THE MORPHOLOGY AND DEVELOPMENT OF "RAGGED" - A MUTANT AFFECTING THE SKIN AND HAIR OF THE HOUSE MOUSE.

Thesis submitted to the Faculty of Science of the University of Edinburgh for the Degree of Doctor of Philosophy

by

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INTRODUCTION

The hair coat is a structure peculiar to mammals; its main functions are those of protection and the maintenance of body temperature. Individual hair fibres are resilient keratinised structures; each fibre grows from a hair follicle which is a specialised downgrowth of the epithelium. Hairs can be classified into types according to their morphology and function, e.g. Danforth (1925) distinguishes:

(1) The tactile hairs, which are long and stiff and grow from specialised follicles containing erectile tissue.

(2) Hairs growing from non-specialised follicles. These hairs form the main coat or pelage of the animal. They can be classified into numerous groups according to their size and rigidity, but the most important two types are:

(a) The relatively coarse and stiffened overhair or guard hairs.
(b) The relatively fine and soft underhairs.

The morphology of most types of hair fibres is basically similar. The external cuticle of the hair consists of overlapping scales of varying size and shape (Hausman 1924). The underlying cortex is composed of long, fusiform, shrunken cells producing a rigid hyaline mass. The internal medulla, which is absent in some types of hair, consists of a cornified epithelium which may be continuous or fragmented to form a discontinuous series of cells or chambers. The type of internal structure so formed is often used as a means of classifying different types of hair fibres (Dry 1926, Fraser 1951).

The embryology and development of hair follicles and fibres has been extensively described in many different mammals. The most
2.

important studies have been made in man (Stohr 1903), in the mouse (Oyama 1904, Gibbs 1941, Hardy 1949, and Balinsky 1950), in the guinea pig (Segall 1918), and in the rat (Fraser 1928, Schamberg and Saleably 1930, Danel 1931, and Butcher 1934 and 1951). Hanson (1947) has discussed the related subject of the development of the epidermis in the rat and the mouse. The main features of hair follicle development are probably similar in all mammals. Development begins by a thickening of the germinative layer of the epidermis and a concentration of nuclei in the subjacent mesenchyme; which of these two modifications actually initiates follicle development is unknown. The basal part of the epidermal thickening grows downwards and differentiates to form the follicle bulb. The more distal regions of the thickening form the epithelial part of the follicle or the external sheath; which even in mature follicles is composed of undifferentiated epidermal cells. The keratinised internal sheath of the follicle is formed by cell division and differentiation; the highly keratinised hair shaft which is formed by division and differentiation of the follicle bulb cells, grows upwards through the previously formed internal sheath and hair canal, and emerges by piercing the superficial stratum corneum of the skin.

After their original development and production of the first hair, the follicles in most mammals enter a period of rest during which there is no hair proliferation. Except in the merino sheep, where hair growth is more or less continuous, all types of follicles undergo successive periods of rest and hair proliferation; a period of hair growth and a period of rest together constitute a complete hair cycle. In many animals the cycles are seasonal; the weasel, mink, stoat and hare having two cycles per year, and the fox, one
In some cases, as in the mouse, the growth of the new hair may not result in the pushing out of the old hair from the follicle concerned.

In the rat and the mouse, each hair cycle is of comparatively short duration and the nature of the cycles has been the subject of considerable study. The growth cycles of individual hair follicles in the mouse have been carefully described by Dry (1926), Chase, Rauch and Smith (1951) and Chase (1954). The period of hair proliferation is known as the anagen stage, during which the hair follicle becomes elongated by proliferation of the external sheath cells and grows down into the adipose layer of the skin. This stage lasts for about 17 days. During the second or catagen stage, the completed hair fibre moves upwards inside the hair follicle until the hair club lies almost opposite the sebaceous gland, and the hair follicle shortens to its original length. This stage of the cycle lasts 2-3 days. The third or telogen stage of the cycle is a resting stage during which there is no change in the hair follicle and no hair proliferation. This stage lasts about 10 days, or longer in old mice. The whole cycle therefore lasts about one month.

Two main kinds of hair growth cycle can be distinguished. First, as in man, where the hair follicles in any region of the body progress independently of each other through the hair growth cycle, so that neighbouring follicles may be in different stages of the growth cycle at the same time. Secondly, as in the rat and the mouse, where neighbouring hair follicles are generally in the same stage of the growth cycle at any specific time, and enter further stages of the cycle synchronously. These two types of hair growth cycle could be termed respectively, autonomous and synchronised.
The synchrony of the latter type, however, although it applies within any specific region of the body, does not operate over the whole body area. Therefore all the hair follicles in one area may be in the same phase of the growth cycle whilst at the same time the hair follicles in a different body area of the same animal may all be in a different phase of growth. This results in gradually progressive waves of hair growth across the body area, a phenomenon which has been described by a number of authors; e.g. Butcher (1934), Haddon, Elson, Roe, Rudall and Timmis (1945), Haddow and Rudall (1945) and Hellinga (1945) in the rat, Collins (1918 and 1923 in the deer mouse, and Dry (1926), Wolbach (1951), and Andreassen (1954) in the house mouse. In the mouse and the rat, only limited and fairly sharply demarcated regions of the body can regenerate hair at any given time. The regenerating areas at all times form a bilaterally symmetrical pattern on the body. In the mouse, the wave of hair growth generally progresses in an anterior-posterior direction, and successive waves of growth occur approximately at monthly intervals. Once initiated in a specific area, each growth wave moves across the body according to its predetermined pattern.

Attempts to discover the cause of the initiation and control of hair growth cycles have resulted in a great deal of experiment and speculation. Probably the most important causal factor is the endocrine release of a hormone or some alteration of the balance of hormones in the bloodstream. There have been many experiments designed to study the effect on hair growth of removing one or other of the endocrine glands or the gonads, or of injecting various hormones; e.g. Butcher (1937, 1939 and 1940), Baker and Whitaker (1948), Baker, Ingle, Li and Evans (1948),
Castor and Baker (1950). Reviews have also been published by Baker (1951) and Houssay (1954). In most of the experiments described rats were used, and in a few, mice. Certain conclusions can be drawn from these experiments: the androgenic and oestrogenic hormones secreted by the gonads inhibit hair growth, the adrenal hormone inhibits hair growth, and the thyroid hormone stimulates hair growth; the hypophysis of the pituitary gland can stimulate hair growth indirectly by activating the thyroid gland, or directly by the secretion of a growth hormone, and it can inhibit hair growth indirectly by the secretion of ACTH and gonadotrophic hormones which activate the adrenal glands and the gonads respectively. In the case of the synchronised type of hair growth cycle such as is found in rats and mice, it seems that hormones may play an important part in initiating the cycles; in the autonomous type of hair growth found in man it is possible that hormones have a less decisive role in this respect.

Inherited defects of the hair causing partial or almost complete nakedness have been reported in very many kinds of mammals. As is pointed out by Thigpen (1951), hairlessness can be caused by a number of different abnormalities: e.g. the partial agenesis of hair follicles as in the rabbit (Kislovsky 1928) and the pig (David 1932b), delay in follicle formation as reported in cattle by Mohr and Wriedt (1928), or excessive keratinisation of the hair follicles as in the rabbit (Drapeau 1933). Other types of abnormality are shown by examples of hypotrichosis in the mouse. In the recessive hairless house mouse (hr) and the rhino house mouse (hr^Rh) hairlessness appears after an apparently normal first pelage. The first mutant has been described by Crew and Mirakaia (1932) and David (1932) who stated that the loss of hair was due to imperfect for-
ation of the hair club, and by Fraser (1946) and Steinberg and Fraser (1946) who ascribed the hair loss to an abnormal widening of the hair canals of the follicles. The second mutant has been studied also by Fraser and by Steinberg and Fraser, and again the main hair follicle abnormality was found to be a widening of the hair canal.

In the case of hypotrichosis juvenilis (hj), a gene reported by Loeffler (1934), only the first, juvenile coat is abnormal. The histological abnormalities are similar to those in hairless (hr) mice. In ichthyotic (ic) mice, described by Carter and Phillips (1950), the hair growth is markedly subnormal, but the cause of this has not been investigated. The histology of the mutant naked (N) in the mouse, has been described by David (1930, 1931, and 1932a) and by Steinberg and Fraser (1946). Hair loss, which occurs periodically after the growth of each successive pelage, was attributed to imperfect keratinisation of the hairs which caused them to break off after a certain period of growth.

For the present study those types of hair defect which are caused by partial follicle agenesis or the delayed formation of follicles are probably the most relevant. The only example of this kind in the mouse is the mutant crinkled (or) which has been described by Falconer, Fraser and King (1951). The abnormalities caused by the crinkled gene can only be fully understood in the light of certain features of hair growth in the normal mouse.

The structure of the mouse coat has been examined by Dry (1926) and by Fraser (1951). Five main types of hair fibre were distinguished; namely, sinus hairs, guard hairs, awls, auchenes and zigzags.

It has been shown by Gibbs (1941) that the hair follicles
of the mouse develop, at least post-natally, in a chronological series of waves which are definable as groups according to their times of development. Moreover, Dry has stated that any particular hair follicle almost always produces a similar type of fibre in successive hair generations.

These last facts became more significant after the analysis of the crinkled mutant, which causes the mouse coat to be totally lacking in guard hairs and zigzags. This condition in the adult is associated with the absence of that group of hair follicles which normally develops in the 14-17 day embryo, and with the apparent absence also of those follicles usually formed after birth. These results gave rise to the concept that each of the adult hair fibre types is associated with one of the successive time groups of developing hair follicles. On this theory, the first group of hair follicles initiated in the 12-14 day embryo gives rise to the simus hairs, the second group of follicles initiated in the 14-17 day embryo produces guard hairs, the third group initiated in the 17-19 day embryo produces awls and auchenes, and the fourth group of follicles initiated after birth produces zigzag fibres.

The mutant ragged (Ra) which is the subject of the present investigation, is a semi-dominant mutation whose gross effects have been described by Carter and Phillips (1954). These authors have mentioned in the heterozygote: shortening of the vibrissae, reduction in the number of zigzag hairs, and agouti colouration changes; and in the homozygote: oedema at birth, shortening and reduction in number of the simus hairs, and quasi-nakedness of the adult. They pointed out that ragged mice partially resemble crinkled mice in lacking some zigzag fibres, but differ in having guard
It was observed that ragged mice provided suitable material for the study of hair growth and for testing the concept that different hair fibre types were associated with different embryological phases of follicle development.

The present thesis describes the adult morphology and the development of the sirus hairs, the coat and the skin of ragged heterozygote and homozygote mice. This work has involved a careful study of the causal connections between different abnormalities; the guiding principle underlying this analysis was that propounded by Grünberg (1943a) as the Unity of Gene Action. This principle postulates that any mutant gene has a single primary action which is either cell-specific or tissue-specific. From the primary action of the gene may result a hierarchy of physiologically related abnormalities, some of which may be more prominent than the primary abnormality. The interpretation of the results of the ragged investigation has been simplified by adherence to Grünberg's postulates.
ROCKS AND GENETICS

As reported by Carter and Phillips (1954), the ragged mutation arose in a female of a cross-bred stock. To provide material for the present work the ragged gene has been kept segregating in this heterogeneous stock so that normal and heterozygous ragged sibs were always available for comparison. For the work on adult morphology, female mice were mostly used.

The backcross breeding data obtained were in accordance with expectation (Table 1a). The intercross data however showed a shortage in the ragged homozygote class (Table 1b); and furthermore, from the intercross matings almost all ragged homozygotes which were born died at birth or soon after. One viable, presumptive ragged homozygote male was eventually reared, and this mouse when mated to normal females produced 37 offspring which were all ragged heterozygotes (Table 1c). The mouse was therefore proved to be a ragged homozygote. It was then mated to heterozygous ragged females and sired other viable ragged homozygotes, both male and female. A number of the R+R+ males so produced reached maturity and proved fertile; they were mated to R+ females (Table 1d). More adult R+R+ mates obtained from these matings were used in the same way. The viability of R+R+ mice produced in this selected stock was improved, but the death rate amongst suckling R+R+ mice was always much higher than in their R+R+ sibs, viz; of 36 R+R+ mice born alive, 33 died before weaning; whereas of 108 R+ littermates born alive only 5 died before weaning.

Approximately as many R+R+ females as males reached maturity. Of these mice three were mated to R+R+ males, and two proved fertile. Their offspring, all of which died at or before birth,
Table 1.
† Segregation Data

<table>
<thead>
<tr>
<th>Parents' Genotype</th>
<th>Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>++</td>
</tr>
<tr>
<td>(a) ++ x Ra+</td>
<td>1216</td>
</tr>
<tr>
<td>(b) Ra+ x Ra+</td>
<td>124</td>
</tr>
<tr>
<td>(c) ++ ♀ x RaRa ♂</td>
<td>0</td>
</tr>
<tr>
<td>(d) Ra+ ♀ x RaRa ♂</td>
<td>0</td>
</tr>
</tbody>
</table>

++/Ra+ classifications were generally made at weaning; RaRa mice were classified at birth.

The segregation data from intercross matings was different from expectation: P < 0.01. This was due to a significant deficiency from the expected number of RaRa mice (P < 0.001).

The shortage from the expected number of RaRa mice from Ra+ x RaRa matings was not significant (P < 0.3 and > 0.2 if the 6 unclassified mice are neglected).

* These mice had died at or soon after birth and had been partially eaten; they were probably predominantly ragged homozygotes.

† This data includes that given by Carter and Phillips (1954).
appeared to be ragged homozygotes.

It has been seen that intercross matings (Ra+ x Ra+) produced less than the expected number of RaRa offspring at birth and even those RaRa mice which were born died soon after birth except in very few cases. Viable RaRa mice could therefore only be obtained consistently from matings of the type: RaRa x Ra+ . This procedure had two disadvantages:

1) The RaRa mice obtained in this way showed a lower-grade expression of the gene than did the still-born RaRa mice from intercross matings. It was not unexpected that the stock selected for higher viability of RaRa mice showed a relatively low-grade expression of the gene.

2) The RaRa mice could be compared with Ra+ sibs but not with ++ sibs.

Despite the efforts made to obtain viable RaRa mice, their high death rate during suckling and the necessity of keeping many of the males for breeding, have limited the number of RaRa adult mice available for histological studies.

The deficiency of RaRa mice found at birth, especially in intercross matings, does not prove that there was an abnormal degree of prenatal mortality in the ragged homozygote class, since even though the litters were classified as soon as possible after birth, some mice which had died at birth may have been eaten before classification.
PART I - ADULT MORPHOLOGY

The Adult Coat.

(1) Gross Appearance.

Normal Mice. (Fig. 1).

The coat of the normal mouse has been studied in detail by Dry (1926). The coat is dense over all the body area and is generally shorter on the venter than on the dorsum. The hair is particularly short on the snout, on the underside of the chin and throat, on the inner sides of the limbs and in the genital region. Hairs growing in a small area close behind and mesial to the ears appear to be finer and less pigmented than in other parts of the coat. The sinus hairs exist as follows: on each side of the face there are about thirty moustache hairs, two supra orbital sinus hairs, one post orbital, and two post oral sinus hairs. Unlike the pelage hairs, the sinus hairs are fairly constant between individuals in their number and spatial relations.

Rag Mice. (Fig. 2).

Although there is considerable variation between individuals, the ragged heterozygotes judged agreed generally with the description given by Carter and Phillips (1954). The coat is more sparse than normal, especially under the lower jaw, on the fore part of the snout, on the inner sides of the fore and hind limbs, on the neck and near the genitalia. The guard hairs appear to stand out more than normally from the rest of the coat. In agouti ragged mice the cheek hair is almost as yellow as normal, but the coat on the back is clearly darker than in normal mice. The tail rings, claws, eyelids and sinus hairs look normal.
Fig. 1. ++ adult mouse

Fig. 2. Ra+ adult mouse

Fig. 3. RαRa adult mouse

Fig. 4. ++ adult mouse

Fig. 5. Ra+ adult mouse

Fig. 6. RaRa adult mouse

Ragga Mice. (Fig. 3).

Homozygous ragged mice are almost naked. Few or no hairs are found on the neck and anterior parts of the body. There is a sparse growth of hair on the rump and posterior regions of the ventrum, near the tail root and genitalia, near the eyes and under the chin. The sinus hairs are reduced in number, and apparently in size. Usually about six moustache hairs are present on each side, growing from the most posterior follicles. Some more anterior follicles are present, but they are less prominent than those from which vibrissae are growing. The presence or absence of the other sinus hairs varies between individuals. There may be one, two, or no supra orbital sinus hairs on each side. There is usually one post orbital sinus hair on each side, and no post oral sinus hairs.

The ears and tail are pigmented though no hairs are visible there. Tail rings are present. The claws seem normal.

Ragga mice look slightly smaller than their Rag+ sibs, and smaller than normal mice of the same age and sex.
(2) **Fibre Morphology.**

(i) **External Morphology.**

(a) **Introduction.**

The external morphology of the fibre types found in the normal mouse coat has been described by Dry (1926) and by Fraser (1951).

Five types of hair fibre are usually distinguished:

1. **Sinus hairs,** which are sensory in function and confined to the upper lip and face of the animal.
2. **Guard hairs,** which are long, straight and project clearly above the rest of the coat.
3. **Awls,** which are also straight but shorter than guard hairs.
4. **Auchenes,** which are similar to awls but possess a single bend or constriction near the middle of the hair.
5. **Zig Zags,** which may be shorter than or as long as awls and have two or more bends in each hair.

Generally, guard hairs comprise about 23% of the coat, awls and auchenes about 26% and zigzags 72%.

(b) **Methods.**

Hair was plucked or shaved from the body area under analysis, sorted into types and examined for gross abnormalities under a low power binocular microscope. Hair from Ra+ and RaRa mice was compared with hair from the same regions of normal mice of comparable age and the same sex. 10 ++, 10 Ra+ and 3 RaRa mice were used.

(c) **Results**

++ Mice. (Fig7)

Hair fibres from normal mice were classified into the five
Camera Lucida drawings (x 6)

Fig. 7. Shows normal hair fibres, Re transitional hairs and typical RaRa hairs.

Fig. 8. Shows abnormal zigzag hairs.
Abnormal Zigzags
Rat Neck

++ Ear

Guard hairs Awls Auchenes Zigzags
Guard hair—Awls

Awl—Zigzags
Rat Neck

RaRa Rump
types described above. Hairs taken from the main body areas of the
dorsum and venter showed no obvious deviations from type. Hairs from
the region close behind and medial to the ears were mainly classifi-
able as zigzags, but were thinner than most zigzag hairs, and often
irregularly constricted (Fig. 3).

In certain short-haired zones of the body, e.g. the under
side of the chin, the inner sides of the limbs and the genital area,
most of the hairs were similar to zigzags but were very short and
often had only one constriction.

Ra+ Mice. (Fig. 7).

The hairs from the main body area of Ra+ mice can be grouped
into the normal classification and all fibre types are present. Some
morphological abnormalities are found. In those regions of Ra+ mice
producing low proportions of zigzag hairs, e.g. the neck, the zig-
zags tend to be finer, smaller, less pigmented and less regularly
constricted than those from the same areas of normal mice. Zigzags
from high zigzag producing areas in Ras mice are generally normal
in shape.

Two types of abnormal 'transitional' hairs are found in Ra+
mice, especially on the anterior part of the back. The first type
is large, possesses a fairly long tip and yet appears slimmer than
the usual type of large awl. It is thus intermediate in size and
shape between guard hairs and awls (Fig. 7). The second type is
intermediate between awls and zigzags and may possess a partial con-
striction (Fig. 7). The constriction separates the distal or earlier
growing part of the hair which is stout, densely pigmented and awl-
like; from the proximal part which is thin and unpigmented like a
badly-formed zigzag hair. These 'transitional' hairs are difficult to classify macroscopically and the occurrence of the guard hair/awl type may have slightly affected the accuracy of the estimations of guard hair and awl frequencies in Ra+ mice.

Hairs from the small area just behind and mesial to the ears cannot always be classified into any of the usual types. They are thinner and may be unconstricted or more irregularly constricted than normal hairs from the same region. Those hairs which are constricted are best classified as zigzags.

**RaRa Mice.** (Fig. 7).

Hairs taken from the dorsum and venter of RaRa mice were generally fine, sometimes twisted and of variable length. They were not classifiable into the normal hair types.
(ii) Fibre Dimensions.

(a) Methods.

Hair samples were taken from the neck and sacrum of dead heterozygous ragged and normal mice (sibs). Hairs at one stage of growth may be carried higher up in the follicles than those at an earlier growth stage; to eliminate differences in length due to this factor the hairs were plucked, not shaved from the mice. The hairs were measured on squared millimetre paper under a low-power binocular microscope; whiskers were plucked from the mice, stretched on an albumenised slide and measured over millimetre paper. The longest whiskers were taken from each side of the moustache of every mouse, so the maximum whisker length of each mouse was always measured.

Hair thickness was estimated by mounting the hairs on slides and measuring them under the microscope at the point of maximum thickness with a calibrated ocular micrometer.

(b) Results.

Hair Length.

Significance tests on the hair length data in Table 2 show that:

(i) The mean length of guard hairs in Ra+ mice is significantly greater than normal in the neck region (P < 0.001) and in the rump region (P < 0.001).

(ii) The mean length of awls in Ra+ mice is significantly less than normal in the neck region, (P < 0.01) and in the rump region (P < 0.001).

(iii) The mean length of zigzags in Ra+ mice is significantly
### Table 2.

Hair Length (mm.)

#### Neck Region

<table>
<thead>
<tr>
<th>Mice</th>
<th>G. hairs</th>
<th>Awls</th>
<th>Zigzags</th>
<th>G. hairs</th>
<th>Awls</th>
<th>Zigzags</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.7 ± 0.2</td>
<td>6.7 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>10.5 ± 0.3</td>
<td>6.4 ± 0.2</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td>2</td>
<td>8.9 ± 0.2</td>
<td>5.8 ± 0.1</td>
<td>5.4 ± 0.1</td>
<td>9.3 ± 0.2</td>
<td>5.8 ± 0.1</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td>10.1 ± 0.3</td>
<td>6.1 ± 0.2</td>
<td>5.1 ± 0.1</td>
<td>10.4 ± 0.3</td>
<td>5.9 ± 0.2</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>8.8 ± 0.2</td>
<td>6.0 ± 0.1</td>
<td>5.0 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>5.3 ± 0.2</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>8.5 ± 0.2</td>
<td>6.0 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>11.0 ± 0.4</td>
<td>5.7 ± 0.2</td>
<td>3.2 ± 0.2</td>
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<tr>
<td><strong>Overall Means</strong></td>
<td>9.2 ± 0.1</td>
<td>6.1 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>10.3 ± 0.2</td>
<td>5.9 ± 0.1</td>
<td>3.9 ± 0.1</td>
</tr>
</tbody>
</table>

#### Rump Region

<table>
<thead>
<tr>
<th>Mice</th>
<th>G. hairs</th>
<th>Awls</th>
<th>Zigzags</th>
<th>G. hairs</th>
<th>Awls</th>
<th>Zigzags</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.2 ± 0.3</td>
<td>8.5 ± 0.1</td>
<td>7.6 ± 0.1</td>
<td>13.2 ± 0.3</td>
<td>7.6 ± 0.3</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td>2</td>
<td>11.1 ± 0.2</td>
<td>7.5 ± 0.1</td>
<td>6.2 ± 0.1</td>
<td>11.7 ± 0.1</td>
<td>7.2 ± 0.2</td>
<td>4.7 ± 0.2</td>
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<tr>
<td>3</td>
<td>11.2 ± 0.3</td>
<td>7.7 ± 0.4</td>
<td>6.7 ± 0.3</td>
<td>12.9 ± 0.2</td>
<td>6.7 ± 0.4</td>
<td>5.9 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>11.0 ± 0.3</td>
<td>6.8 ± 0.3</td>
<td>5.7 ± 0.2</td>
<td>12.4 ± 0.3</td>
<td>6.6 ± 0.4</td>
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<tr>
<td>5</td>
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<td>7.9 ± 0.1</td>
<td>6.7 ± 0.1</td>
<td>13.3 ± 0.2</td>
<td>6.5 ± 0.3</td>
<td>5.3 ± 0.1</td>
</tr>
<tr>
<td><strong>Overall Means</strong></td>
<td>11.6 ± 0.1</td>
<td>7.7 ± 0.1</td>
<td>6.6 ± 0.1</td>
<td>12.7 ± 0.1</td>
<td>6.9 ± 0.1</td>
<td>5.2 ± 0.02</td>
</tr>
</tbody>
</table>

Each figure quoted for guard hairs is the mean length of a sample of about 12 hairs from one mouse. Each figure quoted for the other hair types is the mean length of about 25 hairs from one mouse.
### Table 3.

