated from the follicle. The follicle comes into closer contact with the enlarging papilla and envelops it between the 3rd and 4th d after plucking. Hair production only begins at this time when the dermal papilla is completely enveloped by the growing follicle. On the 7th d in large and on the 11th d in small follicles, dermal papilla cells are extruded and form a cap of connective tissue at the junction with the dermis.

4. The increase in thickness and volume of the p. adiposus, a change in angle of the follicle, and the bending of the follicle bulbs on reaching the p. carnosus allow the follicles to grow much longer than the maximum thickness of the skin.

5. Hair grows most efficiently only after the maximum growth of the follicle is achieved. The large follicles and their hair (guard hairs) initially grow faster than those of the small (undercoat). The growth rates of both types of hair are equal on or about the 11th day after plucking when the follicles have almost reached their maximum size, and is maintained throughout the remainder of the hair growth cycle.

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REFERENCES

ANDREASEN, E. (1953). Cyclic changes in the skin of the mouse. *Acta path. microbiol. scand.* **32**, 157–163.

ASHMORE, H. & UTTLEY, H. (1965). Measurement of the rate of growth of rodent hair using cystine labelled with sulphur-35. *Nature, Lond.* **206**, 108–109.

BREEDIS, C. (1954). Regeneration of hair follicles and sebaceous glands from the epithelium of scars in the rabbit. *Cancer Res.* **14**, 575–579.

BUTCHER, E. O. (1934). The hair cycles in the albino rat. *Anat. Rec.* **61**, 5–19.

BUTCHER, E. O. (1951). Development of the pilary system and the replacement of hair in mammals. *Ann. N.Y. Acad. Sci.* **53**, 508–516.

BUTCHER, E. O. (1965). The specificity of the hair papilla in the rat. *Anat. Rec.* **151**, 231–238.

CATTANEO, S., QUASTLER, H. & SHERMAN, F. (1961). Proliferative cycle in the growing hair follicle of the mouse. *Nature, Lond.* **190**, 923–924.

CARRUTHERS, C., DAVIS, B. & QUEVEDO, W. C. (1960). Influence of hair growth cycle on lipid composition of mouse epidermis and dermis. *Proc. Soc. exp. Biol. Med.* **105**, 259–261.

CHALMERS, T. M. (1964). Structure and metabolism of adipose tissue. In *Progress in the Biological Sciences in Relation to Dermatology* 2, pp. 165–174. Eds. A. Rook and R. H. Champion. Cambridge University Press.

CHASE, H. & EATON, G. (1959). The growth of hair follicles in waves. *Ann. N.Y. Acad. Sci.* **83**, 365–368.

CHASE, H., MONTAGNA, W. & MALONE, J. (1953). Changes in the skin in relation to the hair growth cycle. *Anat. Rec.* **116**, 75–82.

COLLINS, H. H. (1918). Studies of normal moult and of artificially induced regeneration of pelage in *Peromyscus. J. exp. Zool.* **27**, 73–99.

DAVID, L. T. (1934). Expression of genetic hairlessness in the house mouse (*Mus musculus*). I, II, III, IV. *J. exp. Zool.* **68**, 501–518.

DRY, F. W. (1926). The coat of the mouse (*Mus musculus*). *J. Genet.* **16**, 287–340.

EBLING, F. J. (1964). The hair follicle. In *Progress in the Biological Sciences in Relation to Dermatology* 2, pp. 303–324. Eds. A. Rook and R. H. Champion. Cambridge University Press.

GIBBS, H. F. (1941). A study of the post-natal development of the skin and hair of the mouse. *Anat. Rec.* **80**, 61–82.

HUGHES, W. L., BOND, V. P., BRECHER, G., CRONKITE, E. P., PAINTER, R. V., QUASTLER, H. & SHERMAN, F. G. (1958). Cellular proliferation in the mouse as revealed by autoradiography with tritiated thymidine. *Proc. natn. Acad. Sci. U.S.A.* **44**, 476–483.

MESSIER, B. & LEBLOND, C. P. (1957). Preparation of coated radioautographs by dipping sections in fluid emulsion. *Proc. Soc. exp. Biol. Med.* **96**, 7–10.

MONTAGNA, W. (1962). *The Structure and Function of Skin*, p. 244. London: Academic Press.

RYDER, M. L. (1964). Moulting and hair replacement. In *Progress in the Biological Sciences in Relation to Dermatology* 2. Eds. A. Rook and R. H. Champion. Cambridge University Press.

VAN SCOTT, E. J. & EKEL, T. M. (1958). Geometric relationships between the matrix of the hair bulb and its dermal papilla in normal and alopecic scalp. *J. invest. Derm.* **31**, 281–287.