*Coefficients of Variance of Hair Length*

<table>
<thead>
<tr>
<th>Hair type</th>
<th>Mouse</th>
<th>++(neck)</th>
<th>Rat+(neck)</th>
<th>++(rump)</th>
<th>Rat+(rump)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guard hairs</td>
<td>1</td>
<td>7.0</td>
<td>10.2</td>
<td>7.0</td>
<td>7.5</td>
</tr>
<tr>
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<td>2</td>
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<td>4.0</td>
<td>6.4</td>
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<td>10.8</td>
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<tr>
<td>Awls</td>
<td>1</td>
<td>6.8</td>
<td>13.2</td>
<td>7.2</td>
<td>18.0</td>
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<tr>
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<td>2</td>
<td>9.9</td>
<td>13.6</td>
<td>6.0</td>
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<td>12.9</td>
<td>18.1</td>
<td>26.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7.1</td>
<td>15.6</td>
<td>16.5</td>
<td>22.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.8</td>
<td>16.5</td>
<td>8.7</td>
<td>24.6</td>
</tr>
<tr>
<td>Zigzags</td>
<td>1</td>
<td>7.3</td>
<td>4.7</td>
<td>7.2</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.4</td>
<td>8.5</td>
<td>8.3</td>
<td>21.8</td>
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<td></td>
<td>3</td>
<td>11.5</td>
<td>15.6</td>
<td>18.9</td>
<td>13.0</td>
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<td></td>
<td>4</td>
<td>12.0</td>
<td>21.3</td>
<td>15.8</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>8.0</td>
<td>26.9</td>
<td>16.1</td>
<td>10.6</td>
</tr>
</tbody>
</table>

The coefficients of variance were calculated on the hair samples from each individual mouse. They measure the variation in hair length within mice.

* Coefficient of Variance = \( \frac{\text{Standard Deviation}}{\text{Mean}} \times 100 \)
Table 4.

Whisker Length (mm.)

<table>
<thead>
<tr>
<th>Mice</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>27</td>
<td>28</td>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td>Right</td>
<td>28</td>
<td>27</td>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td>Left</td>
<td>29</td>
<td>30</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>29</td>
<td>29</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

Measurements were made on the longest selected moustache hairs from a number of mice (Table 4). These measurements showed no difference in maximum whisker length between heterozygous ragged and normal mice.
less than normal in the neck region, \( (P < 0.001) \) and in the rump region \( (P < 0.001) \).

Finally, it is clear that all hair types in ++ and Ra+ mice are longer in the rump region than in the neck region.

The coefficients of variance (Table 3) suggest that the guard hairs within any Ra+ mouse are not abnormally variable in length, but the awls and often the zigzags within any Ra+ mouse tend to be abnormally variable in length.

**Hair Thickness.**

Data from the measurements of hair thickness are given in Table 5. There is no significant difference in the mean thickness of any of the fibre types between ++ and Ra+ mice. There appears to be a slight tendency however for the zigzags in the neck regions of some Ra+ mice to be thinner than normal.

In normal mice all the fibre types tend to be slightly thicker in the neck region than in the rump region, but the differences are not statistically significant. In Ra+ mice this tendency does not exist except in the case of the awl fibres which are significantly thicker in the neck region than in the rump region \( (P < 0.05) \).
Table 5.

**Hair Thickness (\(\mu\))**

<table>
<thead>
<tr>
<th></th>
<th>Neck Region</th>
<th></th>
<th>Rump Region</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>++</td>
<td>Rat+</td>
<td>++</td>
<td>Rat+</td>
</tr>
<tr>
<td>Mice</td>
<td>G. hairs</td>
<td>Awls</td>
<td>G. hairs</td>
<td>Awls</td>
</tr>
<tr>
<td>1</td>
<td>37</td>
<td>55</td>
<td>27</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>52</td>
<td>25</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>42</td>
<td>22</td>
<td>37</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>43</td>
<td>25</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>39.2 ± 1.1</td>
<td>48.0 ± 3.2</td>
<td>24.8 ± 1.0</td>
<td>36.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>41.7 ± 1.2</td>
<td>23.7 ± 0.7</td>
<td>37.2 ± 1.0</td>
<td>45.5 ± 0.5</td>
</tr>
</tbody>
</table>

Each figure quoted is the average thickness of 8 hairs. Hair samples were taken from 4 mice of each genotype.
(iii) Internal Morphology.

(a) Introduction.

The internal structure of mouse hair has been studied by Dry (1926) and Fraser (1951). These authors have shown that the internal medulla of the hairs is made up of rows of evenly arranged air cells (Fig. 9). On the mid dorsum of normal mice the guard hairs have a thick cortex and two rows of air cells except at the tapered ends of the hairs, where there is a single row of air cells at the proximal end and a fine tip with a solid medulla at the distal end. Awls have three to five rows of air cells at the widest parts of the hairs, and two rows or a single row at the ends. Zigzags have a single row of air cells except at the distal tips and constrictions of the hairs, where there is a "solid" non-septate medulla.

(b) Methods.

Hairs of each fibre type were mounted directly in canada balsam and the internal air cells were studied microscopically. In some cases the hairs were immersed in previously boiled water and then transferred to warm xylol before mounting to remove air from the internal cells.

(c) Results.

++ Mice.

In the dorsal region of normal mice, a few "transitional" hairs occur, which are usually intermediate between the zigzag and awl types. These hairs have a single row of air cells but are thicker than zigzags and possess no constrictions or bends.

In hairs from other body regions of normal mice structural
Camera Lucida drawings of the internal structure of hair fibres (x 160)

Fig. 9. Shows normal hairs and Ra+ transitional hairs.

Fig. 10. Shows Ra+ zigzags and typical RaRa hairs.

Cuticular scales are shown only in one RaRa fibre.
Fig. 11. Camera lucida drawings (x 160), of the internal structure of hair fibres. Cuticular scales are shown in two drawings.
irregularities may occur. Hairs growing closely posterior and mesial to the ears are seen macroscopically to be of the zigzag type, but they are rather thin and little pigmented. They are usually constricted, but less sharply than mid-dorsal zigzags. As the hairs are thin the air cells are smaller than usual and the intervening septa are frequently replaced by a region of continuous medulla over a large part of the hairs.

In certain short-haired regions of the body, e.g. the genital area, the zigzag hairs are very fine and may possess similarly irregular internal structure.

Ra+ Mice.

The guard hairs are normal in Ra+ mice. No defect of structure has been found consistently within any of the other hair types, but certain abnormalities do occur and are listed below.

Class A (Fig. 9): The class of hair described above as intermediate between a guard hair and awl in external appearance has been found to contain the following abnormal types of hair fibre:

(i) Hairs which have a single row of air cells, are thicker than zigzags and have no constrictions. These hairs are usually considerably longer than the "transitional" hairs found in normal mice.

(ii) Hairs of this type have frequent alternations from one to two rows of air cells throughout their lengths. The cortex of these hairs is thinner than that of normal guard hairs.

(iii) Hairs of this type are like awls with three rows of air cells but with abnormally long regions of one- or two-row septation at the ends of the hairs. Macroscopically the hairs appear to have long guard hair-like tips.
Each of these kinds of abnormal hair occurs only rarely, but together they make up a group of hairs which are difficult to classify as either guard hairs or awls. They occur more frequently in the neck than in the rump region of Ra+ mice.

**Class B (Fig. 9):** Another abnormal kind of hair found in Ra+ mice is the type described earlier as intermediate between the awl and zigzag types. The stout awl-like part of these hairs commonly has three, or occasionally two or four rows of air cells, but this region is succeeded very suddenly by a thinner part containing at first two and then one row of air cells. During the growth of these hairs there must be a sudden change in the mode of proliferation from the hair germ matrix, so that a fine single-septulate period of development replaces the previous formation of a multi-septate hair strand with a thick cortex.

**Class C (Fig. 10):** The zigzag hairs of Ra+ mice, particularly those in the anterior zone of the dorsum and in the genital area, often have irregularly arranged air cells, which may in parts of the hairs be replaced by a thin region of continuous medulla.

**Class D (Fig. 11):** The hairs found close behind and medial to the ears are very often not classifiable into any of the usual types. They are fine, have either a single row of air cells or merely a thin pigmented region of continuous medulla which may be absent in parts of the hairs.

**RaRa Mice.**

The hairs on homozygous ragged mice are thin and they occur sparsely. They are not classifiable into the usual groups either
by macroscopic or microscopic examination. Most of the hairs have no constrictions, but a single row of air cells, although some of them are clearly thicker than normal zigzag hairs. Some hairs have a short region of two-row septulation.

It can be concluded from all the above results that conditions for hair growth are less favourable in ragged than in normal mice. In the heterozygote, conditions are assumed to be least favourable where the structural hair defects are most common, i.e. on the head, neck and anterior parts of the dorsum.
(3) The Frequency of Hair Fibre Types.

(a) Introduction.

The sparse appearance of the coats of adult ragged heterozygotes suggested that a high proportion of the zigzag fibres which make up the underfur may be missing. A reduction in number of these fibres, which are always yellow banded in agouti mice (Dry, 1928), could also cause the darkening of the coat observed in agouti ragged heterozygote mice. These two possibilities could be investigated by a quantitative analysis of the relative frequencies of different hair fibre types in normal and ragged heterozygote mice.

Previous work involving the classification into types of mouse hair fibres has been done by Dry (1926) and Fraser (1951). Dry used a number of characters for his classification; the total length of the fibre, the length of the unmedullated tip, the number of rows of internal air cells and the occurrence and number of constrictions in the fibre. Fraser used the easily scored criteria of: the presence and number of constrictions and the total length of the fibre. Dry's classification, although accurate, is laborious, requiring microscopic examination and careful measurement of the fibres.

(b) Methods.

A comparison was made of the differential frequencies of hair fibre types between mice of Ra+ and ++ genotypes. Hair samples were taken to estimate the frequencies of three fibre types: guard hairs, awls and auchenes, and zigzags, in various body regions of normal and heterozygous ragged mice. The awls and auchenes were combined into one class because of difficulty in distinguishing between them. The hairs were classified on their external morphol-
Some samples were taken at defined areas on the neck and rump. A point on the dorsum one-sixth of the distance from the ears to the tail root was arbitrarily selected as the neck region, and a point one-sixth of the way from the tail root to the ears was taken as the region of the rump.

All samples were taken by holding a small tuft of hair in forceps and shaving off the hair close to the skin. Fibres of each of the three classes were separated out and counted under a low-power binocular microscope.

The size of samples varied from 200-800 hairs, and every hair within each sample was classified. Twenty four mice were used for this analysis. Since it was necessary to count a large number of fibres, Fraser's simple criteria of total length and frequency of constrictions were used to separate the hairs into classes.

As the hair lengths were only compared and not measured, an arbitrarily chosen fibre length was not found satisfactory by itself for separating guard hairs and awls. Classification into these types was made by considering in addition the unmedullated fibre tip which is longer and finer in guard hairs and takes up a greater part of the hair length than in awls.

(c) Results.

++ Mice.

Hair samples were taken from normal mice at points along the dorsal mid line from the neck to the rump. The proportions of fibre types found were not significantly different in samples from different parts of the dorsum (Table 6). There was however some variation between individual mice in the proportions of fibre types in any specific area.
Table 6.

*Fibre Type Frequencies - Normal Mice*

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Area Sampled</th>
<th>Number of hairs counted</th>
<th>Guard hairs</th>
<th>Percentage of:</th>
<th>Mean Percentages</th>
<th>Standard Errors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Guard hairs</td>
<td>1.5%</td>
<td>±0.14</td>
</tr>
<tr>
<td>1</td>
<td>Neck</td>
<td>364</td>
<td>1.1</td>
<td>21.7</td>
<td>25.1%</td>
<td>±0.78</td>
</tr>
<tr>
<td>2</td>
<td>Neck</td>
<td>1133</td>
<td>1.3</td>
<td>22.3</td>
<td>73.2%</td>
<td>±0.90</td>
</tr>
<tr>
<td>3</td>
<td>Neck</td>
<td>331</td>
<td>2.5</td>
<td>27.5</td>
<td>71.5%</td>
<td>±0.90</td>
</tr>
<tr>
<td>4</td>
<td>Neck</td>
<td>379</td>
<td>1.6</td>
<td>26.9</td>
<td>71.5%</td>
<td>±0.90</td>
</tr>
<tr>
<td>5</td>
<td>Mid-dorsum</td>
<td>356</td>
<td>1.1</td>
<td>22.4</td>
<td>76.5%</td>
<td>±0.90</td>
</tr>
<tr>
<td>1</td>
<td>Rump</td>
<td>322</td>
<td>1.6</td>
<td>27.9</td>
<td>76.5%</td>
<td>±0.90</td>
</tr>
<tr>
<td>2</td>
<td>Rump</td>
<td>435</td>
<td>1.2</td>
<td>24.6</td>
<td>71.0%</td>
<td>±0.90</td>
</tr>
<tr>
<td>3</td>
<td>Rump</td>
<td>218</td>
<td>2.0</td>
<td>27.0</td>
<td>71.0%</td>
<td>±0.90</td>
</tr>
<tr>
<td>4</td>
<td>Rump</td>
<td>416</td>
<td>1.5</td>
<td>26.4</td>
<td>72.1%</td>
<td>±0.90</td>
</tr>
</tbody>
</table>

*The data in this table are used for the hair density estimates made in the next section.*
Table 7.

*Fibre Type Frequencies – Ragged Heterozygote Mice*

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Area sampled</th>
<th>Number of hairs counted</th>
<th>Guard hairs</th>
<th>Percentage of:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Awls &amp; Auchenes</td>
<td>Zigzags</td>
</tr>
<tr>
<td>1</td>
<td>Neck</td>
<td>309</td>
<td>7.4</td>
<td>86.7</td>
<td>5.8</td>
</tr>
<tr>
<td>2</td>
<td>Neck</td>
<td>296</td>
<td>11.1</td>
<td>86.1</td>
<td>2.7</td>
</tr>
<tr>
<td>3</td>
<td>Neck</td>
<td>265</td>
<td>9.1</td>
<td>88.7</td>
<td>2.2</td>
</tr>
<tr>
<td>4</td>
<td>Neck</td>
<td>310</td>
<td>6.5</td>
<td>87.4</td>
<td>6.1</td>
</tr>
</tbody>
</table>

|       |              |                         |             | Neck Mean %    |  |
|       |              |                         |             | 8.5 ± 0.87     |  |
|       |              |                         |             | 87.2 ± 0.48    |  |
|       |              |                         |             | 4.2 ± 0.44     |  |

|       | Rump         | 526                     | 3.2         | 40.1           | 56.7 |
| 2     | Rump         | 547                     | 1.8         | 34.7           | 63.4 |
| 3     | Rump         | 394                     | 3.3         | 38.1           | 58.6 |
| 4     | Rump         | 438                     | 3.4         | 41.1           | 55.5 |

|       |              |                         |             | Rump Mean %    |  |
|       |              |                         |             | 2.9 ± 0.33     |  |
|       |              |                         |             | 38.5 ± 1.24    |  |
|       |              |                         |             | 58.5 ± 1.50    |  |

*These data were used for the hair density estimates made in the next section.*
The figures given in Table 6 are within the range of those reported by Fraser (1951) except that the proportion of guard hairs found by Fraser was higher than in the present investigation. The percentage of guard hairs in the inbred strains of mice used by Fraser varied from 2.0-2.5%. The divergence between this figure and the present results may be due to differences between the strains of mice used, or to differences in scoring criteria.

In the ventral tail root and genital area there is a significantly higher proportion of zigzag hairs than on the dorsum. These hairs comprise about 88% of the total fibres in the region. They are not precisely typical of the zigzag type, being shorter and often possessing only one constriction.

In a small area close behind and mesial to the ears, about 90% of the hairs are zigzags.

Rat Mice.

In all the body areas examined, ragged mice have a lower proportion of zigzag hairs than normal mice.

Along the dorsal mid line there is an anterior to posterior gradient for increasing proportion of zigzags (Fig. 11a). From 0-3% on the head, the proportion increases to 50-60% on the rump. The overall reduction in the number of zigzag hairs, which in agouti mice are always yellow banded (Dry, 1928), causes darkening of the coat on the backs of ragged agouti mice.

On the ventrum the ragged coat shows a similar deficiency of zigzags, and the proportion of these hairs is again higher at the posterior end.

Those fibre counts summarised in Table 7 show that there is a great deal of variation between mice in the proportion of zigzag
Fig. 11a. The graph shows the antero-posterior gradient of increasing proportion of zigzag hairs in Ra+ mice. Hair samples were taken from about 6 mice at each point on the dorsum.

<p>| | | | | | |</p>
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</tr>
</tbody>
</table>

2 standard errors, one standard error on each side of the mean.
hairs at many regions of the body. Some of this variation might have been reduced had the ragged gene been segregating on an inbred genetic background.

In the small area just behind and medial to the ears there is a high proportion of hairs similar to the zigzag type. The abnormal morphology of these hairs makes them difficult to classify. It is therefore impossible to make accurate fibre type counts, but awl type fibres probably occur more frequently in this region than in normal mice.
The Adult Skin.

(1) Hair Fibre and Follicle Density.

(a) Introduction.

Differential fibre counts in the neck region of Ra+ mice suggested that nearly all the zigzag hairs were missing. Since these hairs normally comprise about 72% of the coat, the results suggested that about 70% of the hair fibres might be absent. The coats of Ra+ mice did not seem as sparse as would have been expected if this were so; but in order to decide whether the density of guard hair and awl fibres was abnormally increased in those areas where the proportion of zigzag hairs was decreased, hair density estimates were necessary. Hair density estimates would also show the exact decrease in number of the zigzags, as distinct from their proportional decrease relative to the other types of fibres. To calculate the density of fibre types from data on their relative proportions, counts of the total hair shaft density in specific areas of skin were needed.

The density of hair follicles in certain breeds of sheep has been calculated by Carter (1943), who used a technique involving the cutting of tangential skin sections. Such a process was not suitable for obtaining quick results; and a fairly complicated technique when first used may not allow uniform treatment of successive skin samples. Non-uniform treatment, causing stretching or distortional shrinking of the material would enable little confidence to be placed in the results. A simpler technique, making use of whole mounts of skin was devised for the present purpose of estimating fibre shaft density. A similar method has been used by Herter (1938).

It was expected from macroscopic observations that the total
hair density in Ra+ mice would be less than normal. Such an abnormality could be due to one of three possible causes:

(i) There are fewer hair follicles in Ra+ than in ++ mice.

(ii) There are no fewer follicles in Ra+ mice, but some follicles do not produce hairs.

(iii) A combination of (i) and (ii).

In order to solve this problem it was necessary to estimate the follicle density as well as the hair fibre density in ++ and Ra+ mice.

(b) Methods.

To estimate the hair density in ++ and Ra+ mice, skin was taken from the neck and rump areas of the mice. The skin was shaved and then removed, scraped free of connective tissue, placed perfectly flat in a petri dish with the epidermis upwards and fixed with Bouin's fluid. It was stained in Delafield's haematoxylin, differentiated in acid alcohol, dehydrated, and cleared in methyl benzoate. Whole mounts of the skin were made in which the hair follicles were stained with haematoxylin and the hair shafts were stained yellow with Bouin's fluid. Skin for these whole mounts was taken from the same regions of the same individual mice that had been used for previous hair fibre type counts (Tables 6 and 7). These fibre frequency counts had been made on samples taken from points one-sixth of the distance from ears to tail root - i.e. the neck region, and one-sixth of the distance from tail root to ears - i.e. the rump region. The two skin samples from each mouse were removed so that each of these points was located in the centre of the skin sample taken from its respective region.
Fig. 12. ++ skin whole mount (neck region). Note: even size and uniform orientation of follicles and hair fibres.

Fig. 13. Rat skin whole mount (neck region). Note: variable size of follicles; the presence of follicles not producing hairs; and abnormally low hair fibre density.
per unit area of skin were counted by using a microscope, with ocular graticule, focussed near the centre of the skin samples.

Several pairs of skin samples from ++ and Ra+ mice were treated simultaneously; each sample was treated exactly alike so that any shrinkage or stretching of the material should have been uniform between samples.

Although the hair shafts were clearly visible on the skin whole mounts, the follicles themselves were not. In Ra+ and RaRa skin it seemed that there might be some follicles which were incompletely developed and which carried no hairs; the density of these follicles could not be estimated from the skin whole mounts. A comparison of the total follicle density in ++, Ra+, and RaRa skin, including those follicles not producing hairs, was obtained from sagittal skin sections by estimating the average number of follicles per microscope field.

(c) Results.

The total hair shaft counts (Table 8) were made on the whole mounts of skin taken from the same areas of the body and the same individual mice as those used for the fibre type counts. The shaft number was calculated by averaging figures from about 8 similar microscopic fields in the required area.
Table 8.

Mean Number of Fibre Shafts per Unit Area of Skin.

(0.432 sq. mm.)

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Neck</th>
<th>Rump</th>
<th>Neck</th>
<th>Rump</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56 ± 1.6</td>
<td>68 ± 2.5</td>
<td>19 ± 1.2</td>
<td>36 ± 1.6</td>
</tr>
<tr>
<td>2</td>
<td>34 ± 2.0</td>
<td>52 ± 2.3</td>
<td>19 ± 1.3</td>
<td>48 ± 1.6</td>
</tr>
<tr>
<td>3</td>
<td>44 ± 1.5</td>
<td>61 ± 3.0</td>
<td>19 ± 1.4</td>
<td>49 ± 2.4</td>
</tr>
<tr>
<td>4</td>
<td>39 ± 1.8</td>
<td>64 ± 1.0</td>
<td>23 ± 2.4</td>
<td>46 ± 1.3</td>
</tr>
</tbody>
</table>

From the data on the relative frequencies of fibre types (Tables 6 and 7), and the data on the total hair shaft density in the same areas of the same mice (Table 8), the density of each fibre type on each mouse was calculated (Table 9).

These results establish the following facts:

(i) The density of zigzag hairs is everywhere lower in Ra+ mice than in the corresponding position in ++ mice, and it is lower in the Ra+ neck region than in the rump region.

(ii) The density of guard hairs and of awls and auchenes is significantly greater on the necks of Ra+ mice than on the necks of normal mice. These hair types tend also to be denser on the Ra+ rump than on the normal rump, but this difference is not significant at the 5% level. It seems clear that in those areas of Ra+ mice which lack most zigzag hairs there is a greater than normal density of awls and auchenes, and of guard hairs.

(iii) In normal mice the density of all hair types is greater on the sacrum than in the neck region.
## Table 9.

**Density of Fibre Types.** *(Fibres/unit area of skin = 0.432 sq. mm.)*

<table>
<thead>
<tr>
<th>Hair type</th>
<th>Variance</th>
<th>Neck</th>
<th>Rump</th>
<th>Variance</th>
<th>Mice</th>
<th>Density at Rat+ neck greater than ++ neck</th>
<th>Density at Rat+ rump not greater than ++ rump</th>
<th>Density at ++ neck less than ++ rump</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guard hairs</td>
<td>++</td>
<td>0.73</td>
<td>0.82</td>
<td></td>
<td>1</td>
<td></td>
<td>P &lt; 0.01</td>
<td>P = 0.1</td>
</tr>
<tr>
<td></td>
<td>σ = 1.3</td>
<td>0.85</td>
<td>1.04</td>
<td>σ = 0.8</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cv = 194</td>
<td>0.48</td>
<td>0.98</td>
<td>cv = 118.7</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.62</td>
<td>0.96</td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awls and auchenes</td>
<td>++</td>
<td>12.5</td>
<td>16.7</td>
<td></td>
<td>1</td>
<td></td>
<td>P &lt; 0.01</td>
<td>P = 0.4</td>
</tr>
<tr>
<td></td>
<td>σ = 1.2</td>
<td>9.4</td>
<td>11.0</td>
<td>σ = 1.2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cv = 11.42</td>
<td>9.6</td>
<td>17.0</td>
<td>cv = 7.45</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.5</td>
<td>16.9</td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zigzags</td>
<td>++</td>
<td>16.5</td>
<td>14.4</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>σ = 1.5</td>
<td>16.3</td>
<td>16.8</td>
<td>σ = 1.8</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cv = 8.62</td>
<td>16.9</td>
<td>18.7</td>
<td>cv = 10.46</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.1</td>
<td>18.9</td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>42.8</td>
<td>50.4</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>σ = 7.2</td>
<td>23.8</td>
<td>36.9</td>
<td>σ = 5.0</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cv = 22.4</td>
<td>34.0</td>
<td>43.0</td>
<td>cv = 11.33</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>27.9</td>
<td>46.1</td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>1.2</td>
<td>20.5</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>σ = 0.42</td>
<td>0.57</td>
<td>0.2</td>
<td>σ = 3.7</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cv = 47.1</td>
<td>0.4</td>
<td>28.7</td>
<td>cv = 14.12</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.4</td>
<td>25.5</td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

σ = Standard deviation.  

\(\text{cv} = \frac{\sigma}{\text{Mean}} \times 100\).  

It includes variation in density within mice and between mice.
Table 10.
Follicle Density Estimates from Sagittal Skin Sections
Follicle Density per Microscope Field

<table>
<thead>
<tr>
<th>Mice</th>
<th>Neck Region</th>
<th>Rump Region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>++</td>
<td>Rat</td>
</tr>
<tr>
<td>1</td>
<td>3.9 ± 0.2</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>4.0 ± 0.3</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>5.6 ± 0.2</td>
<td>7.4 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>5.1 ± 0.2</td>
<td>5.9 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>5.5 ± 0.4</td>
<td>-</td>
</tr>
<tr>
<td>Overall Means</td>
<td>4.8 ± 0.2</td>
<td>6.0 ± 0.2</td>
</tr>
</tbody>
</table>

Each figure quoted is the mean of 10 measurements on serial skin sections from one mouse.
(iv) Coefficients of variance show that the density of zigzag hairs is quite variable in the neck and rump zones of both Ra+ and normal mice; but the density of awls and auchenees is more constant. (The variable density attributed to guard hairs is probably due to sampling errors since their numbers are small). Since the guard hair density is low, and the awl density fairly constant, total hair density is dependent upon, and varies closely with zigzag density. In both Ra+ and normal mice, a higher zigzag density is associated with a higher total hair density.

Finally, there is a clear tendency in normal mice for all hair types to be more variable in density at the neck than in the rump region.

Estimates of total hair follicle density in the neck and rump regions of ++, Ra+ and RaRa mice are given in Table 10. Statistical tests show that:

(i) In the neck region, Ra+ follicle density is significantly greater than normal ($P < 0.001$).

(ii) In the rump region, Ra+ follicle density is significantly greater than normal ($P < 0.01$).

(iii) In normal mice, the follicle density is significantly greater on the rump than in the neck region ($P < 0.001$).

(iv) In Ra+ mice, the follicle density is significantly greater on the rump than in the neck region ($P < 0.01$).

(v) In the neck region, RaRa follicle density is significantly less than normal ($P < 0.001$).

(vi) In the rump region, RaRa follicle density is significantly less than normal ($P < 0.001$).
(vii) In RaRa mice, there is no significant difference between the follicle density on the rump and in the neck region.

It is clear from these results that:

(a) The abnormally low hair density in Ra+ mice is not due to agenesis of any hair follicles, but is due to the failure of some hair follicles to produce hairs.

(b) Since the low follicle density in RaRa mice is proportionately less abnormally low than the hair fibre density in these mice, it seems likely that the low hair density in RaRa mice is due to both agenesis of some hair follicles, and the failure of some other hair follicles to produce hairs.

Despite the results of the statistical tests, the conclusion that the follicle density in Ra+ mice is higher than normal must be accepted with reserve. The apparent density of Ra+ follicles was probably spuriously high for one or more of three reasons: first, as will be shown in the following section on follicle morphology, Ra+ follicles are often sharply bent or curved and some parts of such follicles are more likely to appear on a sagittal skin section than are normal straight-growing follicles; second, many of the structures scored as follicles in Ra+ skin were not true follicles but incompletely formed ones; third, these incompletely formed follicles may in some cases have split into several separate structures.
(2) Follicle and Skin Morphology.

(a) Introduction.

The skin of the normal mouse has been described by a number of authors, e.g. Montagna (1952). It consists of four well-defined layers. The outside layer is the epidermis, then the dermis, the adipose layer and lastly a layer of muscle - the panniculus carnosus. The epidermis can be subdivided into the stratum germinativum or Malpighian layer, which is next to the dermis, and a horny superficial layer - the stratum corneum. In adult mice, the Malpighian layer is only one to three cells thick, and so the separate basal, spinous and granular layers which in most other mammals can be seen within the Malpighian layer, are not distinguishable.

The cells of the Malpighian layer and those cells which form the external sheaths of hair follicles are apparently the same, and form a continuous layer. The hair follicles are evenly spaced and orientated, and are fairly uniform in size at any particular stage of inactivity or growth. They are seen in sagittal sections to be set at an angle of about 45° to the plane of the skin's surface.

The periodic loss and regeneration of hair in the mouse has already been described as occurring in a series of similar cycles. The appearance of any hair follicles depends partly upon the stage of development which they have reached within one of these cycles. A cycle can be divided into three stages; each normal hair follicle passes progressively through these stages (Dry, 1926). First is the anagen stage, when the hair follicles grow down deeply into the adipose layer of the skin and reach their maximum length. Chase, Montagna and Malone (1953) have reported that at the same time the dermis and adipose layers increase in thickness by 50% and 300% re-
spectively. During this period hair proliferation occurs. Second, the catagen stage, during which the completely formed hair moves up the follicle until its base lies just below the sebaceous gland. During the same time the hair follicle shortens until it reaches its original length and is confined entirely to the epidermis and dermis, and the dermis and adipose layers return to their original thicknesses. Changes in the thickness of the epidermis are smaller, and after the early stages of anagen are in the opposite direction from the simultaneous changes in the other skin layers. Third, the telogen or resting stage, during which there is no change in follicle morphology or in the thickness of the skin. After the resting period the whole cycle is repeated. A complete cycle lasts about one month.

According to Bullough (1942), progressive changes in the thickness of the epidermis occur in female mice during the oestrous cycle. The epidermis thickness fluctuates according to the rate of mitosis in the germinative layer, which in turn varies according to the stage of the oestrous cycle. The changes in thickness are not very great (range, 21.3-14.4 in the neck region; and 18.7-12.2 in the rump region) and are probably often masked by the changes resulting from the hair growth cycle.

(b) Methods.

Serial sections were made of skin from the neck and rump regions of ++, Ra+ and RaRa mice. The skin was shaved, removed without scraping, fixed in Bouin's fluid, dehydrated, cleared in methyl benzoate and impregnated in wax (54°C m.p.) for about 8 hours. Sagittal sections were cut at 7μ, and stained with Delafield's haematoxylin and eosin.
Figs. 14 and 15. Sagittal skin sections (Camera lucida drawings).

Note: Uniform orientation, absence of curvature and even size of adjacent ++ follicles. Ra+ follicles show non-uniform orientation, curvature in lateral and longitudinal planes, varying size of adjacent follicles (which are in different stages of growth) and the presence of incompletely developed follicles. RaRa skin shows additional abnormalities, viz., the presence of vacuoles and an abnormally thick epidermis. A comparison of drawings A and G (at the same magnification) shows typically the difference in follicle length and skin thickness between the telogen and anagen stages respectively.

The skin layers shown in these diagrams are: first, the epidermis without stratum corneum, second, the dermis, third, the adiposus, fourth, the panniculus muscle layer.
Five normal mice, four heterozygous ragged mice and four homozygous ragged mice were used to provide skin samples from both the neck and rump zones of each mouse.

The thickness of the skin layers and the lengths of hair follicles were measured by using a graduated microscope eyepiece. Measurements of the stratum corneum layer were taken only from those regions of the skin which appeared not to have been excessively scraped during shaving.

(c) Results.

(1) Ragged Heterozygote and Normal Skin.

++ Mice (sagittal skin sections).

The examination of skin sections from normal mice confirmed the morphological relations described above. The hair follicles were evenly arranged and uniformly orientated; they were not generally bent or curved, except for some of the long anagen-stage follicles, which were slightly bent in a posterior direction just above the hair bulbs. Neighbouring hair follicles were usually in the same stage of growth and were all growing in the same plane relative to the surface of the skin. No case of a hair follicle not producing a hair fibre was observed in normal skin.

Ra+ Mice (sagittal skin sections).

In heterozygous ragged mice, all the normal skin layers are present. Comparative measurements were made of the thickness of these layers in ++ and Ra+ mice, but due to the periodic changes in skin thickness which occur, at least in ++ mice, during the hair growth cycles and the oestrous cycle, these comparisons may not only signify differences between ++ and Ra+ mice (Table 11). However,
Table 11.
Average Thickness (\(\mu\))

++ (5 mice)  

<table>
<thead>
<tr>
<th></th>
<th>Neck</th>
<th>Sacrum</th>
<th>Neck</th>
<th>Sacrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Corneum</td>
<td>25 (\pm) 2.7</td>
<td>15 (\pm) negligible</td>
<td>35 (\pm) 8.3</td>
<td>23 (\pm) 2.4</td>
</tr>
<tr>
<td>Rest of epidermis</td>
<td>17 (\pm) 0.8</td>
<td>13 (\pm) 1.2</td>
<td>16 (\pm) 0.8</td>
<td>14 (\pm) 0.5</td>
</tr>
<tr>
<td>Dermis</td>
<td>112 (\pm) 19.5</td>
<td>97 (\pm) 4.0</td>
<td>112 (\pm) 12.9</td>
<td>128 (\pm) 5.8</td>
</tr>
</tbody>
</table>

Table 12.
Range of Thickness (\(\mu\))

++ mice (4 mice)  

<table>
<thead>
<tr>
<th></th>
<th>Neck</th>
<th>Sacrum</th>
<th>Neck</th>
<th>Sacrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiposus</td>
<td>0 - 450</td>
<td>0 - 315</td>
<td>90 - 225</td>
<td>54 - 225</td>
</tr>
<tr>
<td>Dermis</td>
<td>90 - 180</td>
<td>90 - 108</td>
<td>72 - 135</td>
<td>108 - 135</td>
</tr>
</tbody>
</table>
the results seem to indicate that in both normal and Ra+ mice the stratum corneum layer of the epidermis is thicker in the neck than the rump region; the stratum corneum of Ra+ mice is apparently slightly thicker than normal in both these regions. The measurements of stratum corneum thickness may however be unreliable due to the skin having been shaved for the preparation of sections.

The ranges of thickness of the dermis and adipose layer found in a number of ++ and Ra+ mice are shown in Table 12. The variation in thickness which is found between mice is probably partly due to the progressive changes in skin thickness which occur during the hair growth cycles. However, it is significant that in both the neck and rump areas the adipose layer seems to be more constant in thickness in Ra+ than in normal mice.

Numerous abnormalities are observed in Ra+ skin sections; these are in nearly every case more extreme in the neck region than at the rump, and in the following account this will be assumed unless it is stated otherwise.

Hair follicles are present but their incidence is more irregular than in normal skin. The follicles found can be divided into two classes; those which produce hairs and those which do not. As in normal skin, there are two types of hair-producing follicles. The larger type is at the anagen growth stage and projects deeply into the adipose layer of the skin; the small type is in catagen or telogen stages and does not project below the dermis. Among those follicles which do not produce hairs there are also two types. First there are a few completely developed follicles which, except for the fact that they do not possess hairs, are grossly normal in structure. The follicle papillae are never visible as these follicles
Sagittal skin sections (x 75)

Fig. 16. ++ neck region - anagen stage.

Fig. 17. ++ neck region - telogen stage.

Fig. 18. Ra+ rump region - showing some follicles in the telogen stage (T.F.), some incompletely formed follicles (arrowed) and one large anagen stage follicle orientated parallel to the surface of the skin.

Note: (1) the great difference in skin thickness and follicle length between anagen and telogen stages in ++ skin.
(2) adjacent ++ follicles are mostly in the same phase of growth, not so Ra+ follicles.
Sagittal skin sections (x 75)

Fig. 19. ++ neck region - catagen stage.

Fig. 20. Rat+ neck region; showing one large, proximally curved follicle in anagen stage (A.F.) and other adjacent follicles in late catagen or telogen stages.

Note: ++ adjacent follicles are evenly orientated and in the same growth phase. Rat+ adjacent follicles are not evenly orientated and are in different growth phases from each other.
Sagittal skin sections (x 238)

Fig. 21. Neck region - telogen stage; showing even size and uniform orientation of follicles.

Fig. 22. Rat neck region; showing a mis-orientated hair follicle which has formed a capsule, enclosing layers of keratin and a hair fragment.

Fig. 23. Rat neck region; showing keratin "plugs" in the hair canals of two follicles which have not produced hairs.
Fig. 24. **rump region - late catagen stage.**

Fig. 25. **rump region; showing one incompletely formed follicle (I,F.), one curved follicle with hair fibre (centre) and an open keratin-filled vacuole (right).**

Fig. 26. **rump region; showing a keratin-filled vacuole (K) and a mis-orientated follicle with hair fibre growing parallel with and close to the epidermis.**
**Sagittal skin sections (x 150)**

**Fig. 27.** ++ rump region - late catagen phase.
Note: even size, uniform orientation and absence of curvature of the follicles.

**Fig. 28.** Rat+ rump region. Two follicles are shown, both carrying hair fibres. Since these are sagittal sections the non-appearance of the central region of each follicle shaft shows lateral curvature. The follicles are also curved in the longitudinal plane.

**Fig. 29.** Rat+ neck region. The two large follicles are in anagen stage and the right hand one shows apparent compression of the partially keratinised hair shaft. The two smaller left hand follicles (C.F.) are in the catagen phase.

Note: Neighbouring follicles are in different phases of growth.
are apparently always in the resting stage of the growth cycle. Secondly, there is a considerable number of incompletely formed follicles in Ra+ skin. These are isolated groups of external sheath or Malpighian layer cells, forming clumps or elongated streaks in the dermis (Fig. 8). These cell aggregates are of varying size and shape but are always smaller than fully developed hair follicles. They never achieve the structure of follicles and lack the hair germ, papilla, inner sheath and hair canal, and are never associated with sebaceous glands. All the completely developed follicles, whether or not they produce hairs, are associated with sebaceous glands and possess the normal follicle structure of outer and inner root sheaths, hair canal, hair germ, and hair bulb.

The orientation of hair follicles with respect to each other and with respect to the epidermis is frequently non-uniform or otherwise abnormal in Ra+ skin.

The lateral component of the angle of the follicles to the plane of the skin's surface is often other than the normal 90°, and may be different in neighbouring follicles. Neighbouring follicles therefore do not always lie in the same plane, as they do normally. The longitudinal component of the angle of the follicles to the plane of the skin's surface may vary from the normal 45°, e.g. large follicles in anagen phase may lie almost parallel to the plane of the skin's surface for much of their length (Fig. 13).

Gross morphological abnormalities of individual hair follicles also occur in Ra+ mice. These generally take the form of waving or undulation and curving of the follicles and hairs (Fig. 28). The bulb of the large anagen phase type of follicle is often bent round to lie against the panniculus carnosus muscle layer.
This causes a hockey-stick shaped follicle unless, as in some cases, the rest of the follicle lies nearly parallel to the plane of the skin's surface. Such follicles may also curve or wave in the lateral and longitudinal planes.

In general, anagen stage follicles in Ra+ mice are considerably shorter than the corresponding follicles in normal mice; they are not uniformly orientated at the normal angle of 45° to the plane of the skin's surface, and they are curved or undulated to an abnormal degree. These facts and the data reporting a less than normal maximum thickness of the adipose layer in Ra+ skin (Table 12), suggest that the large anagen phase follicles in Ra+ mice often have not sufficient depth of skin to grow to the normal length, despite the compensating effects of curving of the follicles and their altered angles of growth. In Ra+ mice there seems to be insufficient depth of skin to accommodate anagen phase follicles unless they are curved, shortened or otherwise distorted, because the adipose layer does not expand to the same extent as in normal mice.

The follicles at catagen or telogen stages in Ra+ skin, which have retracted and are therefore shorter than during the anagen phase of their growth, may also be undulated or curved in both planes.

The shafts of hair follicles in the Ra+ neck region tend to be wider than those of normal follicles and the external sheath cells of the Ra+ follicles are often diffusely arranged.

In general, Ra+ hair follicles at all phases of growth are stouter and less neat, often curved and undulated, and less evenly arranged and orientated than comparable normal follicles.
++ and Ra+ mice (from whole skin mounts).

Microscopic examination was made of the stained whole mounts of skin taken from the neck and rump of four pairs of heterozygous ragged and normal mice. The results confirm some of the observations made on the skin sections. The hair fibre shafts and the hair follicles could be seen on some of these whole mounts (Figs. 30, 31). In the skin from normal mice all the follicles carried hair fibres. The fibres and follicles were uniformly orientated and arranged in precise rows. Individual follicles did not appear to be bent or curved.

In skin from the necks of Ra+ mice a proportion, in some cases as great as 20%, of the follicles apparently did not carry hair shafts. The Ra+ hair fibres and follicles in the neck region were not uniformly orientated, nor were they arranged consistently in rows (Fig. 31). A number of large follicles were seen which appeared similar to the anagen phase follicles observed in sections of ragged heterozygote skin. Some of these large follicles showed a pronounced curve near the hair bulbs, giving a hockey-stick appearance which was not seen in normal follicles.

These differences from normal were much less apparent in the skin from the rump of Ra+ mice.

(ii) Hair Growth Cycles in Ragged Heterozygote and Normal Skin.

(a) Results.

Hair Follicles.

Neighbouring hair follicles in the skin of normal mice are generally in the same phase of growth. On all skin samples except one from the neck and sacral regions of normal mice, the hair
**Fig. 30.** Rat neck region, whole skin mount. 
Note: even size, uniform orientation of follicles and hair shafts. All follicles carry hair fibres.

**Fig. 31.** Rat neck region, whole skin mount. 
Note: some follicles (anagen phase) which are larger than their neighbours; non-uniform orientation of hair shafts. Some follicles do not carry hairs.
follicles were in catagen or telogen stages and were confined entirely to the epidermis and dermis. In these sections follicles were very rarely seen to be out of phase with their neighbours and to project into the adipose layer of the skin. The total thickness of the skin (measured from the epidermis to the panniculus layer of muscle) varied from 101 \mu m - 416 \mu m, and the adipose layer on these sections varied in thickness from 0 to 315 \mu m.

On the one normal skin sample where the follicles were not confined to the epidermis and dermis, almost all the follicles were in the anagen phase of growth and projected deeply into the adipose layer. The total thickness of the skin during the anagen stage averaged 630 \mu m, and the thickness of the adipose layer was about 450 \mu m. These results are in agreement with those of Chase et al. (1953) who described the increase in skin thickness during the anagen stage of the follicle growth cycle. The normal anagen follicles were as usual uniformly orientated and straight, except for in some cases a slight curve just distal from the follicle bulb; and they grew down consistently at a longitudinal component angle of about 45\(^\circ\) to the skin's surface.

The average length of these normal follicles was 1116 \mu m (i.e. less than twice the total thickness of the skin, which was 630 \mu m). (Table 13).

Important differences from the normal were observed in Ra+ skin. Neighbouring hair follicles in the same skin sample might simultaneously be in different stages of the growth cycle (Fig. 10). Frequently about half the follicles on one section projected down into the adipose layer (i.e. were in anagen phase) whereas the rest of the follicles were shorter and confined to the epidermis and
Table 13.
The Average Length of Anagen Stage Follicles (Neck region), and Accompanying Skin Thickness (\(\mu\)).

<table>
<thead>
<tr>
<th></th>
<th>++</th>
<th>Rat+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicle Length</td>
<td>1116 ± 27</td>
<td>630 ± 30</td>
</tr>
<tr>
<td>Total Skin Thickness</td>
<td>630 ± negligible</td>
<td>270 ± 10</td>
</tr>
<tr>
<td>Adiposus Thickness</td>
<td>450 ± negligible</td>
<td>126 ± 10</td>
</tr>
</tbody>
</table>

The total skin thickness was measured from the epidermis to the base of the adipose layer inclusively.

Note that the skin thickness available for anagen follicles in ++ skin is over half the average follicle length, whereas the skin thickness available for anagen follicles in Rat+ skin is less than half the average follicle length.
and dermis. This overlapping of follicle growth phases was seen clearly on all skin sections from the neck region of Ra+ mice, and on all skin sections except one series from the rump region; such skin samples could not be classified as belonging to any one of the normal growth phases.

The total skin thickness on these sections varied from 180 to 378 $\mu m$, and the thickness of the adipose layer remained at a moderate level between $54 \mu m$ and $225 \mu m$ (Table 12). Although some large anagen phase follicles were always present in all these skin samples, both the total skin thickness and the thickness of the adipose layer were often less than they were in normal skin during the stages when no anagen follicles were present. When the skin thickness was small the large follicles were seen to grow at an acute angle to, or parallel with the surface of the skin; or they were curved and undulating in shape, and reduced in length.

**Epidermis.**

It was observed in both ++ and Ra+ mice that the cytological condition of the epidermis tended to vary according to the skin thickness and stage of growth of the hair follicles. Near the commencement of periods of increasing skin thickness and/or active follicle growth the basal Malpighian cells of the epidermis were often relatively numerous and their nuclei were large and oval in shape. During resting stages of the hair cycle when the skin thickness was small, the Malpighian cells of the epidermis seemed less numerous, their nuclei were often flattened and pyknotic, and the germinative layer of the epidermis was relatively thin.
Discussion.

From the evidence cited above it seems that in Ra+ mice the hair follicles can be out of phase with each other in their growth cycles, i.e. neighbouring follicles may be at different stages of growth. This means that some of the follicles must be out of phase with the periodic increase and decrease of skin thickness which normally accompanies the follicle growth cycle. Furthermore, in Ra+ mice the range of this variation in skin thickness seems less than normal. Many large follicles in Ra+ skin were observed to be in the anagen stage while the skin had only maintained a moderate thickness. The curved shape of these follicles and the fact that they were frequently orientated almost parallel with the plane of the skin's surface were probably due to the abnormal thinness of the skin; this had resulted in a restriction of the space normally available for the downward growth of anagen follicles.

These anagen follicles, although large and projecting deeply into the adipose layer of the skin, were yet shorter than the corresponding normal anagen follicles (Table 13). The average length of normal anagen follicles was 1116 μ when the skin thickness was 630 μ; whereas the average length of Ra+ anagen follicles was 630 μ when the skin thickness was only 270 μ. It seems possible therefore that the proportionally smaller thickness of skin available for the growth of the large Ra+ follicles may cause:

(i) Morphological abnormalities of the follicles,
(ii) Abnormal orientation of the follicles,
(iii) The attainment by these follicles of a less than normal maximum length.

Most individual hair follicles in Ra+ mice will have been
affected in one or all of these ways during the anagen phase of their growth cycles. When these follicles shorten and reach the catagen and telogen stages of the cycle, their curved structure and altered orientation may be retained to some extent. All the observed morphological abnormalities of Ra+ hair follicles can thus be explained on the above hypothesis.

(iii) Ragged Homozygote Skin (from sagittal skin sections).

(a) Results.

All the normal skin layers are present in some RaRa mice; in others the adipose layer is absent. The thickness of the skin layers varies considerably between individuals, but always the average thickness of the dermis is greater than normal and the average thickness of the adipose layer is less than normal. The thickness of the skin layers also varies a great deal within some individual RaRa mice due to excessive wrinkling and folding of the skin (Table 14).

Although the phenotypes of all adult ragged homozygotes appear grossly similar, there is considerable variation in the histological condition of the skin of different individuals. During the present work, two main types of RaRa mice were distinguished with respect to their skin histology; these will be described separately.

Type (1).

Mice Nos. 1 and 4 in Table 14 are of this type. The skin is abnormally folded and the thickness of the skin layers is therefore variable. However, the average thickness of the epidermis is greater than normal, and the adipose layer is thinner than normal.

Epidermis: In the neck region there are 1–5 (average 3) rows of basal Malpighian cells; in the rump region there are 2–6
Table 14.
The Average Thickness of Skin Layers in RaRa mice (μ).

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Neck S. corneum</th>
<th>Epidermis</th>
<th>Dermis</th>
<th>Adiposus</th>
<th>S. corneum</th>
<th>Epidermis</th>
<th>Dermis</th>
<th>Adiposus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8(0-30)</td>
<td>29(8-45)</td>
<td>153(45-324)</td>
<td>25(0-180)</td>
<td>18(0-60)</td>
<td>29(10-80)</td>
<td>135(45-297)</td>
<td>5(0-60)</td>
</tr>
<tr>
<td>2</td>
<td>15(5-54)</td>
<td>9(5-20)</td>
<td>180(108-270)</td>
<td>0</td>
<td>9(-)</td>
<td>14(4-27)</td>
<td>180(108-225)</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>9(0-30)</td>
<td>13(5-27)</td>
<td>225(180-270)</td>
<td>0</td>
<td>18(9-18)</td>
<td>15(4-45)</td>
<td>270(225-360)</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>8(0-36)</td>
<td>25(7-36)</td>
<td>126(72-180)</td>
<td>27(0-54)</td>
<td>9(5-27)</td>
<td>29(14-45)</td>
<td>126(63-198)</td>
<td>0</td>
</tr>
</tbody>
</table>

++ (average for 5 mice)

<table>
<thead>
<tr>
<th>Neck</th>
<th>Rump</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>112(90-180)</td>
<td>97(90-108)</td>
</tr>
<tr>
<td>172(0-450)</td>
<td>140(0-315)</td>
</tr>
</tbody>
</table>

The bracketed figures are the maximum ranges of thickness which were observed.
Sagittal skin sections (x 150)

Fig. 32. **↔** neck region - catagen phase.

Fig. 32. RaRa (type 1) neck region. The large central follicle carries no hair fibre and has a dilated vacuolate hair canal. This follicle appears to have just completed an active growth phase and is at a stage of growth equivalent to the normal catagen. The incomplete follicles (arrowed) are merely clumps of Malpighian cells.

Fig. 33. RaRa (type 1) rump region; showing a local area of epidermal hyperplasia and one incompletely formed follicle. Note: (1) virtual absence of the adipose layer in RaRa skin although Fig. 33 shows probably an equivalent catagen phase; (2) thick, actively proliferating germinative region in the RaRa epidermis.
Fig. 25. RaRa (type 1) rump region; showing an encapsulated hair fragment (H.F.) and an incompletely developed follicle (I.F.).

Fig. 26. RaRa (type 1) rump region; showing 3 incompletely developed follicles and one functional but mis-orientated follicle with hair fibre (F.).

Fig. 27. RaRa (type 2) rump region; showing 2 small, incompletely developed follicles which are merely clumps of Malpighian cells.

Note: (i) Excessive folding of type (1) RaRa skin,
(ii) Thick, proliferative epidermis of type (1) and thin, apparently inactive epidermis of type (2) RaRa skin,
(iii) Small thickness of adipose layer in type (1) and absence of adipose layer in type (2) RaRa skin.
Fig. 38. RaRa (type 1) rump region; showing an incompletely developed follicle (extreme right), a large vacuole containing keratin (K) and a hyperplastic region of follicle external sheath tissue (E).

Fig. 39. RaRa (type 1) rump region; showing one large, almost normally developed follicle with a large sebaceous gland, and a small isolated clump of Malpighian cells.

Fig. 40. RaRa (type 2) rump region; showing one incompletely developed follicle and an abnormally large superficial blood vessel (V).

Note: (i) Thick, proliferating germinative layer of type (1) epidermis - cf. thin, apparently inactive epidermis of type (2).
(ii) Abnormal folding and hyperkeratinisation of type (1) RaRa skin.
(average 4) rows of basal Malpighian cells. of 1-4 (average 2) rows of basal Malpighian cells in normal skin. The Malpighian cells are numerous and often closely packed together; their nuclei are regularly oval in shape and are not deeply stained or pyknotic. Mitotic figures are frequent in this layer of the epidermis. Unlike the normal adult epidermis there are cells forming an incomplete stratum intermedium layer, there are keratohyalin granules and a stratum lucidum. These layers are normally only present in the immature epidermis.

In skin from the rump area there are a few local regions where marked hyperplasia of the germinative layer increases the epidermal thickness up to 80 μ (Fig. 34), but every where the epidermis is thicker than normal.

Follicles: Fully developed hair follicles occur rarely in ragged homozygote skin. Some of these fully developed follicles produce hairs in the rump region, but none do so in the neck region, where no hairs are found. Whether a hair has been produced or not, large sebaceous glands are always associated with these follicles. The follicles never project more than slightly below the dermis; they usually have wider external sheaths than normal follicles, and are often curved or undulated in both lateral and longitudinal planes of growth. The orientation of RaRa follicles is not consistent, and when hairs are produced they may emerge at abnormal angles. Follicles carrying hairs are otherwise normal. Those follicles not carrying hairs are grossly similar in structure except that the distal part usually becomes bulbous and vacuolate (Fig. 33). The vacuoles, which sometimes contain cornified epithelium, are formed by a distension of the hair canal and flattening of the follicle inner sheath.
Follicles, whether or not they carry hairs, are often plugged at the mouth by flaking cornified epithelium.

In ragged homozygote skin there are, in addition, numerous incompletely formed follicles which are confined to the epidermis and dermis. These "follicles" are different sized groups or elongated streaks of Malpighian or external sheath cells (Fig. 36); the cell groups are often quite large and the distal ends may join the epidermis, but they never possess the structure of normal follicles, viz. hair canal, outer and inner sheath, etc., and are not associated with sebaceous glands.

Vacuoles not associated with hair follicles and usually containing cornified epithelium are found in the dermis (Fig. 38). Some of these vacuoles enclose hairs or fragments of hair which do not emerge from the skin.

Finally, in skin sections from the rump of a 36-day old RaRa mouse, a number of epidermal thickenings were observed. These were morphologically identical with the hair follicle primordia which normally appear in embryos and neonatal mice (Fig. 41). It is possible that they were true primordia, since the epidermis is still fully differentiated at this stage in RaRa mice.

In summary it can be said that type (1) ragged homozygote skin differs from normal in the following respects: excessive wrinkling and folding, few hair follicles and fewer hairs, numerous incompletely formed follicles, localised hyperkeratosis and hyperplasia of the epidermis; and possibly the belated appearance of follicle primordia.

**Type (2):**

Mice Nos. 2 and 3 in Table 14 are of this type. The skin
Fig. 41. Rara rump region, showing the development of a follicle primordium (F.). This material was taken from a 36 day old mouse. The epidermis appears actively proliferative.

Sagittal Section (x 260).
is not excessively folded and the thicknesses of the skin layers are less variable within individuals. The epidermis is usually thinner than normal and considerably thinner than in RaRa type (1) mice. The dermis is thicker than normal but the adipose layer is absent.

Epidermis: There are 0-4 (average 2) rows of sparsely arranged Malpighian cells as in normal skin, but the nuclei are usually small, vertically flattened, pyknotic and irregular in shape. Mitotic figures are rarely seen. There is no stratum intermedium, or stratum lucidum, and there are no keratohyalin granules, except in a few locally hyperplastic regions (Fig. 40). The epidermis generally seems in a state of far less active proliferation than in RaRa type (1) skin; it is similar to the normal, apparently non-proliferative epidermis as it appears during part of the resting stage of the hair growth cycle.

Follicles: There are no fully developed hair follicles in the neck region. In the rump area there are fewer fully developed follicles and fewer incompletely formed follicles or groups of Malpighian cells than in RaRa type (1). Many of the follicles and most of the groups of Malpighian cells which are seen in the dermis are smaller than in RaRa type (1); otherwise the hair follicle abnormalities are similar.

Types (1) and (2).

In both types of RaRa skin taken from the neck region there is a number of abnormally large blood vessels. These vessels are located in the adipose layer or lower part of the dermis; their cross sectional area is large but they are usually packed with red blood corpuscles (Fig. 40).
(b) Discussion.

It is clear from the foregoing description that the skin of adult RαRa mice can vary in its histological and cytological characteristics. The external phenotypes of RαRa mice are similar however. It therefore seems possible that the skin of RαRa mice undergoes cyclic changes equivalent to those in normal mice, even though a normal hair coat is not present. Two main types of RαRa skin have been described above. It is concluded that these two types correspond to normal skin, first during the active growth phase and second, during the resting phase of the hair cycle.

Type (1) RαRa skin is characterised by: the presence of an adipose layer, comparatively large follicles, and a thick and in some areas hyperplastic germinative layer of the epidermis which contains numerous closely packed Malpighian cells with oval, non-pyknotic nuclei. Mitotic figures are numerous in the stratum germinativum.

Type (2) RαRa skin is characterised by: The absence of an adipose layer, and comparatively small follicles. The epidermis is thin; the Malpighian cells are sparse and have flattened, pyknotic nuclei, and the stratum intermedium and stratum lucidum layers are entirely absent. Mitotic figures are rarely seen in the epidermis.

It is concluded that the type (1) RαRa skin is equivalent to normal adult skin during the anagen stage of follicle growth and during a phase of active proliferation of the Malpighian layer. The type (2) RαRa skin is equivalent to the normal telogen or resting stage of the hair follicle cycle, and the Malpighian layer is non-proliferative.

Even during the "anagen" phase in type (1) RαRa skin the adipose layer is much thinner than in normal anagen phase skin.
It is considered that this fact may be related to the absence of most of the functional hair-producing follicles in RaRa skin. The down-growth of the follicle population in normal skin may be the cause of, or a necessary pre-condition for expansion of the adipose layer. The morphological abnormalities of the few completely developed follicles in RaRa skin may be due to the lack of space for follicle down-growth resulting from the partial absence of the adipose layer.

A number of important questions remain to be considered:

(i) Why is there so great a difference in the average thickness, the cell population and the proliferative activity of the epidermis of the types (1) and (2) RaRa skin? These differences are greater than those between the anagen and telogen phases in normal skin.

(ii) What is the cause of the local areas of hyperplasia of the epidermal Malpighian layer, and the local regions of hyperkeratosis (evidenced by keratin-plugged follicles, and the presence of vacuoles containing cornified epithelium) which appear mainly in the type (1) RaRa skin?

(iii) What is the cause of the excessive wrinkling and folding of type (1) RaRa skin?

It has been stated by Chase et al. (1951) that there is a basic cellular similarity between the epidermis and the hair bulbs and external follicle sheaths. A follicle external sheath and hair bulb is an extension of the epidermal Malpighian layer folded down over a group of dermal cells. Just as the epidermal Malpighian layer gives rise to a layer of cornified cells, the stratum corneum; so the hair bulbs of follicles produce cornified internal sheath
cells and the keratinised material of the hair shaft.

It may be that in ragged homozygote and in normal mouse skin there is a potential for a certain increased degree of mitotic activity in Malpighian epidermal cells and their derivatives in the hair follicles during the anagen phase of the hair cycle. Since only a few hair follicles are formed in ragged homozygote mice, this potential for cell division may be diverted, causing abnormal hyperkeratosis in local areas instead of hair formation; and general thickening and local hyperplasia of the epidermis instead of the normal follicle down-growth by the division of external sheath cells. Such a diversion of mitotic activity could be visualised in terms of competition between the hair follicles and the Malpighian layer of the epidermis for a substrate supplying energy for cell division. (Competition between different skin tissues, including hair follicles and the epidermis, for a carbohydrate substrate which limits cell division, has been discussed by Bullough (1952). Again, competition between hair follicles for a fibre-forming substrate has been postulated by Fraser (1952a, b, c)).

The abnormal conditions obtaining in the type (1) RaRa skin would on this interpretation, be the result of an abnormal decrease in tissue competition due to partial suppression of hair and hair follicle formation. The potential for cell division is therefore diverted, and produces local hyperkeratosis and hypernormal numbers of epidermal Malpighian cells. (This situation may be to some extent paralleled by that in experiments on skin grafting, where it has been observed that newly grafted skin frequently shows hyperplasia of the epidermis and hyperkeratosis until new hair growth has commenced).
The excessive wrinkling found in type (1) RaRa skin may be due to the absence of those hair follicles which normally incorporate many epidermal Malpighian cells in their external sheaths. The absence of the external sheaths, which are considered as folded down regions of the epidermis, might force the RaRa epidermis to become convoluted in order to incorporate the increased number of Malpighian cells which are produced during the anagen phase of the hair cycle.

The hypothesis elaborated above makes use of a new concept which seems to be the only one consistent with the facts. The anagen phase of the hair cycle is characteristically a period of rapid cell division, causing down-growth of the hair follicles; but it now appears that the response to the commencement of the anagen phase is a function not of the hair follicles as such, but of the germinative epithelial cells which compose the basal Malpighian layer of the epidermis and the external sheaths of hair follicles. The commencement of the anagen phase therefore normally produces an increased rate of mitosis in the germinative epithelial cells which causes down-growth of the follicles by elongation of the external sheaths, and also a temporary increase in thickness of the germinative layer of the epidermis. In normal skin the most obvious effect is the down-growth of follicles, but there is some evidence of increased proliferation in the epidermis also. In ragged homozygote skin the absence of many hair follicles means that the most obvious effect of the anagen phase is epidermal hyperplasia.

This concept states implicitly that the primary response to the stimulus which initiates cycles of hair growth in the mouse occurs in a definitive cell layer and not in the hair follicles as such, which are morphologically differentiated structures embodying
various different types of cells.

The concept may help to clarify the important problem of the cause of the periodic hair growth cycles in rodents.

Finally, it has been observed by Chase et al. (1953) and partially confirmed during the present work, that the epidermis increases in thickness at the commencement of the anagen phase in normal mice and then for the rest of the anagen phase decreases to a thickness even less than that maintained in the telogen phase.

It is considered that these facts can be understood on the basis of a two-fold hypothesis; first, that the commencement of anagen produces an increased mitosis rate in the whole germinative epithelium; and secondly, that competition exists between the different skin layers. In connection with the latter part of the hypothesis, it has been concluded by Bullough (1952) that a sequence of priorities exists in the skin, such that the deeper layers have an advantage in securing nutrients or other substances necessary for cell division. For example, Bullough has remarked that when the hair roots become vascularised, it is at the expense of the epidermis; and Mottan (1945) has observed that waves of hair growth inhibit epidermal mitosis. It seems that competition of some kind probably exists between the different skin layers.

In the normal mouse, when the anagen phase begins, the rate of mitosis of all germinative epithelium cells increases and the epidermis increases in thickness. During the later part of the anagen phase the downward growth of the hair follicles enables them to achieve priority in obtaining substances necessary for the high rate of mitosis occurring in the hair bulbs; correspondingly, the epidermis suffers a lack of these substances and shows a decreased
rate of mitosis in the germinative layer, and diminished thickness.

In the RαRα mice examined, the almost complete absence of competition from down-growing hair follicles apparently enables the epidermis to maintain a high rate of mitosis and therefore an abnormally great thickness throughout the anagen phase.

In conclusion, it should be acknowledged that the fact that mitosis is stimulated in the whole of the germinative epithelium at the beginning of the anagen phase of follicle growth in normal mice, has been independently mentioned by Chase (1954).
PART II - EMBRYOLOGY AND DEVELOPMENT

(1) **External Morphology.**

(1) **External Morphology of Embryos.**

(a) **Introduction.**

The external morphology of ragged and normal embryos was compared in order to study the development of the phenotypic abnormalities caused by the ragged gene and to establish the time of their first appearance in the embryo. It was also desirable to compare the prenatal viability of ragged and normal embryos, especially since from most intercross matings less than the expected number of ragged homozygotes was found at birth.

During the study of the external morphology, the association of varying grades of oedema with different degrees of abnormality in the sima and pelage hair follicles was carefully recorded. (In the following account, the phrase "erupting" of sima hairs or follicles refers to the presence on the follicles of a definitive point or tip due to the growing but still invisible hair pushing out the epidermis above the follicle; the phrase "emergence" of sima hairs implies the appearance of the hair shaft itself outside the follicle).

(b) **Methods.**

Intercross matings - Ra+ x Ra+, and backcross matings - Ra+ x ++ were set up to provide embryos. The intercross matings were intended to allow comparisons between ++, Ra+ and RaRa embryos where possible; the backcross matings would allow comparisons between ++ and Ra+ embryos only, but it was thought that classification of these two genotypes could be more certain here than in the inter-
cross matings since in the latter there might be cases of overlapping between the Ra+ and RaRa classes.

Timing of Embryos:
Males were always mated with non-suckling females so that the ages of embryos could be estimated by the time of appearance of the vaginal plug in the mother. The females were examined for plugs daily between 9.00 and 9.30 a.m. The estimated age was checked by observing the stage of development of the external features of the embryos (Grüneberg, 1943b). Copulation usually occurs near midnight; embryos were dissected out of the females between 9 a.m. and midday and so their ages were reckoned as the number of days since a vaginal plug had been found plus half a day.

Dissection and Fixation of Embryos: (Carter 1954).

Pregnant females were killed and the uterus was removed, pinned out and washed in Ringer's saline. The uterine horns were slit and the distribution of viable and inviable embryos in each horn was noted. Each embryo, within its still unbroken membranes and with its placenta, was removed and placed in an embryo cup containing saline at room temperature. The yolk sac and amnion membranes were torn near the placenta and carefully pulled off the embryo with fine forceps.

The morphology of the embryos was examined under a low-power binocular microscope and they were classified as viable or inviable and also, where possible, into the three phenotypes ++, Ra+, RaRa. After an hour or so the vitelline and umbilical vessels could be cut without the occurrence of much bleeding. The embryos were then washed in saline and fixed in Bouin's fluid. After fixation the embryos were measured with a travelling microscope and outline
drawings were made with a camera lucida of those ones required for sectioning. 492 viable embryos were dissected out and examined in this way. All dead embryos were classified according to their estimated time of death; this classification was based upon their external features where these were recognisable, or otherwise upon the size of the placenta. Those embryos which had decomposed too much for their time of death to be estimated were classified as "moles".

The mice used in this part of the work were from the original heterogeneous stock in which the ragged gene arose. No selection (e.g. for increased viability) had been practised upon this stock.

(c) Results.

In backcross and intercross matings it is possible to distinguish heterozygous ragged from normal embryos after 16½-17½ days' gestation, but not before this age. The heterozygous ragged embryos show delayed sinus hair development compared with the normal. In intercross matings homozygous ragged embryos are distinguishable from either normal or heterozygous ragged embryos at or after 13½ days' gestation. The ragged homozygotes show a generalised oedema and abnormalities of sinus hair and follicle development.

The morphology of the heterozygous and homozygous ragged embryos was studied in detail.

Rat embryos. (Observations from backcross matings).

Before 16½ days' gestation no consistent differences to distinguish Ra+ from ++ embryos were detectable. There were however a few embryos from backcross matings which showed evidence of slight oedema. At 14½ days' gestation this oedema manifested itself
as a swelling in the dorsal neck region of the embryos. At 16½ days there was slight swelling in the same region and the skin appeared puffy in the oedematous embryos. Only a few of the litters examined at these ages showed any examples of oedema and there were no cases of oedema in litters examined at other ages. Consequently, oedematous embryos comprised only a very small proportion of the total embryos examined at any given age. There were 2/19 oedematous embryos at 14½ days, 0/26 at 15½ days, 5/44 at 16½ days.

Half of all embryos examined from backcross matings would be expected to be ragged heterozygotes, and therefore even if the oedematous embryos were ragged heterozygotes there must probably be other ragged heterozygotes which did not show oedema. In any event it is clear that the ragged gene in the heterozygous state causes no more than low-grade oedema.

From 16½ days until term, putative Ra+ embryos are characterised by abnormally slow development of the sinus hairs, and can be clearly identified.

At 16½ days' gestation:

Normally all the whiskers are clearly erupting. In Ra+ embryos only half the whiskers are erupting. In normal embryos one or both of the supra orbital sinus hairs and the post orbital sinus hairs are erupting, whereas in Ra+ embryos the posterior supra orbital sinus hair only is faintly erupting.

At 17½ days' gestation:

In normal embryos about 6 whiskers per side have now clearly emerged; in Ra+ embryos only 0-3 whiskers per side have emerged. Normally the posterior supra orbital sinus hairs and the post orbital sinus hairs have emerged; in Ra+ embryos only the posterior supra
orbital sinus hairs have emerged.

At 13½ days' gestation.

Normally many of the whiskers have clearly emerged and some are fairly long. In Ra+ embryos the whiskers are considerably shorter. In normal embryos both supra orbital sinus hairs have emerged (the anterior one being shorter) and also the post orbital sinus hairs, whereas in Ra+ embryos the anterior supra orbital sinus hairs are still absent and the post orbital sinus hairs are shorter than normal.

Full term Ra+ embryos are characterised by abnormally short or absent anterior supra orbital sinus hairs, and abnormally short post orbital sinus hairs and whiskers.

All the data on sinus hair emergence at specific ages are subject to considerable "between litter" variation and the above observations are averaged from a number of litters. It seems, however, that the criteria used do allow of accurate genotypic classification first, because from backcross litters of ages 16½-18½ days, 20 ++ and 22 Ra+ embryos were identified (the expected number would be 21++ and 21 Ra+ embryos); and secondly because the characteristics distinguishing ++ and Ra+ embryos showed continuity from day to day and when used for post-birth classifications could be checked by genetic testing of the animals concerned.

RaRa embryos: (Observations from intercross matings).

Ragged homozygote embryos cannot be identified with confidence before 13½ days' gestation; it is possible however that at 12½ days the rows of developing whisker follicles are slightly less prominent than normal. After 13½ days, putative RaRa embryos were distinguished mainly by the occurrence of oedema. The correctness
of the criteria for identifying RaRa embryos will be discussed later.

**At 13½ days' gestation:**

**++** The normal mouse embryo shows developing whisker follicles and possibly a few early initiated pelage follicles on the flanks (Grüneberg, 1943). It is not oedematous.

**RaRa:** Two forms of oedema are seen in presumptive RaRa embryos:

(a) A slight form which is exemplified by puffy swelling of the embryo in the region of the neck and anterior dorsum. There is little or no retardation in growth. The pelage follicles if present appear normal.

(b) The more extreme form of oedema is exemplified by broad laterodorsal bulges on the sides of the embryo which stretch longitudinally from the level of the eyes to the hind legs. There may be fewer pelage follicles present than normally and the whisker follicles are less clear and more uneven in development than normal. Occasionally the supra orbital sinus follicles are not split into two as they are in the normal embryo.

**At 14½ days' gestation:**

**++** The pelage follicles are now more numerous and widespread over the body.

**RaRa:** Oedema is again found, and as at 13½ days, high and low grades of expression can be distinguished. In all oedematous embryos the body follicles, i.e. the pelage follicles, are present but are less clear and less well developed than normal; there is also less clear and more uneven development of whisker follicles. The density of the pelage follicles may be slightly less than normal. In very highly oedematous embryos the post oral sinus follicles are sometimes split into three; these follicles are not split in normal
**Fig. 12.** Embryos from a litter of 16½ days' gestation. Normal on the left; putative RαRa, showing high-grade oedema, on the right.
The body changes are denoted by lesions prominent on the skin. The normal epidermis shows a reddening staining of the skin.

At 15th day, Reabsorption

And may be slightly indurated.

Reason the skin on the face is always less wrinkled than normal.

Prominent by the occasion of the epidermis caused by the occlusion. As appears through loss than normal but this may be due to some dermographic appearance is slight less than normal, in some cases they develop even more marked than the skin normal. In some cases the dermographic appearances are present that are smaller and less well developed.

The body changes on the other surfaces of the body are not as prominent as on the face.

Generally only the posterior part of the body shows abnormal changes.

Few or none of the wrinkles are usually there to be seen.

May be (4.4.4.4) and the mat of the body or the epidermis and the mat of the body are more prominent on the face than between the neck and the head.

The two main types of occlusion are known. The hard occlusion.

Changes is becoming wrinkled on the skin on the body at all the other sites. Wrinkles are eminently hard and most of the body appear. Most of the wrinkles and head and most of the body appear on the neck.

The pressure wrinkles are rarest dense and are present on the body. Localized suppurated lesions are occasionally found in the body.
Ralla: Widely variable high and low grades of oedema are found. The appearance of the embryos is similar to that at 15½ days, although the skin haematomata are more common. The body follicles are smaller, less well developed and more uniform in size than normal. The development of all the sinus hair follicles is affected to a varying extent. In slightly or moderately oedematous embryos the sinus follicles are less abnormal than in the highly oedematous embryos. The whisker follicles are generally flatter and less uniformly arranged than normal; from none to half of them may be erupting, depending upon the severity of the oedema. None of the sinus hairs, or only the supra orbital sinus hairs, may be erupting. The slighter grades of oedema seem to be associated with an effect upon sinus hair development which is very similar to the effect found in the ragged heterozygote embryo. In highly oedematous embryos there is often some retardation in the development of such external features as the toes, fingers and pinna of the ear.

At 17½ days' gestation.

Ralla: Compared with the 15½-day embryo, wrinkling of the skin is now more marked. The pelage follicles are less prominent except on the head. All the whiskers are erupting and about 6 per side have usually emerged. The posterior supra orbital and the post orbital sinus hairs have emerged.

Ralla: The very high grades of oedema are less frequent than at earlier ages. There is in most cases a swelling of the neck and throat region which may extend posteriorly along the dorsum. Superficial skin haematomata are usually present. The body follicles are smaller and less prominent than normal. The whisker follicles are also less prominent than normal; about 3-6 whisker follicles
may be erupting but no whiskers are emerging. Of the other sinuses follicles none, or just the posterior supra orbital sinus follicles, are erupting. The abnormalities of the sinus hair follicles seem less variable than at earlier ages.

At 18½ days’ gestation:

The skin is still extensively wrinkled; the pelage follicles are inconspicuous. Many of the whiskers have clearly emerged and some are fairly long. Both the supra orbital sinus hairs and the post orbital sinus hairs have emerged.

ReHa: There are no cases of very high grades of oedema. Oedematous embryos show puffy skin and areas of swelling near the throat, neck and anterior dorsum. Superficial haematomata are usually found. The body follicles are even less conspicuous than in the normal embryo; their development is probably retarded. None of the whiskers have emerged at this age and few of the follicles are erupting. None of the other sinus hairs have emerged. The whisker follicles are flatter and less well developed than normal and so the region of the moustache or upper lip has an abnormally smooth appearance.

At full term:

There is little difference in the normal embryo from the 18½-day stage except that those whiskers and sinus hairs which have emerged are longer.

ReRa: The oedematous embryos are the same as at 18½ days except that in some cases up to 3 whiskers may have emerged.

The Incidence of Oedema.

The proportion of oedematous embryos in backcross and intercross matings is given in Table 15.
Table 15.

The incidence of oedema in backcross (Ra+ x ++) and intercross (Ra+ x Ra+) matings.

<table>
<thead>
<tr>
<th>Age of embryos in days</th>
<th>Grade of oedema</th>
<th>No. of embryos</th>
<th>Proportion of oedematous embryos in intercross matings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ra+ x ++</td>
<td>Ra+ x Ra+</td>
</tr>
<tr>
<td>12½</td>
<td>Not</td>
<td>12</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Slight</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very high</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>13½</td>
<td>Not</td>
<td>35</td>
<td>5/40</td>
</tr>
<tr>
<td></td>
<td>Slight</td>
<td>0</td>
<td>The deficiency from expected ratio is not significant</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>4</td>
<td>(P &lt; 0.170.05) at the 5% level</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very high</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>14½</td>
<td>Not</td>
<td>17</td>
<td>8/23</td>
</tr>
<tr>
<td></td>
<td>Slight</td>
<td>2</td>
<td>(No deficiency from $\frac{1}{2}$ ratio)</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very high</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>15½</td>
<td>Not</td>
<td>26</td>
<td>9/33</td>
</tr>
<tr>
<td></td>
<td>Slight</td>
<td>2</td>
<td>(No deficiency from $\frac{1}{2}$ ratio)</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very high</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>16½</td>
<td>Not</td>
<td>39</td>
<td>14/53</td>
</tr>
<tr>
<td></td>
<td>Slight</td>
<td>5</td>
<td>(No deficiency from $\frac{1}{2}$ ratio)</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very high</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>17½</td>
<td>Not</td>
<td>42</td>
<td>*6/46</td>
</tr>
<tr>
<td></td>
<td>Slight</td>
<td>0</td>
<td>The deficiency from expected ratio is not significant at</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>0</td>
<td>the 5% level (P &lt; 0.1 &gt; 0.05)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very high</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>18½</td>
<td>Not</td>
<td>19</td>
<td>*7/42</td>
</tr>
<tr>
<td></td>
<td>Slight</td>
<td>0</td>
<td>The deficiency from expected ratio is not significant at</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>0</td>
<td>the 5% level (P &lt; 0.3 &gt; 0.2)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very high</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*If the data for these ages are combined, giving a ratio of $\frac{13}{88}$, the deficiency from a $\frac{1}{2}$ ratio is significant (P < 0.05).
Out of 237 post 12-day intercross embryos, 49 were classified as oedematous (a proportion of 1/4.8). If all RaRa embryos were normally viable and if they were all assumed to show oedema after 12 days' gestation, a proportion of 1/8 oedematous embryos would be expected. There is therefore a difference from the expected number of oedematous embryos, but the deficiency is not significant at the 5% level ($\text{Po}0.1$).

Additionally, 93 viable embryos of ages 14½, 15½, 16½, 17½ and 18½ days were obtained from control matings (++Ra x ++Ra) in the ragged stock; none of these embryos was oedematous.

The data on the incidence of oedema can be summarised as follows:

(i) No oedema was found in embryos from control matings within the ragged stock.

(ii) Low-grade oedema was found in a few embryos of 14½ and 16½ days from backcross matings; these embryos may have been ragged heterozygotes.

(iii) Oedema was found in a certain proportion of 13½-18½ day embryos recovered from ragged intercross matings, but it did not visibly occur in embryos before 13½ days' gestation.

(iv) The oedema found was variable in its expression.

(v) The pelage and sinus hair follicles were sometimes abnormal and always retarded in their development in oedematous embryos. Higher grades of oedema seemed to be associated with more extreme retardation of follicle development.

(vi) A 1/4 proportion of embryos recovered from intercross matings might be expected to be ragged homozygotes. The ratio of oedematous embryos recovered is sufficiently nearly 1/4 as to suggest that oedema
is a usual if not invariable characteristic of RaRa embryos. Deficiencies from the expected $\frac{1}{4}$ ratio of oedematous embryos were found at $13\frac{1}{2}$, $17\frac{1}{2}$ and $18\frac{1}{2}$ days. These deficiencies were not statistically significant, but this may be because the class numbers were insufficiently large, since if the data for the $17\frac{1}{2}$ and $18\frac{1}{2}$ day embryos are combined a significant deficiency of oedematous embryos is revealed.

**Prenatal Deaths.**

The proportions of dead and viable embryos found at different ages in the control, backcross and intercross matings is shown in Table 16.

It is clear that the proportion of embryos which died in intercross matings was about twice the proportion which had died in backcross and control matings. This difference was highly significant ($P < 0.001$). There was no difference in the proportion of dead embryos found in backcross and control matings. The only class of embryo occurring in intercross matings but not in the backcross or control matings is the ragged homozygote. It must be concluded that an abnormally high proportion of RaRa embryos suffer prenatal death, but that a Ra+ embryo is no more likely to die before birth than a ++ embryo. It is probable that the class of embryos which shows high grade oedema is selectively liable to suffer prenatal death, since many embryos of this kind which were examined appeared to be moribund.

The fact that less than the expected number of RaRa mice is found at birth from intercross matings could now be explained on the basis of preferential prenatal death of RaRa embryos.
Table 16

Deto. = estimated age of death of embryo - so D8 = died at 8 days gestation.

**EMBRYONIC DEATHS**

**Control matings (++ x ++)**

<table>
<thead>
<tr>
<th>Days</th>
<th>12½</th>
<th>13½</th>
<th>14½</th>
<th>15½</th>
<th>16½</th>
<th>17½</th>
<th>18½</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable</td>
<td>-</td>
<td>-</td>
<td>14</td>
<td>19</td>
<td>12</td>
<td>14</td>
<td>34</td>
</tr>
<tr>
<td>Moles</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>
| Total 93 viable; 10 dead \( (1/10 \text{ embryos dead}) \)

**Backcross matings (Ra+ x ++)**

<table>
<thead>
<tr>
<th>Days</th>
<th>12½</th>
<th>13½</th>
<th>14½</th>
<th>15½</th>
<th>16½</th>
<th>17½</th>
<th>18½</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable</td>
<td>19</td>
<td>26</td>
<td>44</td>
<td>42</td>
<td>19</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moles</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D12</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D15</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
| Total 150 viable; 17 dead \( (1/10 \text{ embryos dead}) \)

**Intercross matings (Ra+ x Ra-)**

<table>
<thead>
<tr>
<th>Days</th>
<th>12½</th>
<th>13½</th>
<th>14½</th>
<th>15½</th>
<th>16½</th>
<th>17½</th>
<th>18½</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable</td>
<td>12</td>
<td>40</td>
<td>23</td>
<td>33</td>
<td>53</td>
<td>46</td>
<td>42</td>
</tr>
<tr>
<td>Moles</td>
<td>6</td>
<td>5</td>
<td>9</td>
<td>7</td>
<td>10</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>D8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D11</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D12</td>
<td>-</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D13</td>
<td>-</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>D14</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>
| Total 249 viable; 64 dead \( (1/5 \text{ embryos dead}) \)

Total number of embryos dissected out: 492 viable 91 dead.
**Fig. 43.** Diagram representing the frequency of oedematous embryos of ++, Ra+ and RaRa genotypes.
(d) **Discussion.**

**The occurrence of oedema.**

The data describing the occurrence of oedema can be summarised as follows:

(i) No oedematosus embryos occur in ++ x ++ matings within the stock in which the ragged gene is segregating.

(ii) A few slightly oedematosus embryos, near the threshold of recognition, occur at certain ages in Ra+ x ++ matings.

(iii) All grades of oedema occur in Ra+ x Ra+ matings; the proportion of oedematosus embryos is similar to the expected number of RaRa embryos.

It is concluded from this evidence that:

(a) All embryos showing high grade oedema are ragged homozygotes.

(b) All slightly oedematosus embryos are either ragged homozygotes or ragged heterozygotes, but most of them are homozygotes.

(c) Embryos showing no oedema could be of ++, Ra+, or RaRa genotype: but it is unlikely that many are RaRa. Fig. 43 is a hypothetical representation of this scheme, based on the supposition that oedema is a continuous variable.

**Identification of genotypes.**

The main criteria used to classify embryos as Ra+ or RaRa were: delay in sinus hair growth in Ra+, and oedema and retarded pelage and sinus follicle development in RaRa. It is considered that these criteria were adequate for two reasons: (a) The results of classification on these lines were in close agreement with the theoretically expected numbers of Ra+ and RaRa embryos from backcross and intercross matings. (The slight deficiencies of RaRa embryos at some ages could be explained by the selective prenatal
deaths of these embryos which was shown to occur - Table 16).

(b) The abnormalities used to classify Ra+ and RaRa embryos were similar to those found in neonatal and older Ra+ and RaRa mice. Such mice in many cases matured and their genotypes were established genetically.

It will be seen from this discussion and from the data presented in the next section that the identification of the higher grade oedematous embryos as ragged homozygotes, rests on the following argument:

(i) Such oedematous embryos occur only in intercross matings and they occur in the expected Mendelian proportions.

(ii) They correspond in appearance per continuatum with the oedematous newborn mice from intercross matings which have similar sinus hair defects but which die at birth. It has been shown elsewhere that these newborn mice were probably ragged homozygotes.

(iii) Lower grade oedematous newborn mice with sinus hair defects similar to those in the oedematous embryos were produced in a stock selected for high viability of ragged homozygotes. They lived to be proven RaRa by breeding tests.

The cause of oedema in RaRa embryos.

The observational data do not throw much light upon this question. The frequent association of high grade oedema and skin haematomata, which are presumably caused by the breakage of superficial capillary blood vessels, may be important. This suggests as one possibility that oedema might be the result of abnormally high hydrostatic pressure in the capillaries which could result in the breakage of some capillaries. The fact that the oedema is general rather than spatially localised in the embryos is in favour of this
The effects of oedema in ReRa embryos.

Oedema may be the cause of the retarded and uneven growth of the sinus follicles, although this is certainly not proved. It is probably significant that the most severe cases of follicle abnormality were found in embryos showing the highest grades of oedema.

In 14½- and 15½-day embryos showing high grade oedema the post oral sinus follicles were sometimes split into three parts. This may be due to an exaggerated stretching or tension in the skin caused by the oedema. A similar effect has been reported by Grünberg (1943a). The fact that oedema is most conspicuous in the neck and throat regions may explain why the post oral sinus follicles seem most sensitive to interference. No embryos were seen at later ages with this kind of sinus follicle abnormality. This may be because only embryos with high grade oedema show sinus follicle abnormalities at 14½-15½ days and such embryos may be very liable to die in the 15½-17½ day period.

Oedema may be the cause of the retarded growth of pelage follicles with which it seems to be associated. For example, it is possible that the abnormal presence of extra-cellular sub-epidermal fluid may interfere with cell nutrition and therefore with mitosis, thus delaying follicle growth. It may be significant that oedema first becomes visible in the embryo at 13½ days, which is near the time of visible initiation of the first pelage follicles. Although oedematous embryos show a retardation of the development of all follicles, there is no evidence suggesting a delay in the commencement of follicle initiation.
Oedema and Pre-natal death.

From intercross matings, embryos with both high and low grade oedema were found. There were deficiencies from the expected \( \frac{1}{2} \) ratio of oedematous embryos at 13\( \frac{1}{2} \), 17\( \frac{1}{2} \) and 18\( \frac{1}{2} \) days' gestation. It is possible that the deficiency of oedematous embryos at 13\( \frac{1}{2} \) days may be due to incomplete penetrance at that age, so that not all ReRea embryos would show oedema at such an early stage. The most likely explanation for the deficiency of oedematous embryos at 17\( \frac{1}{2} \) and 18\( \frac{1}{2} \) days seems to be that there was preferential perinatal death of oedematous embryos, occurring especially between 16\( \frac{1}{2} \) and 17\( \frac{1}{2} \) days' gestation. This theory is supported first, by the data on embryonic deaths (Table 16) which demonstrate an abnormally high rate of pre-natal death in ragged intercross matings at this period of gestation, and secondly, by the fact that it is the higher grades of oedema which are less common in 17\( \frac{1}{2} \)- and 18\( \frac{1}{2} \)-day embryos than in younger embryos; pre-natal death would be expected to remove selectively the embryos showing very high grades of oedema, whereas embryos showing lower grades of oedema might live until birth. A second possibility is that superficial oedema may be more difficult to recognise in older post 17\( \frac{1}{2} \)-day embryos whose skin possesses a rigid and less easily distorted structure than that of younger embryos. This would imply a partial transience of oedema manifestation since penetrance is apparently complete at 14\( \frac{1}{2} \)-16\( \frac{1}{2} \) days' gestation.

(a) Introduction.

The external morphology of the sinus hairs and follicles and the pelage follicles has been studied in embryos from ragged backcross and intercross matings. It was desirable to continue this type of examination in neonatal animals so that the presumptive classes of ++, Ra+ and RaRa could be definitely distinguished. Also, it was necessary to be able to identify Ra+ and RaRa mice at birth with accuracy in order to obtain skin for histological examination from ++, Ra+ and RaRa sibs.

Most of the sinus hairs have generally emerged and are visible in normal newborn mice. There are numerous moustache hairs or whiskers; and of the other sinus hairs there are on each side of the face: an anterior and posterior supra-orbital sinus hair, a post-orbital sinus hair, and two post-oral sinus hairs.

The pelage hairs do not usually appear until one day after birth.

(b) Methods.

Neonatal ++, Ra+ and RaRa litter mates were examined under a low power binocular microscope. Particular attention was paid to the morphology and time of appearance of the sinus and pelage hairs.

Generally, ++ and Ra+ sibs, and Ra+ and RaRa sibs were available for comparison. 10 litters were examined from Ra+ x ++, and RaRa x Ra+ matings, solely in order to study the ++, Ra+, RaRa phenotypes. The results obtained were confirmed by the examination of many more litters during the course of subsequent work.
The descriptions of Ra+ and RaRa mice given in Table 17 generally include only their differences from the normal. Of the pelage hairs, only those on the dorsum were considered. These hairs were viewed from the side, with the mice silhouetted at eye level against a suitable background.

Observations on RaRa mice made later than the day of birth were of necessity confined to the viable type of ragged homozygotes produced from RaRa x Ra+ matings. Observations at birth, however, included the still-born homozygotes.

(c) Results.

New-born RaRa Mice.

New-born ragged homozygote mice can be divided into two classes: (1) Those which die at, or soon after birth. This type is produced in the unselected heterogeneous stock in which the ragged gene first arose; these inviable new-born mice are therefore equivalent to the presumptive RaRa oedematous embryos described in the previous section. (2) Those which are viable at birth and live through part of the suckling period, or else to maturity. These mice are produced in the stock selected for high viability of ragged homozygotes; they show a lower grade expression of the gene than do the inviable type, but they are the only kind of ragged homozygotes whose morphology can be described after birth.

General appearance: The inviable type of homozygotes show a generalised oedema. In some cases, just before death, they may emit a mucus-like fluid from the mouth or nostrils.

The viable homozygotes often show a lower grade of oedema; they do not emit fluid from the mouth or nostrils.
<table>
<thead>
<tr>
<th>Table 17. COMPARATIVE EXTERNAL MORPHOLOGY OF JUVENILE MICE.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whiskers</strong></td>
</tr>
<tr>
<td>Fairly straight</td>
</tr>
<tr>
<td>Other sinus hairs:</td>
</tr>
<tr>
<td>Supra orbitals</td>
</tr>
<tr>
<td>Post orbitals</td>
</tr>
<tr>
<td>Post orals</td>
</tr>
<tr>
<td>Pelage hairs</td>
</tr>
<tr>
<td>Whiskers</td>
</tr>
<tr>
<td>Other sinus hairs:</td>
</tr>
<tr>
<td>Supra orbitals</td>
</tr>
<tr>
<td>Post orbitals</td>
</tr>
<tr>
<td>Post orals</td>
</tr>
<tr>
<td>Pelage hairs</td>
</tr>
<tr>
<td>Whiskers</td>
</tr>
<tr>
<td>Other sinus hairs:</td>
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<td>Supra orbitals</td>
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<td>Pelage hairs</td>
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<td>Dorsum darkly coloured in mice with pigmented hair</td>
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<td>Whiskers</td>
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<td>Other sinus hairs:</td>
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<td>Pelage hairs</td>
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<td>Shorter hairs visible growing between the longer ones. Dorsum darkly coloured.</td>
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In those viable mice showing a considerable degree of oedema there sometimes appears to be an abnormal displacement of the stomach or intestines. Such mice invariably die during suckling.

Whiskers: In the inviable mice there are from 0-3 very short whiskers per side. In the viable type there are from 1-6 (average 4) whiskers per side. These whiskers are longer than those in the inviable homozygotes but shorter than normal. Those whiskers present are always growing from the most posterior column of moustachefollicles, which are the most advanced in embryological development. No other sinus hairs are found. No pelage hairs are present.

The rest of the descriptive data are given in Table 17.

(d) Discussion.

Identification of ++, Ra+ and RaRa phenotypes.

In the preceding sections both embryos and neonatal mice were classified as ++, Ra+ or RaRa. The evidence that these classifications were correct is of two kinds, viz: (1) Continuity of phenotype; (2) Agreement with Mendelian segregation ratios.

It has been seen that some of the criteria used for classifying neonatal mice, namely development of the whiskers and other sinus hairs, were the same as those used for classifying embryos. The phenotypic abnormalities considered to be typical of the ++, Ra+ and RaRa genotypes in embryos and suckling mice can often be followed continuously until the mice are mature. In adult mice the phenotypic classifications have been proved correct by genetic testing.
In the case of Ra+ x ++ matings the numbers of ++ and Ra+ embryos and neonatal mice were as expected on the basis of Mendelian segregation ratios. In the case of Ra+ x Ra+ matings, there was a deficiency of RaRa embryos and of RaRa still-born mice. It is not considered, however, that this shows the criteria for classification to be incorrect for the following reasons:

(a) An abnormally high rate of prenatal deaths was shown to occur in Ra+ x Ra+ matings and the evidence indicated that most of these deaths occurred among the oedematous embryos which were putatively RaRa.

(b) In the unselected stock, all RaRa mice died at birth; some of these may have been eaten by their mothers before classification and so a deficiency from the expected number of RaRa mice would arise.

(c) In the selected stock, the viable type of putative RaRa mice showed little or no deficiency from the expected numbers at birth. These mice, although showing a lower grade expression of the Ra gene than the inviable homozygotes, had similarly abnormal sinus hair development. Many of these mice matured and were proved to be ragged homozygotes by genetic testing.

(2) Juvenile Follicle and Skin Morphology.

General Introduction.

A detailed study of the development of the first pelage from its morphological beginning in the 14-day embryo until completion at the age of three weeks, was made in normal, heterozygous ragged and homozygous ragged mice. It was realised that the absence of many hair fibres, which is the most important characteristic of
of adult ragged mice, could result from any one of a number of abnormalities in young ragged mice or embryos, viz:

(i)a. No initiation or (i)b. delayed initiation of some hair follicles.

(ii)a. No growth or (ii)b. abnormally slow growth of some hair follicles.

(iii) Abnormally early cessation of follicle initiation.

(iv) Abnormally early cessation of follicle growth.

(v) Abnormal morphology of hair follicles or skin.

(vi) Degeneration of some hair follicles.

Any one of these causes or any combination of them could give rise to the absence of some hair follicles in the adult or to the failure of some follicles to produce hairs. The study of adult material has shown that in the ragged heterozygote the follicle density is at least as high as normal, but there is a large number of incomplete or abnormal follicles present which carry no hairs. Causes (i)b, (ii)b, (iii), (iv) or (v) could give such a result.

In the adult ragged homozygote there is less than the normal number of follicles and some of the follicles present are morphologically abnormal. This could be the result of any of the above causes. A study of the development of the skin and the first coat was expected to classify these problems and to throw some light on the normal growth of the skin and hair in the mouse.

The development of hair follicles and skin in the rat has been studied by Fraser (1928), Daneel (1931) and Butcher (1934); and in the mouse by Oyama (1903) and Gibbs (1941).
Fig. 44. (After Hardy 1951). Diagrammatic representation of normal hair follicle development. Stages 1a to 2 are classified as primordia, stages 3 and 4 as small follicles, stages 5 and 6a, as medium follicles, stage 6b as medium-large follicles and stage 6c as large follicles.
(a) Introduction.

The pre-natal development of the mouse epidermis is similar to that described in the rat by Fraser (1928). Before the 13½-day embryo stage, a single layer of extremely flattened, vertically compressed cells differentiates superficially to form the periderm. The periderm is regionally equivalent to the cornified layers of the fully differentiated epidermis. Beneath the periderm, several rows of cells have differentiated by 13½ days. These cells have oval, basophilic nuclei; they are equivalent to the stratum germinativum of the epidermis from which develop the hair follicle primordia. By 17½ days' gestation the epidermis has further differentiated and can be divided into the stratum corneum, stratum granulosum, stratum intermedium and Malpighian layers.

The morphological development of hair follicles can be briefly summarised as follows:

Stage 1 is a thickening of the basal layer of the epidermis due to a convex aggregation of Malpighian cells (Fig. 44).

Stage 2 is the down-growth of this structure by cell division.

Stage 3 is the widening of the proximal region of this down-growing structure to enclose a number of dermal cells which have collected as a mesenchymal condensation, thus forming the follicle bulb.

Stage 4 is further down-growth of the follicle and the formation in the bulb of a matrix or region of proliferation.

Stage 5 is further growth and differentiation, with the formation of a keratinised cone or inner sheath within the follicle.

Stage 6 is proliferation from the matrix and within the inner sheath, to form the hair shaft which pierces the inner sheath, and eventually emerges. During the following descriptions of
follicle morphology, the follicles are classified as large, medium, small or primordial, according to their stage of growth (Fig. 44).

Soon after birth, there are four histological layers recognizable in the skin. These are, as in adult mice, the epidermis, dermis, adiposus and panniculus carnosus (muscle).

In late embryos and in young suckling mice, the epidermis can, unlike the adult epidermis, be divided into four distinct cytological layers (Gibbs 1941): the stratum corneum, which is a layer of flattened keratinised cells; the stratum granulosum, which comprises several rows of long vertically compressed cells containing keratohyalin granules; the stratum intermedium, comprising several rows of large irregular cells with oval nuclei; and the stratum cylindricum or basal Malpighian layer, consisting of a varying number of rows of flattened cells with large, oval, dark-staining nuclei. The stratum intermedium and the basal Malpighian layer together make up the germinative layer of the epidermis. The stratum intermedium and stratum granulosum layers are absent in the mature epidermis.

The phasic development of the hair follicles after their initiation and early down-growth is the same as that which occurs during adult hair cycles, i.e. Anagen, the stage of down-growth and hair proliferation; Catagen, the stage of retraction or shortening of follicles; and Telogen, the resting stage.

Observations on the cytological changes and the changes in thickness of the skin layers during development, and the relationship of these changes to synchronous alteration in total skin thickness are described in ++, Ra+ and RaRa mice.
Methods.

For pre-natal histological study, embryos of 13½-18½ days' gestation were dissected out of the uterus as described earlier. Complete embryos (sibs) aged from 13½-16½ days were fixed in Bouin's fluid, measured with a travelling microscope and drawn in outline with the help of a camera lucida. The embryos were then dehydrated, cleared in methyl benzoate, embedded and orientated in paraffin wax (m.p. 54°C) and sectioned transversely at 10 μ. 10 embryos were sectioned serially from head to tail but in most cases only sample lengths of ribbon were selected and mounted serially. The sections were stained in Delafield's haematoxylin and eosin. For this series, embryos which had been classified as oedematous were compared with non-oedematous litter mates at ages - 13½, 14½, 15½ and 16½ days. The oedematous embryos were presumptive ragged homozygotes and the non-oedematous embryos were either ragged heterozygotes or normal. The nature of the oedema and its apparent effects were observed.

For the older embryos the 3 genotypes - ++, Ra+ and RaRa - could all be identified. Skin was taken from the neck and rump regions of litter mates of these three genotypes at 17½ and 18½ days. The skin samples were removed from already fixed embryos, dehydrated, cleared in methyl benzoate, trimmed rectangular so that sagittal sections could be cut, and impregnated in paraffin wax (m.p. 54°C) for about 7 hours. Serial sections were cut at 7 μ and stained with Delafield's haematoxylin and eosin. The development of the skin and hair follicles and the histology and cytology of the skin layers was studied microscopically. Measurements of the thickness of the skin and its definitive layers were made by using a micrometer eyepiece at a standard magnification (204 x). Measurements of the
epidermis were made non-inclusive of the stratum corneum since comparisons were to be made with suckling mice and in some cases their stratum corneum layer was partially removed during shaving.

For the post-natal histological study, serial sections were made of skin taken from the neck and rump of ++, Ra+ and RaRa mice. ++/Ra+ comparisons were all made between litter mates; RaRa mice were in some cases compared with Ra+ sibs, but normal sibs were not available, since viable RaRa mice were not obtainable from intercross matings and had to be produced from backcross matings of the type: Ra+ x RaRa. For comparisons at birth, however, RaRa still-born mice from intercross matings could be used; for comparisons at later ages RaRa mice were taken from the backcross matings in a stock which had been selected for increased viability of ragged homozygotes.

The skin was shaved if necessary, removed without scraping, fixed in Bouin's fluid, dehydrated, cleared in methyl benzoate, trimmed rectangular and impregnated in wax for 8 hours. Careful trimming and orientation of the material enabled sagittal sections to be cut parallel to the plane of slope of the hair follicles. Serial sections were cut at 7μ and stained with Delafield's haematoxylin and eosin. Skin was taken from normal mice at ages: ½, 1½, 3½, 4½, 5½, 7½, 9½, 12½, 15½, 18½, 21½ days after birth; from Ra+ mice at the same ages; and from RaRa mice at ages: ½, 1½, 7½, 12½, 18½ days after birth. Either one or two mice of each genotype were taken at each age. Microscopic examination of the material was carried out as described for the sagittal sections of pre-natal skin.
(c) Results.

(i) From Transverse Sections of Whole Embryos (13½-16½ days).

The Nature of the Oedema:

As described above, oedema was first visible in whole embryos from intercross matings at 13½ days' gestation. The classifications for intensity of oedema which were made from the transverse sections were mainly in agreement with those made on the same embryos under the low power microscope immediately after dissection. Oedema is characterised by the presence of large extra-cellular spaces, and undifferentiated mesenchyme tissue forming a broad translucent region of tissue beneath the epidermis (Fig. 13). The oedema is usually superficial and it is fairly generalised but more extreme anterodorsally and dorso-laterally than ventrally. Oedema can generally be seen in transverse sections from a region anterior to the eye to as far posterior as the tail region, but it is most prominent in the area of the neck and anterior dorsum.

The extra-cellular spaces may in some cases be formed by gross dilation of peripheral blood vessels; the resulting vacuities sometimes contain red blood corpuscles.

The Effects of the Oedema.

A number of abnormalities were observed in 13½-16½ day embryos which are almost certainly peculiar to the Re/Ra genotype and which may be the direct result of the oedema which characterises these embryos.

(a) There is abnormal development of the sinus follicles in oedematous embryos. The development of the whisker follicles is uneven so that neighbouring follicles show greater differences in their stages.
Transverse sections of whole embryos at 14¼ days' age (region of the liver). (x 18).

**Fig. 45.** Non oedematous embryo.

**Fig. 46.** Oedematous embryo (putative RaRa), showing sub-epidermal oedema.

Note: (i) Thinner developing epidermis, and (ii) Smaller follicle primordia (arrowed) in the oedematous embryo.
Transverse sections of whole embryos at 15½ days' age (region of the lungs and heart). (x 18).

Fig. 47. Non-oedematous embryo.

Fig. 48. Highly oedematous embryo (putative RaRa), showing a wide region of sub-epidermal oedema and a dilated superficial blood vessel (B) with apparent haemo-concentration.

Note: The thinner epidermis of the oedematous embryo, especially in dorsal areas.
Transverse sections of whole embryos at 16½ days' age (tongue region). (x 13).

Fig. 49. Non-oedematous embryo, showing large superficial vessel or sinus (V).

Fig. 50. Oedematous embryo (putative RaKa), showing an abnormally large vessel or sinus (V) and a superficial blood vessel with apparent haemoconcentration, (B).
Transverse sections of the epidermis of 15½ day embryos (x 260).

Fig. 51. Non-oedematous embryo, showing a follicle primordium (F).

Fig. 52. Highly oedematous embryo, showing a follicle primordium (F) and a dilated superficial blood vessel with haemo-concentration (V).

Note: (i) The retarded development of the follicle primordium, and (ii) The thin, little differentiated epidermis, in the oedematous embryo.
of growth and differentiation than is normal. In oedematous embryos the whisker follicles are also retarded in their development compared with the normal. In the 15½-day embryo the whisker follicles normally show hair bulb, papilla and often inner sheath development whereas these follicles in oedematous embryos have no differentiation of the inner sheath and less clear bulb and papilla differentiation. There may be fewer developing whisker follicles in oedematous embryos.

(b) There is slower differentiation and thickening of the epidermis in oedematous embryos. This may be partially due to a stretching action of the oedema upon the periderm. At 13½ days the abnormalities of the epidermis are small, and the periderm is normal. The Malpighian layer of the epidermis is, however, thinner in oedematous embryos, especially laterally. In the lateral neck and rump regions of normal embryos there are 2-5 rows of Malpighian cells in the epidermis, compared with 2-3 rows in oedematous embryos. At 14½ days the difference is most marked in the neck region. In the oedematous embryos there is no complete layer of Malpighian cells dorsally, compared with 1-2 rows in the normal; and there are 1-2 rows laterally, compared with 3-4 rows in the normal. The periderm is normal. At 15½ days the abnormalities are greater in oedematous embryos. In the neck region the epidermis is thinner both dorsally and laterally. Dorsally there may be 0-3 rows of Malpighian cells compared with 4 rows in the normal; laterally there are 1-3 rows of Malpighian cells compared with 4-5 rows in the normal. In the rump region the differences are similar but less extreme.

In severely oedematous embryos of 15½ days, epidermal blebs may be seen. These cause a breakdown of epidermal structure in the
germinative region and produce vacuities between the peridam and the Malpighian cell layer.

(c) There is retarded growth and differentiation of hair follicles in oedematous embryos. The follicles first appear at the normal time in 13½-14 day embryos; but the aggregates of Malpighian cells which form the epidermal thickenings seem smaller, flatter and more diffuse in oedematous than in other embryos. At 15½-16½ days the normal follicles are clearly demarcated, round and in transverse section appear to be cut off or separated from the epidermis due to their longitudinal slope (Fig. 51). In oedematous embryos the follicles are smaller, flatter and usually do not seem separated from the epidermis (Fig. 52). The normal follicles often show further differentiation of the bulb, papilla and region of the inner sheath than do follicles from the oedematous embryos. Very highly oedematous embryos may, unlike the normal, have better development of follicles on the posterior part of the embryos than anteriorly where the oedema is more severe. It appears therefore that follicle growth is retarded in oedematous embryos but there is no evidence for any mechanical prevention of follicle initiation or follicle induction due to the presence of sub-epidermal oedema, since follicle development frequently occurs over oedematous tissue.

(d) In the 15½-day embryo, oedema may be the cause of an abnormal delay in the condensation of mesenchyme which gives rise to superficial muscle layers.

(e) In highly oedematous embryos of 15½-16½ days many of the blood vessels appear to be abnormally dilated, especially in peripheral regions (Fig. 48); but they are nevertheless packed with red
blood corpuscles. This seems to have resulted in the breakage of superficial blood vessels with local haemorrhage in some cases. The fact that these abnormalities are only seen in highly oedematous embryos suggests that there may be a causal relationship between the occurrence of oedema and abnormally distended blood vessels.

(f) No consistent abnormalities of the viscera were observed in oedematous embryos. The presence of excessive extra-cellular fluid in the pleural and cardiac cavities was, however, suspected in some $15\frac{1}{2}$- and $16\frac{1}{2}$-day oedematous embryos.

(ii) From Sagittal Sections of Skin.

Throughout the histological study of ++, Ra+ and RaRa skin, the degree of wrinkling or folding of the skin and its total thickness (measured inclusively from the epidermis to the panniculus muscle layer) were carefully observed. For the sake of brevity these observations will not be included in the day to day descriptions of skin morphology. Although there was individual variation from day to day in the degree of folding of the skin, no important differences between the three genotypes (++, Ra+ and RaRa) were noted. The results of the skin thickness measurements are graphically presented elsewhere. It seems that skin thickness increases continuously from the beginning of follicle development until the period of maximum follicle growth after birth; thereafter it steadily declines. The increase in skin thickness during follicle growth is considerably less than normal in Ra+ and RaRa mice.

At 17½ days' gestation:

++ neck: There is no differentiation of the dermis and adipose layers and the panniculus muscle layer is not always clear.
Sagittal skin sections from 17½ day embryos - neck region (x 100).

**Fig. 53.** ++; showing several follicle primordia and one larger follicle.

**Fig. 54.** Ra++; showing several primordia - one fairly large.

**Fig. 55.** RaRa; showing high-grade sub-epidermal oedema, a dilated superficial blood vessel (v) and two follicle primordia (f).

**Note:** (i) Incomplete differentiation of the dermis and adipose layers in all three skin types, but especially in RaRa where there is also no differentiation of the panniculus muscle layer.

(ii) Apparently retarded follicle development in Ra+ and especially in RaRa skin.
**Epidermis:** There are 1-2 rows of basal Malpighian cells with 1-2 rows of stratum intermedium cells. The stratum granulosum is wide and the keratohyalin granules clear and prominent; there are locally hyperplastic regions of this layer.

**Follicles:** A few medium-small sized hair follicles having bulb, papilla and slight inner sheath development are seen. There are small follicles with bulb differentiation only, and primordia of varying sizes. The superficial blood vessels are fairly small and thick walled (maximum diameter 50 μ). They are not packed with erythrocytes.

**Rat neck:** There is no differentiation of the dermis and adiposus layers. The epidermis is normal.

**Follicles:** The same types of hair follicles are present as in normal skin, but the small follicle primordia make up a greater proportion of the total number of follicles. There are fewer small bulbed follicles than normal. These effects are due to a retardation of the growth of these follicles initiated at about 16 days' gestation. The superficial blood vessels are normal.

**Rabbits neck:** There is no differentiation of the dermis and adipose layers, or of the panniculus carnosus muscle layer. No estimates of total skin thickness could therefore be made. High-grade oedema may be present immediately beneath the region of presumptive dermis; this is probably the cause of the partial failure of skin differentiation (Fig. 55).

**Epidermis:** There is usually only 1 row of basal Malpighian cells. There are 2-4 rows of stratum intermedium cells. The stratum granulosum is wide and the keratohyalin granules are visible but not clearly so. There are local regions of extreme hyperplasia of
the stratum intermedium and the stratum granulosum.

**Follicles:** Only primordia are found and they are mostly small. The superficial blood vessels are larger than normal. Vessels of 90-130 μ diameter are frequently observed, and there are a few of maximum diameter 300 μ. These vessels are frequently packed with erythrocytes. Some larger vessels are found less superficially, in the oedematous regions; these do not contain erythrocytes and may be lymphatic vessels.

++**Rump.** There is no differentiation of the dermis and adipose layers. The epidermis is the same as in the neck region, but there are no regions of hyperplasia.

**Follicles:** The hair follicle development is the same as that in the neck region except that the medium-small sized follicles show no inner sheath differentiation. The blood vessels are fairly numerous and very thick walled. The superficial vessels are about 45 μ in diameter; the less superficial vessels may be 140 μ in diameter. Most of the vessels contain a low density of erythrocytes.

**Rear Rump:** There is no differentiation of the dermis and adipose layers. The epidermis is normal.

**Follicles:** The hair follicles are normal except that there are fewer showing bulb and papilla development. The blood vessels are normal.

**Rear Rump:** There is no differentiation of the dermis and adipose layers. The epidermis is normal.

**Follicles:** Only hair follicle primordia are present and they are mostly small. The blood vessels are larger than normal. Vessels of up to 270 μ maximum diameter are found superficially. They are often packed with erythrocytes. The walls of many of these vessels
appear to be thinner than normal. However, the gross abnormalities of the skin are clearly less in the rump than in the neck regions of the ragged homozygotes.

At 13½ days' gestation:

++ neck: Differentiation of the dermis and adipose layers is not very clear.

**Epidermis:** There are 2-3 rows of basal Malpighian cells; a few of their nuclei are irregularly shaped but they are mostly oval, and not flattened or pyknotic. There is a single row of stratum intermedium cells. The stratum granulosum is wide and the keratohyalin granules fairly clear.

**Follicles:** There are some medium-small sized follicles which apparently stretch the full width of the presumptive dermis. These follicles have bulbs and papillae and may have some inner sheath differentiation. The remaining follicles are primordia of varying sizes. The blood vessels are generally small and inconspicuous but have fairly thick walls.

**Ra+ neck:** The differentiation of the skin is normal. The epidermis is normal.

**Follicles:** The same types of hair follicles are present as in the normal skin. There are fewer medium-small follicles and these rarely show any development of the inner sheath. Of the primordia, the smaller types are abnormally numerous; they here comprise the majority of all follicle types. This again suggests that there is a retardation of the growth of hair follicles in Ra+ mice. The blood vessels are normal.

**RaRa neck:** The differentiation of the dermis and adipose layers is probably even less distinct than normal. In the material examined
oedema was much less marked than at 17.5 days. There was some differentation of the panniculus muscle layer. These differences may be due to the very variable expressivity of oedema abnormalities in ragged homozygote embryos.

**Endermis:** There are 1-2 rows of basal Malpighian cells and 2-3 rows of stratum intermedium cells. The epidermis is otherwise normal.

**Follicles:** Only hair follicle primordia of varying sizes are found. Some of the primordia are merely indistinct thickenings of the epidermis. Large superficial blood vessels are more numerous than normal, and a few abnormally large blood vessels of maximum diameter 380 µ are found. Some of the vessels may have thinner walls than normal.

**Rt. rump:** Separate dermis and adipose layers are not differentiated.

**Endermis:** The epidermis is similar to that in the neck region. There are 2-3 rows of basal Malpighian cells and 1-2 rows of stratum intermedium cells.

**Follicles:** There are fewer medium-small sized follicles than in the neck region and these follicles do not show inner sheath differentiation. The primordia are of varying sizes.

**Rt. rump:** The epidermis is normal.

**Follicles:** The development of the hair follicles is probably not abnormal but the smaller types of primordia may comprise a higher than normal proportion of the follicles. The blood vessels are normal.

**Rtl. rump:** Separate dermis and adipose layers are not differentiated.
Epidermis: The epidermis is hardly abnormal except for regions where there is only a single row of basal Malpighian cells. Only follicle primordia are found; most of them are small. The blood vessels are larger and perhaps more numerous than normal. They appear to be rather thin-walled in some cases, but this may only be a result of the dilation of the vessels.

At 3 day after birth.

Follicles: The hair follicles are out of phase with each other, i.e. neighbouring follicles are frequently at very different stages of development. Three types of follicles are seen, of relative lengths: medium, small and primordial. The medium follicle stretches into the adipose layer, has clear differentiation of the bulb and papilla and a developing inner sheath, but no hair shaft. The small type of follicle stretches to the base of the dermis or
Sagittal skin sections at 1/4 a day after birth - neck region (X 100).

Fig. 56. ++; showing one fairly large follicle with advanced inner sheath development and a number of small follicles and primordia.

Fig. 57. Rat; showing one fairly large follicle and several primordia.

Fig. 58. RaRa; showing follicle primordia only, and an abnormally large superficial blood vessel with apparent haemoconcentration (V).

Note: (i) Incomplete differentiation of the dermis and adipose layers in all types of skin.
(ii) That follicle development is less far advanced than normal in Rat and especially in RaRa skin.
slightly below, and has bulb and papilla differentiation only. The primordia are of varying sizes. A few are merely small thickenings of the basal layers of the epidermis. These thickenings are in the form of radially shaped aggregations of Malpighian cells which are convex towards the dermis. Many blood vessels are seen in the adipose layer. A few large vessels of maximum diameter about 300 \( \mu \) are present. Others of about 40 \( \mu \) diameter are more frequent. The walls of the vessels are fairly thick. The vessels usually contain erythrocytes but not so many as to block the vessel lumen entirely.

**Boca neck:** The dermis and adipose layers are, as in normal skin, not clearly differentiated.

**Epidermis:** The epidermis is normal except that the basal Malpighian cells are slightly less closely packed together.

**Follicles:** The hair follicles are out of phase in their development and can be divided into three types as in normal skin, viz: medium, small, primordial. The medium follicles stretch into the adipose layer, and probably because of the thinness of the skin, they may curve excessively or be in contact with the panniculus carnosus muscle layer at their proximal ends. These follicles have normal bulb, papilla and inner sheath differentiation; they are found much less frequently than in normal skin. The "small" follicles are normal. The primordia are of varying sizes; those in the form of epidermal thickenings, showing little downward proliferation, are very much more frequent than in normal skin. The blood vessels are normal.

**Boca neck:** Separate dermis and adipose layers are not distinguishable.

**Epidermis:** The epidermis is thinner than normal. There are
the normal 1-2 rows of basal Malpighian cells in many regions but these cells are abnormally sparse in other areas. Their nuclei are rounder and less darkly stained than normal. There are only 1-2 rows of stratum intermedium cells as compared with 2-3 rows normally.

**Follicles:** Only primordia of the small "epidermal thickening" type are present. The arrangement of Malpighian cells within the primordia is less orderly than in normal or Ra+ skin and a less clearly radial shape results. Large superficial blood vessels in the dermis-adipose region are about ten times more numerous than in normal skin. Vessels of maximum diameter 300μ are rare, but those of 50-150μ diameter are common. In nearly every case the vessel lumen is blocked with erythrocytes. In a few cases the vessel walls seem to have ruptured, causing superficial haemorrhage. The presence of vacuities or regions showing break-down of the histological structure of the dermis-adipose suggests the occurrence of oedema.

**++ rump:** The dermis and adipose layers are still not clearly differentiated.

**Epidermis:** The only difference from the epidermis of the neck region is that the basal Malpighian cells are more numerous and closely packed together and more often columnar in their orientation. A greater proliferative activity of the germinative layer is indicated.

**Follicles:** The same three types are present. The medium follicles are much more rarely seen than in the neck region; the small follicles and small primordia are more frequent. The blood vessels are numerous, but small, thick-walled and not packed with erythrocytes.

**Ra+ rump:** The dermis and adiposeus layers are not clearly differentiated. The epidermis is normal.
Follicles: The medium follicles are more rare than normal, and a greater proportion of the primordia are small epidermal thickenings. The blood vessels are normal. 

Rara rump: There is no differentiation of the dermis and adipose layers. 

Epidermis: In most regions there is a single row of sparsely occurring basal Malpighian cells instead of the 1-2 rows of closely packed cells found normally. There are generally 2 rows of sparse stratum intermedium cells. The epidermis is therefore thinner than normal, and the proliferative activity of the germinative layer is small. 

Follicles: Most of the follicles are primordia of varying sizes, but there are a few larger follicles with bulb, papilla and some inner sheath development. The fact that follicle development is further advanced in the rump than the neck region of ragged homozygotes itself constitutes an abnormality. The superficial blood vessels are not abnormal in size, but appear to be rather thin-walled and are often packed with erythrocytes. There is some evidence of oedema which is similar to but less marked than that in the neck region. 

At 1½ days after birth. 

++ neck: 

Epidermis: There are 1-2 rows of basal Malpighian cells which are not closely packed together and not columnar in orientation. There are 1-2 rows of stratum intermedium cells. Otherwise the epidermis is the same as at the ½ day stage. 

Follicles: The hair follicles are still out of phase in
their development, but less so than at the \( \frac{1}{2} \) day age. They can be divided into two classes: medium and small. The medium follicles stretch to the base of the adipose layer; they each possess a bulb, papilla, hair matrix, inner sheath and have commenced the period of hair proliferation. They are about three times more frequent than at the \( \frac{1}{2} \) day age. The small follicles stretch about to the base of the dermis; they show bulb development only. No follicle primordia are seen. The orientation of follicles, i.e. the consistency of their angle of incidence to the surface of the skin, is moderately uniform. No follicles show excessive slope. The blood vessels are small and thick-walled.

Rat neck: Dermis and adipose layers are distinguishable. The epidermis is normal.

Follicles: The follicles are more out of phase in development than normal, i.e. there is a greater than normal range between the largest and smallest types found. This is due to a retardation in the development of the smaller follicles and not to advanced growth of the larger. Three types are distinguished: medium, small, primordial. The medium follicles are normal except that hair proliferation is rarely occurring; these follicles occur about \( \frac{1}{2} \) as frequently as normal. The small follicles are normal. The primordia are not numerous here but are more numerous than normally. The orientation of follicles is not very uniform. The blood vessels are normal.

Rat neck: The dermis and adipose differentiation is not very clear. The epidermis is little different from normal.

Follicles: There are a few very small follicles which stretch to the base of the dermis, but they have no bulbs or other structure.
Nearly all the follicles seen are fairly large primordia. They are formed by rather diffuse aggregations of Malpighian cells. The blood vessels are normal. (N.B. Ralta material examined at this and later ages is from the viable type of Ralta mice with lower-grade abnormality. No further abnormalities of the blood vessels were seen).

*Rump:* The dermis and adipous layers are differentiated but not very clearly.

*Epidermis:* The basal Malpighian cells are fairly closely packed and columnar in some regions. Apart from this evidence of slightly more proliferative activity, the epidermis is the same as in the neck region.

*Follicles:* Hair follicles are out of phase to a moderate extent. Two types are distinguished: large-medium and small-medium. The large-medium follicles are fully developed and have mostly produced hairs. The largest are bent or curved parallel to the surface of the skin apparently owing to an insufficient depth of skin being available for their growth. The small-medium follicles stretch to the base of the dermis or slightly into the adipose layer; structurally, they are cords of Malpighian cells with bulb and papilla structure only. It is sometimes possible to see on each section of a series a sharp division of the skin into two separate zones. In one zone the skin is comparatively thicker, more folded and the epidermis shows more proliferation than in the other zone. Such zones show that waves of growth or clearly demarcated areas of proliferation occur in normal skin at this age.

*Rump:* The dermis and adipose differentiation is not very
clear. The epidermis is normal.

**Follicles:** The hair follicles are clearly out of phase in their development. Three types are distinguished: medium, small, and primordial. The medium follicles stretch well into the adipose layer and show bulb, papilla and inner sheath differentiation. None produce hairs. They are less common than in normal skin. The small follicles stretch to the base of the dermis and show bulb development only. The primordia are more common than normally.

**ReRa rump:** Separate dermis and adipose layers are hardly distinguishable.

**Epidermis:** There are 1-4 rows of closely packed basal Malpighian cells. There are generally about 2 rows of stratum intermediate cells as in the normal skin, but these cells may become interspersed with Malpighian cells so that the two layers are less clearly differentiated than normal.

**Follicles:** Two types of follicles are seen: small and primordial. The small follicles stretch to the base of the dermis but have little bulb development. Some of the primordia are merely small and diffuse groups of Malpighian cells and are hardly distinguishable.

**At 3+ days after birth.**

**Neck:** The differentiation of the dermis and adipose layers is now clear.

**Epidermis:** There are 1-2 rows of rather sparsely arranged basal Malpighian cells. The cell nuclei are in some cases flat and slightly pyknotic, or irregular in outline. There are 1-2 rows of stratum intermedium cells. The stratum granulosum region is thinner
than at the 1\(\frac{1}{2}\) day age and the keratohyalin granules though clear, are not prominent.

**Follicles:** The hair follicles are becoming more closely in phase in their development. Over half the follicles stretch to the base of the adipose layer. These are fully developed and carry hairs. The rest of the follicles are of medium size and stretch to the base of the dermis or below. They show differentiation of the bulb and papilla and hair matrix, and of the inner sheath in some cases. Follicle primordia are not seen.

**Rump:** The differentiation of the dermis and adipose layers is clear. The epidermis is normal.

**Follicles:** The hair follicles are more out of phase in development than normal. This is due to a retardation in the growth of the later initiated follicles, so that many follicles are smaller and less far developed than any in normal skin. Closely adjacent follicles vary in size from the fully developed type (less numerous than normally) which stretch to the base of the adipose layer, to small down-growths of Malpighian cells which are classifiable as follicle primordia. Follicle orientation is not uniform but hardly abnormal. The angle of growth of some follicles is, however, more parallel to the surface of the skin than normal; probably this is due to the smaller depth of skin available for their growth.

**Rump:** The skin is more than usually folded in some regions, and only slightly folded in other regions. The thickness is variable, being very much greater in the folded areas. The differentiation of the dermis and adipose layers is clear.

**Epidermis:** In the folded regions of the skin there are 1-2 rows of fairly closely packed Malpighian cells with oval nuclei of
regular outline. There are 2 rows of stratum intermediate cells and the keratohyalin granules of the stratum granulosum are clear and prominent. In the regions where the skin is much less folded the Malpighian cells are sparser and their nuclei often flat or irregular in shape. The keratohyalin granules are smaller and less in evidence. The epidermis in these areas is obviously thinner and in a less active state of proliferation than in the folded skin areas.

Follicles: The hair follicles are more out of phase in development than in the neck region. There are long, fully developed follicles with hairs, medium sized follicles at or near the commencement of hair proliferation and small follicles with some bulb development only, which stretch hardly to the base of the dermis. There are a few peg-like down-growths of Malpighian cells, classifiable as follicle primordia. The orientation of the follicles is fairly uniform. The thick and considerably folded zones of skin where the epidermis is actively proliferating, represent fairly clearly defined areas of more active hair growth than in the surrounding regions of skin.

Rearrump: The skin is considerably folded in some regions but not in others. The thickness is variable, being much greater in the folded zones. The differentiation of the dermis and adipose layer is fairly clear.

The epidermis is the same as in normal skin at this age. The same differences are seen between the folded, relatively active zones and the thinner, unfolded zones of less active proliferation.

Follicles: The hair follicles are rather more out of phase than is normal. Normal follicle types are present, but the small follicles and the primordia are generally more numerous and retarded
in development than those in normal skin.

At 4 days after birth.

++ neck:

**Epidermis:** There are 1-2 rows of basal Malpighian cells which are fairly closely packed in some areas. Their nuclei are oval and regularly shaped. There are 2 rows of stratum intermedium cells. The keratohyalin granules are clear but not prominent. It seems that the epidermis shows a moderate degree of proliferation.

**Follicles:** The hair follicles are only slightly out of phase in development. The large follicles are fully developed, carry hairs and stretch to the base of the adipose layer. There are some follicles of medium size which stretch below the base of the dermis, and up to half way into the adipose layer. These follicles have full differentiation of the bulb, papilla, and hair matrix, but little or no inner sheath development. No small follicles or primordia are found. The orientation of follicles is fairly uniform.

++ neck: The epidermis is normal.

**Follicles:** The hair follicles are more out of phase in development than normal. The long follicles are normal in their size and development. They may be abnormally bent, however, or their angle of growth may be more parallel to the surface of the skin due to the insufficient depth of skin available for normal downward growth. The smaller follicles stretch no further than to the base of the dermis. They may show no differentiation at all or merely differentiation of the bulb. There are fewer large follicles than in normal skin.

++ rump:

**Epidermis:** The epidermis is similar to that in the neck
region except that the basal Malpighian cells are more closely packed together, and the keratohyalin granules are more prominent. This probably implies greater proliferative activity of the germinative layer of the epidermis.

**Follicles:** The hair follicles are more out of phase in development than in the neck region. There are long, fully developed follicles; medium follicles with slight inner sheath development which stretch a short distance into the adipose layer; and a few smaller follicles with bulb development only which do not stretch below the dermis. Orientation of the follicles is uniform; they are rarely curved and do not grow parallel to the surface of the skin since the depth of skin is sufficient to allow normal downward growth.

**Rat rump:**

**Epidermis:** The epidermis is normal, the germinative region being fairly proliferative.

**Follicles:** The follicles are more clearly out of phase than normal. The same types are present as in normal skin, but the smallest are shorter and less developed than normal and they comprise a higher proportion of the total number of follicles. The long follicles are often bent and may grow almost parallel to the surface of the skin due probably to the depth of the skin being less than normal. Orientation of the follicles is less uniform than normal.

**At 5½ days after birth:**

**++ neck:**

**Epidermis:** The germinative layer is rather thinner than at 4½ days. There are 1-2 rows of fairly sparsely occurring basal
Fig. 59. ++. The follicles are all closely in phase in their development (mid-anagen stage); they are elongated and even in size.

Fig. 60. ++. The follicles are out of phase in their development. Elongated, normally developed follicles are shown (the right-hand one is curved, probably because of the abnormal thinness of the adiposus), and there are several follicles whose development is retarded (I. F.).
Fig. 61. The follicles are fairly closely in phase in their development (mid-anagen phase); they are elongated and even in size.

Fig. 62. Adjacent follicles are out of phase in development. Two large, normally developed follicles are shown growing at an acute angle to the surface of the skin (probably because the adipose layer is thinner than normal). There are many incomplete follicles (I.F.) whose development has been retarded.
Malpighian cells whose nuclei are oval and regular in shape. There are 2 rows of stratum intermediate cells. The stratum granulosum is clear but rather thinner than at 3½-4½ days. The keratohyalin granules are clear. It therefore seems that the proliferative activity of the epidermis is less than at 4½ days.

**Follicles:** The hair follicles are now very closely in phase in their growth. They are all fully developed, carry hairs and stretch approximately to the base of the adipose layer. The later-initiated follicles appear to have developed faster than those initiated early and have now reached almost the same phase of growth. Follicle orientation is very uniform, and none are bent or curved.

**Rat neck:** The epidermis is normal.

**Follicles:** The hair follicles are abnormally out of phase in their development. There are long fully grown follicles, follicles of medium size, and smaller follicles varying in size from primordia to down-growing cords of Malpighian cells which stretch to the base of the dermis. Follicle orientation is moderately uniform but many large follicles are curved or tend to grow parallel to the surface of the skin.

**Rump:** Skin thickness is now very great but not even; there are clearly demarcated zones of greater and lesser thickness.

**Epidermis:** The epidermis is generally thicker than in the neck region. The basal Malpighian cells are columnar and are more closely packed together in the zones of thicker skin than elsewhere. More proliferation seems to be occurring in the areas of thicker skin.

**Follicles:** The follicles are only slightly out of phase in growth. In addition to long, fully developed follicles there are
some of medium length which stretch only into the upper part of the adipose layer. These follicles have developing inner sheaths but no hairs. The orientation of the follicles is uniform and they are not bent or curved.

Rat rump: The epidermis is normal.

Follicles: The hair follicles are abnormally out of phase in development. There are long fully developed follicles, medium-small follicles with bulb development only which stretch to the base of the dermis, and small follicles with very little bulb differentiation which stretch less than the full width of the dermis. Clearly the smaller follicles are abnormally retarded in growth. The large follicles are often severely curved and tend to grow parallel to the surface of the skin. Follicle orientation is not very uniform.

At 7½ days after birth.

++ neck:

Epidermis: The epidermis is thinner than at earlier ages. There is only 1 row of basal Malpighian cells which are not closely packed together. Their nuclei are in a few cases irregularly shaped, or flattened and pyknotic. There is only 1 row of stratum intermedium cells. The stratum granulosum is thinner and the keratohyalin granules less clearly visible than at earlier ages. It seems that the trend towards less proliferation in the epidermis which was first noted at the 4-5 day ages in the skin from the neck region is now becoming marked. The epidermis is becoming similar to its normal condition in the adult, where the stratum granulosum and stratum intermedium are absent.

Follicles: The hair follicles are closely in phase with each
Sagittal skin sections at 7½ days after birth - rump region (x 100)

**Fig. 63.** The follicles are closely in phase developmentally (late-anagen stage); they are all equally elongated, uniformly orientated and none are curved.

**Fig. 64.** Adjacent follicles are out of phase in development. The bulb regions of 2 large normally developed follicles are seen - sharply curved probably because of the sub-normal depth of the adiposus. There are also a number of incomplete follicles (I.F.) whose development has been retarded.

**Fig. 65.** A number of small follicles and primordia are seen, whose development has been excessively retarded. One larger follicle is severely curved probably because of the small skin thickness available for its growth. The adipose layer is almost absent.
other in their development. All the follicles are long, fully developed and stretch approximately to the base of the epidermis. The later-initiated follicles are more slender, but as far developed as those initiated early. The situation is similar to that in a normal anagen phase in adult skin where all neighbouring follicles are in the same growth phase. Orientation of the follicles is very uniform; they are not bent or curved and grow consistently at the normal angle of about $45^\circ$ to the surface of the skin.

Rat neck:

Epidermis: The epidermis is normal; its proliferative activity appears small and its morphology is approaching the adult condition.

Follicles: The hair follicles are still abnormally out of phase in their growth. There are long fully developed follicles which have produced hairs; there are slim cords of Malpighian cells showing bulb differentiation only, which stretch just into the adipose layer, and there are smaller pegs of Malpighian cells which show little bulb development and do not stretch below the dermis. These last two types seem unlikely to develop into complete functional follicles. It appears that follicle and epidermis proliferation has decreased at the normal time in Rat skin, but owing to retarded growth, the later-initiated follicles seem unlikely to complete their development.

Rata neck: The adipose layer is almost completely absent.

Epidermis: The epidermis is similar to that in normal skin. There are some flattened, irregular pyknotic nuclei in the basal Malpighian layer. Proliferation is probably not great although mitoses can be observed.
Follicles: Two main types of follicles are seen. There are a few of medium-small size with bulb and papilla development only. These follicles stretch to the base of the dermis. In addition there is a large number of follicle primordia. Although the total follicle density at this stage is little different from normal it is probable that few of these follicles will be able to complete their development due to their retarded growth.

Epidermis: There is a single row of basal Malpighian cells which are moderately closely packed and whose nuclei are clear, oval and regular in shape. There is a single row of stratum intermedium cells. The stratum granulosum is thin; the keratohyalin granules are clear but not prominent. Proliferative activity seems therefore greater than in the neck region but less than in the rump area at earlier ages.

Follicles: The hair follicles are fairly closely in phase in their development. They are all long, fully developed and stretch approximately to the base of the adipose layer. Orientation of the follicles is very uniform; they are not bent and grow at an angle of about 45° to the surface of the skin.

Rat rump: The epidermis is normal.

Follicles: The hair follicles are still out of phase in growth. There are large normally developed follicles, and small cords of Malpighian cells with bulb and papilla differentiation only, which stretch to the base of the dermis. Follicles of the last type look unlikely to complete their development. Orientation of the follicles is less uniform than normal. The large follicles are frequently bent or have grown parallel to the surface of the skin.
The adipose layer is extremely thin or absent in some areas.

**Epidermis:** There are 2 rows of generally columnar basal Malpighian cells. These cells are closely packed together and their nuclei are oval, clear and regular in shape. There are 1-2 rows of stratum intermedium cells. The keratohyalin granules are clear and prominent. More proliferation seems to be occurring than in normal skin of the same age.

**Follicles:** Three types of follicles are distinguished. Those of medium-small size stretch to the base of the dermis; they have bulb and papilla structure and usually show inner sheath development but do not carry hairs. The small follicles stretch at least half way across the dermis; they are stout down-growths of Malpighian cells and show bulb development only. In addition to these two types, there are follicle primordia of widely varying sizes. It seems that all follicles have been retarded in their development and it is probable that only some of the first type described here will complete their development and produce hairs.

**At 3½ days after birth.**

**Neck:**

**Epidermis:** There are 2 rows of fairly closely packed basal Malpighian cells. Their nuclei are oval, non-pyknotic and regular in shape. Stratum intermedium cells are rarely found, and do not form a continuous row. The stratum granulosum is fairly thin; the keratohyalin granules are present but not clearly visible. The epidermis is still proliferating but is approaching the mature cytological state where it is largely made up of the basal Malpighian
cell layer.

**Follicles**: The hair follicles are at their maximum length, stretching to the base of the adiposus. They are not bent or curved, and their orientation is very uniform. This is a typical late anagen stage of growth.

**Rat neck**:

**Epidermis**: The epidermis seems slightly less proliferative than normal; the basal Malpighian cells are less closely packed together.

**Follicles**: The hair follicles show the same abnormalities as in the 7½-day material. The small down-growths of Malpighian cells have not developed over the last 2 days and will clearly not form complete follicles. The long fully developed follicles are frequently bent or growing parallel to the surface of the skin, due apparently to the abnormally small depth of skin available for their growth. Follicle orientation is not very uniform. Several large follicles show abnormal keratinisation and widening of the hair canal.

**Thump**:

**Epidermis**: There are 1-2 rows of basal Malpighian cells. The cells are fairly close-packed; a few of the nuclei are flattened and pyknotic or irregular in shape. There is a single row of stratum intermedium cells. Keratohyalin granules are present, but not prominent.

**Follicles**: The hair follicles are all long and fully developed; some of them are slightly bent just above the bulb. Their orientation is uniform.
The epidermis is normal.

**Follicles:** Hair follicle development is little different from that at 7½ days. The large follicles are bent or tend to grow parallel to the surface of the skin. This is probably because although their maximum length is almost normal, i.e. 1080 μ, the maximum skin thickness is about half the normal thickness.

The smallest Malpighian cell down-growths will obviously not complete their development; they appear to be less numerous, however, than at 7½ days and therefore some of the follicles which were incompletely developed at that stage may have been able to advance their growth. This would confirm the view that follicle growth can proceed longer in the rump than in the neck region.

**At 12½ days after birth.**

**+ neck:**

**Epidermis:** There are 1-2 rows of basal Malpighian cells, which are not closely packed together. Some of the nuclei of these cells are vertically flattened and pyknotic. Stratum intermedium cells are very rare. The stratum granulosum layer is almost absent and no keratohyalin granules are visible. Little proliferation is occurring in the epidermis.

**Follicles:** All the hair follicles are fully developed. Most of them still stretch to the bottom of the adipose layer, but a few are slightly shorter. Since the skin is thinner than at 9½ days, this probably indicates the beginning of the catagen stage of follicle development, during which the follicles shorten and cease hair proliferation. Orientation of the follicles is uniform and they are not bent or curved.
**Erat neck:**

**Epidermis:** The epidermis is normal except that the stratum granulosum layer is clearer and keratohyalin granules are still visible. The cytological dedifferentiation of the epidermis is therefore slightly delayed in comparison with the normal.

**Follicles:** There are long normally developed follicles, and small follicles with bulb and papilla development only, which do not stretch below the dermis. These small follicles or Malpighian cell down-growths are no longer growing and will not complete their development. The large follicles are as long or slightly longer than normal although the skin is thinner; they are therefore often bent or aligned parallel to the surface of the skin, and their orientation is non-uniform. The shortage of space for the downgrowth of Ra+ follicles can be seen from these figures; viz. a follicle length of 1080 μm may occur at a skin thickness of 315 μm; whereas in normal skin, follicles of 1035 μm grow in an average skin thickness of 495 μm. The Ra+ follicles are longer than normal because the catagen phase has not yet commenced in Ra+ skin.

**RaRa neck:** There is no adipose layer.

**Epidermis:** There are 1-2 rows of basal Malpighian cells which are fairly closely packed together. There are no flattened or pyknotic nuclei such as are seen in normal skin. Stratum intermedium cells form a discontinuous row. Keratohyalin granules are present but not prominent. The epidermis seems rather less cytologically dedifferentiated than normal.

**Follicles:** There are both medium and small sized follicles. The medium follicles stretch to the base of the dermis (i.e. the full depth of the skin); they possess bulb, papilla and some inner
sheath differentiation and are often considerably curved due probably to the small depth of skin available for their growth. These follicles do not carry hairs but the hair canals are in some cases plugged with keratin. The small follicles vary in size from stout down-growths of Malpighian cells with some bulb and papilla structure to small epidermal thickenings or primordia.

**Rump:**

**Epdidermis:** There are 2 rows of basal Malpighian cells; the nuclei of many are irregular or flattened and pyknotic. The keratohyalin granules are absent in most regions. Stratum intermedium cells are rare.

**Follicles:** The maximum follicle length is now slightly less than at 9½ days (viz. 990 μ compared with 1030 μ at 9½ days). This fact and the evidence that the skin thickness has decreased since 9½ days show that the follicles are entering the catagen stage. Few of the follicles are curved and their orientation is fairly uniform.

**Rat rump:**

**Epdidermis:** The epidermis is normal except that fewer flattened or pyknotic Malpighian cell nuclei are seen. It seems that the epidermis may be slightly later in ceasing active proliferation than normal.

**Follicles:** The fully developed follicles have a maximum length of about 1080 μ which is rather longer than normal at this stage. Since the skin thickness is less than normal they are frequently bent or aligned parallel to the surface of the skin. The follicles are as long and the skin is as thick as at 9½ days and it therefore appears that the follicles are, unlike the normal, not yet
entering the catagen stage. Orientation of these follicles is not uniform. The smaller incompletely developed follicles, as at 9½ days, do not stretch below the base of the dermis. Their degree of differentiation varies from bulb development only to some development of the inner sheath. Probably none of these follicles will become functional.

**Rtta rump:** There is no adipose layer.

**Epidermis:** There are 2 rows of fairly closely packed basal Malpighian cells. The cell nuclei are not pyknotic and rarely flattened. Stratum intermedium cells are frequently seen but they do not form a continuous layer. The stratum granulosum is thicker than normal and the keratohyalin granules are clear and prominent. The epidermis is abnormally late therefore in ceasing proliferation.

**Follicles:** The hair follicles are the same as those in Rtta skin from the neck region. There are medium sized follicles with structural differentiation which have not produced hairs; and there are smaller follicle primordia and Malpighian cell down-growths.

**At 15½ days after birth.**

**++ neck:**

**Epidermis:** There are about 2 rows of Malpighian cells. They are not closely packed together and the nuclei are frequently irregular or pyknotic. Stratum intermedium cells are very rarely seen. The stratum granulosum is generally absent and there are no keratohyalin granules. The epidermis has almost reached the adult cytological state.

**Follicles:** The hair follicles are now all in the catagen stage of development. Some are in early catagen and still stretch
over half way across the adipose layer, others are in slightly later stages and are further retracted. They are therefore very slightly out of phase in development. All the follicles are fully differentiated and carry hairs. Some are slightly curved but their orientation is uniform.

**Rat neck:**

**Epidermis:** There are 1-2 rows of basal Malpighian cells which are not closely packed together. Their nuclei are oval, regular in shape and non-pyknontic. Stratum intermedium cells are common but they do not form a continuous row. The stratum granulosum is thin; keratohyalin granules are present but only faintly visible. Again, therefore, the epidermis is cytologically less mature or less differentiated than normal.

**Follicles:** Some of the fully developed follicles stretch almost to the base of the adipose layer and appear to be still in the anagen stage of the growth cycle. Other follicles have clearly retracted almost entirely into the dermis and are in the catagen stage. These follicles are abnormally out of phase in their development; many of them are also curved and not uniformly orientated. The remaining follicles are straight down-growthsm of Malpighian cells of varying sizes. These structures show no change in development since the 12½-day stage.

**Rump:**

**Epidermis:** There are 1-2 rows of basal Malpighian cells. The cells are not closely packed together, and their nuclei are flattened or irregular and pyknontic in some areas; in these areas the epidermis is thinner than elsewhere. Stratum intermedium cells are very rare. The stratum granulosum is very thin, and keratohyalin
granules are absent in most regions.

**Follicles:** The follicles are apparently still in the anagen phase of growth. They are not curved or bent and are uniformly orientated.

**B*at r*ump:**

**E*idermis:** There are 1-3 rows of basal Malpighian cells which are in some regions fairly closely packed together. The nuclei are irregular and slightly pyknotic in some areas. Stratum intermedium cells are fairly common in the thicker regions of the epidermis but they do not form a continuous row. Keratohyalin granules are faintly visible in the thicker epidermal regions only.

**F*ollicles:** The hair follicles show little change in development from that at 12½ days. The completely developed follicles are in late anagen stage of growth. The smaller follicles showing incomplete structural differentiation have ceased their development. The functional follicles are themselves apparently out of phase in their development, since only some of them have clearly entered the catagen phase.

At 13½ days after birth:

**++ neck:**

**E*idermis:** The epidermis now consists entirely of stratum corneum and Malpighian cells of the basal layer. This is the adult condition. There are 2 rows of Malpighian cells; their nuclei are rather irregular and pyknotic in certain areas.

**F*ollicles:** The hair follicles are closely in phase in their development. They are all in a late catagen stage and they have retracted into the dermis. Some of the follicles are curved in the
Fig. 66. The follicles are closely in phase (late-catagen stage); they are fully retracted into the dermis; they are evenly orientated and not curved.

Fig. 67. The 2 large follicles (C, V.) shown are very severely curved - possibly they have not retracted sufficiently to allow for the contraction in skin thickness during the catagen stage.

Fig. 68. The only large follicle shown is curved, probably because of the continuously sub-normal skin thickness.
lateral plane; this is probably due to their contraction during catagen.

Rear neck:

Epidermis: There are 1-3 rows of basal Malpighian cells which with the stratum corneum comprise most of the epidermis. Some of the Malpighian cells have irregularly shaped nuclei and a few nuclei are pyknotic. A few stratum intermedium cells are present in some areas. The stratum granulosum is very thin and the keratohyalin granules are only faintly visible. Although the epidermis is approaching the adult state, it is less differentiated than normal, i.e. it probably ceases proliferation later than normal.

Follicles: The large fully developed follicles are in late anagen or early catagen stages. They are to some extent out of phase in their development and those follicles which appear late in entering the catagen phase are severely bent and compressed, apparently due to the considerably diminished skin thickness. These follicles are apparently out of phase with the contraction of the skin which occurs during the catagen stage, because of their retarded development and consequent delay in commencing to shorten or retract. The incompletely developed "follicles" showing varying degrees of differentiation are still present in the dermis; the longer structures which are partially differentiated cords of Malpighian cells, show some signs of curvature and retraction. This may be due to these follicle structures retracting autonomously or it may be the result of changing tension of the skin due to contraction of the dermis which does occur during the catagen phase.

Rear neck: The adipose layer is absent.

Endermis: There is generally a single row of basal Malpighian
cells (but there may be 0-3 rows in different regions); the nuclei are not pyknotic and they are not flattened or irregularly shaped. Stratum intermedium cells are common and form an almost continuous row. Keratohyalin granules are present but not clearly visible. The epidermis shows abnormally delayed dedifferentiation.

**Follicles:** There are a few medium-large sized follicles which possess full structural differentiation, viz. bulb, papilla, hair canal, inner sheaths; no hair shafts are present but the hair canals may be plugged with keratin. These follicles are severely bent or curved. There are also smaller follicle structures or cords of Malpighian cells which vary in size from undifferentiated primordia to structures stretching almost the full depth of the dermis.

**Flank:**

**Epidermis:** There are 1 or 2 rows of fairly sparsely arranged basal Malpighian cells. Some of their nuclei are slightly irregular or pyknotic. Stratum intermedium cells are rare. There is no continuous stratum granulosum and keratohyalin granules are only faintly visible.

**Follicles:** The hair follicles are all in the catagen stage of development. They are slightly out of phase since some have retracted to half way up the adipose layer and others have shortened to lie entirely within the dermis. Orientation of the follicles is uniform and they are rarely bent or curved.

**Rat rump:**

**Epidermis:** There is a fairly continuous single row of basal Malpighian cells which are not closely packed together. The nuclei are not pyknotic, flattened or irregular in shape. There is a single continuous row of stratum intermedium cells. The stratum
granulosum is thin; keratohyalin granules are present but not clearly visible. The epidermis is again less mature and therefore thicker than normal.

**Follicles:** The long, fully developed follicles are in late anagen or early catagen stages. Most of them have not begun to shorten appreciably and they are therefore later than normal in reaching this stage of development. Their orientation is not uniform and they are frequently bent or aligned parallel to the surface of the skin. The small incompletely formed follicles are still present as at earlier ages.

**R*Ra rump:**

**Epidermis:** There are 2-3 rows of basal Malpighian cells which are fairly closely packed together. Some regions even show local hyperplasia. The cell nuclei are not flattened, pyknotic, or irregular in shape except in a few very thin regions of the epidermis. Stratum intermedium cells are common but do not form a continuous row. Keratohyalin granules are prominent in the thick proliferative regions of the epidermis and only faintly visible in other regions.

**Follicles:** The hair follicles are similar to those in the R*Ra*Ra neck region at this stage. But the medium-large structurally complete follicles are rather more common here, and the smaller incompletely formed follicles are less common than in the neck region. The hair canals of the larger follicles are often wider than normal and plugged with keratin.

At 21½ days after birth.

**++ neck:** The adipose layer is generally absent.
Enidermis: There are 1-2 rows of basal Malpighian cells which are not closely packed together. Some of the nuclei are irregular in shape and a few are pyknotic. Stratum intermedium cells are rare. The keratohyalin granules are absent or only faintly visible.

Follicles: All the hair follicles are fully retracted into the dermis. They are in the telogen or resting phase. There is some curvature of the follicles in the lateral plane; this is probably the result of contraction during catagen. Orientation of the follicles is uniform.

Fat neck: The adipose layer is generally absent.

Enidermis: There are 1-2 rows of basal Malpighian cells which are not closely packed together. The nuclei are not flattened or irregular in shape and they are not pyknotic. Stratum intermedium cells are present but they do not form a continuous row. The stratum granulosum is thin; keratohyalin granules are faintly visible.

Follicles: Many of the fully developed follicles seem to be in the catagen stage. Some, however, stretch below the dermis to the panniculus carnosus muscle layer. The skin has contracted a great deal in thickness but the larger follicles are apparently out of phase with the skin contraction and they have hardly begun to shorten. The follicles are therefore severely bent at the base or they are aligned parallel to the surface of the skin (Fig.). The small incompletely formed follicles show little change since the 18½-day stage. The catagen stage of follicle development seems to begin later and proceed more slowly than in normal mice, probably because the follicles are retarded in their development.
++ rumn: The adipose layer is present but very thin.

Epidermis: There are generally 2 rows of basal Malpighian cells which are rather sparsely arranged. Some of the nuclei are irregularly shaped or flattened and pyknotic. There are no stratum intermedium cells and no keratohyalin granules. This is the mature state of the epidermis.

Follicles: The hair follicles are closely in phase in their development. They are all in late catagen phase. The bases of the hair shafts are not yet in their highest resting position just below the sebaceous gland but the papilla rests are visible below the follicles. Follicle orientation is very uniform and there is no curving or parallel growth of the follicles.

++ rumn: The adipose layer is thin.

Epidermis: There are 2-4 rows of basal Malpighian cells which are fairly closely packed together. The nuclei are neither irregular nor flattened and pyknotic. Stratum intermedium cells are common but do not form a continuous row. The stratum granulosum is thin, and the keratohyalin granules only faintly visible. The epidermis is cytologically less mature than normal.

Follicles: The fully developed hair follicles are still out of phase to some extent. Some are in late anagen or early catagen, and others in late catagen stages. This is demonstrated by the fact that some follicles have retracted completely within the dermis and others have not shortened from an extended position against the panniculus muscle layer. The smaller, incompletely formed follicles show no change in development. Fairly long cords of Malpighian cells, with bulb differentiation only are the type most commonly observed.
The observations made from the 15½-21½ day material enable two important conclusions to be drawn:

(i) The processes of follicle development and hair growth are later in reaching completion in the rump region than in the neck region of normal mice.

(ii) Follicle development and hair growth in Ra+ mice are later than normal in reaching completion in the neck and rump regions; but the retardation seems greater in the neck region.
Sagittal skin sections at 5½ days after birth - rump region (x 260)

**Fig. 69.** ++; showing the epidermis fully differentiated and actively proliferating. All the follicles are fully grown.

**Fig. 70.** Rat+. The epidermis is normal - the germinative layer being highly proliferative. All the follicles shown are however retarded in development - the stage of bulb differentiation has hardly commenced.

**Notes:** In the ++ and Rat+ epidermis all the separate layers can be seen, viz., from the distal side: stratum corneum (wide), stratum lucidum (narrow), stratum granulosum (with clear rows of kerato-hyalin granules) stratum intermedium (large, lightly stained cell nuclei) and the Malpighian layer (closely packed, darker stained cell nuclei).
Sagittal skin sections at 21½ days after birth - rump region (x 260)

**Fig. 7a.** showing the epidermis fully de-differentiated. Only the Malpighian layer is clearly distinguishable, but the cells are sparse and relatively non-proliferative. Note that many of the nuclei are pyknotic.

**Fig. 7b.** Rat; showing the epidermis slightly less de-differentiated than normal. The stratum corneum is more in evidence than normal and the cells of the Malpighian layer are less sparse and probably more actively proliferative; few of their nuclei are pyknotic.
(iii) From Macroscopic Observations.

(a) It was noticed during the shaving of 9½-12½ day old mice that the Ra+ skin seemed hyperkeratotic compared with normal skin. Abnormally large quantities of superficial keratin were loosened and removed during the shaving of Ra+ mice.

(b) During the removal of skin samples, it was noted that before 9½ days age in normal agouti mice, skin from the neck region was thicker and looked more darkly pigmented than that from the rump. The thickness and pigmentation was due to hair proliferation in the skin. By 12½ days age the skin from the neck is similar to that from the rump. After 12½ days the rump appears to be the main region of hair proliferation. Judging by these criteria, Ra+ neck skin showed less active hair proliferation than normal, whereas Ra+ rump skin was not noticeably sub-normal in activity. RaRa skin from both areas never seemed thickened or pigmented.

Since these conclusions are in agreement with the histological data, it seems to be possible to estimate, by macroscopic observation, the stage of the hair growth cycle reached by any specimen of skin.
(iv) From Skin Thickness Measurements.

Results of the measurements of the thickness of the main skin layers are presented graphically in Figs. 73 to 80. On each graph measurements are given from a single animal of each genotype at each age. For the 17½- and 18½-day embryo measurements the ++, Ra+ and RaRa animals used were all sibs. For the post-partum measurements only the ++ and Ra+ animals used were sibs; from 1½ to 7½ days, all the ++ and Ra+ measurements were taken from mice from the same litter, and from 9½ to 21½ days all the ++ and Ra+ measurements were taken from mice of another single litter. The graphs seem to establish that:

(a) The thickness of the epidermis of ++, Ra+ and RaRa mice is relatively high from before birth until 4½ days after birth, but thereafter slowly diminishes.

(b) There is little difference in the thickness of the epidermis in ragged and normal mice at any stage. It seems possible, however, that the period of active proliferation, as measured by the thickness of the epidermis, persists slightly longer than normal in ragged mice.

(c) The total skin thickness of normal mice gradually increases from the commencement of follicle down-growth. It reaches a maximum between 7½ days and 9½ days after birth, when the follicles are at the point of maximum elongation; and thereafter declines steadily.

(d) These changes in total skin thickness depend largely upon the thickness of the adipose layer which greatly increases during the stages of maximum follicle growth in normal mice, and in-
creases similarly but to a lesser extent in Ra+ mice.

(e) The total thickness of the skin in Ra+ mice is therefore very much less than normal during the period of follicle down-growth. This difference is clearly due to the failure of the adipose layer in Ra+ mice to expand to the normal extent. In RaRa mice the adipose layer is absent or almost absent at all stages; the total skin thickness is therefore markedly sub-normal.

(f) The thickness of the adipose layer and therefore the total skin thickness reaches a higher maximum and is more nearly normal in the rump region than in the neck region of Ra+ mice. It will be suggested later that this may be due to the more normal development of hair follicles in the posterior regions of Ra+ mice.

(g) The standard errors shown on the graphs indicate the variation in thickness of the skin layers within mice. Large standard errors generally indicate a high degree of folding of the skin.
2 Standard Errors, one standard error on each side of the mean.

Fig. 73 and Fig. 74.
= 2 Standard Errors, one standard error on each side of the mean.

Fig. 75 and Fig. 76.
= 2 Standard Errors, one standard error on each side of the mean.

Fig. 77 and Fig. 78.
= 2 Standard Errors, one standard error on each side of the mean.

Figs. 79 and Fig. 80.
Disc.ussion.

The rather complicated data on follicle and skin morphology can best be discussed by subdivision into sections.

Epidermis:

Periodic phases of active cell division and of relative inactivity were detected in the Malpighian layer of the epidermis. Generally the Malpighian layer seemed to be active when the hair follicles were being initiated and inactive during the stages of late-anagen, catagen and telogen. The epidermis was assumed to be highly proliferative when there were numerous close-packed cells in the basal layer and when the cell nuclei of the whole germinative layer were clear, round-oval in shape and non-pyknotic. During inactive phases cell division seemed to have slowed down; the basal Malpighian cells were relatively sparse and their nuclei were flattened or irregular in shape and often pyknotic. During the phases of active proliferation in the epidermis the skin was usually considerably folded, and the germinative layer was much thicker than during inactive phases.

After birth, the epidermis remained proliferative in Ra+ and RaRa mice just as long or longer than in the ++ mice. Since hair follicles are initiated and developed from the germinative layer of the epidermis, this is circumstantial evidence for the view that there is no abnormally early cessation of follicle initiation in Ra+ and RaRa mice.

It seems from the descriptive data and the graphs showing changes in epidermal thickness, that the epidermis is relatively thick whilst it is actively proliferating, and thinner during periods of little proliferation. Similarly, Spain (1915) and Erick-
son (1931) have shown that a thick stratum germinativum of the epidermis is associated with a high rate of proliferation.

The present work has shown, first, that the germinative layer of the epidermis is fairly thick and actively proliferating until about 4½ days after birth, in ++ and Ra+ mice; thereafter, the epidermis gradually becomes thinner and its rate of proliferation appears to decline. Secondly, it is clear that in normal mice, by 4½ days after birth, a high proportion of the hair follicles have grown down almost to the base of the adipose layer, and a high rate of cell division is occurring in the follicle bulbs.

These facts can be interpreted on the hypothesis developed in the section on adult morphology; that hair follicle growth may be initiated by a stimulus causing an increased rate of mitosis in all germinative epithelial cells; but that later the Malpighian cells of elongating hair follicles may compete successfully against the Malpighian cells in the epidermis for substances favouring a high rate of cell division. During the growth of the first pelage in ++ and Ra+ mice, one would expect therefore a high mitosis rate in all germinative epithelia from the commencement of follicle development until so many of the follicles become elongated as to inhibit cell division in the germinative layer of the epidermis. Such a process seems to occur, and the results of the competition are first visible in the diminishing thickness of the epidermis from 4½ days after birth.

In RaRa mice, the epidermis, especially in the rump region, remains thick and actively proliferative much longer after birth than normal. On the present hypothesis, this would be due to lack of competition from developing follicles. The epidermis of the
The neck region is, however, less abnormally active; this may be because the early development of the epidermis has been delayed by oedema, which is generally more severe in the anterior regions.

At the end of the anagen phase of follicle growth the follicles begin to shorten. This occurs between 12$\frac{1}{2}$ and 15$\frac{1}{2}$ days after birth in normal mice, and slightly later in Ra+ mice. During this catagen phase, the epidermis de-differentiates, so the separate stratum intermedium, stratum granulosum and stratum lucidum disappear. This process of de-differentiation is very much delayed and often incomplete in RaRa mice.

**Hair Follicles.**

The histological data which have been presented demonstrate clearly the cause of the absence of many zig-zag fibres in the adult Ra+ mouse. A high proportion of these hairs is missing in the adult, because the rate of growth of the hair follicles, especially the late-initiated follicles, is abnormally slow in Ra+ mice. Since the hair follicles in Ra+ mice stop growing nearly at the normal time, many of the late-initiated follicles which normally produce zig-zag fibres have not completed their development by the time follicle growth ceases. These follicles are therefore non-functional and do not produce hair fibres. Follicle growth is less abnormally retarded on the rump than on the neck in Ra+ mice and there is therefore a smaller proportion of non-functional follicles on the rump.

The slow growth and differentiation of late-initiated hair follicles in Ra+ mice also has a second effect, namely, that the follicles become abnormally out of phase in their growth. In the normal mouse, by 5-6 days after birth, all neighbouring hair follicles in a specific region of skin have reached a similar stage of...
development, and all the follicles stretch downwards a similar distance into the adipose layer. The follicles therefore all enter the catagen stage simultaneously and retract into the dermis at the same time. This synchronous behaviour of neighbouring hair follicles persists in later hair cycles as has been shown in the section on adult morphology. In Ra+ mice, however, the delay in the growth of the later initiated follicles seems to result in their being out of phase with their earlier initiated neighbours. These 'delayed' follicles, only some of which become non-functional, are later than the other follicles in entering each of the developmental stages of anagen, catagen and telogen. These differences continue to exist in adult mice and affect the later cycles of hair growth so that neighbouring follicles can be in different stages of the hair cycle.

**Follicle Initiation.**

It is quite clear that pelage follicle initiation in Ra+ and RaRa mice commences at the normal time, in the 14-day embryo, and the morphological process of initiation appears to be normal. It has been observed by Balinsky (1950) that the morphological initiation of hair follicles by the appearance of primordia in the germinative layer of the epidermis is produced by cell migration and not by localised foci of increased cell division. A process which may include cell division has been seen during the present study to initiate the formation of hair follicle primordia. Closely packed cells of the proliferating germinative region of the epidermis form radial, proximally convex thickenings of the basal layer of the epidermis (Fig. 5). Such thickenings were the first visible signs of follicle primordia. If these structures are
formed by cell migration and not by local increases in the rate of mitosis, aggregation of many cells within these localised thickenings must cause a fall in cell density in the surrounding regions of the basal layer of the epidermis. It is possible that such an effect could produce an interference mechanism such that the initiation of a follicle at a specific point would prevent the initiation of other follicles within a certain area surrounding that point. Under such a system there would in fact be competition for Malpighian cells of the basal layer of the epidermis, so that a concentration of such cells within a given focus must prevent any similar concentration arising within a specific area around that focus. An interference system of some kind has previously been suggested by a number of authors, e.g. Hardy (1949), as necessary to explain the orderly initiation of hair follicles in mammals; but no mechanism for such a system has been put forward previously. If the system of follicle interference which has been described does actually operate, then it is clear that follicle initiation must be controlled by the epidermis. Therefore the primary stage of follicle initiation is the thickening of the epidermis and not the concentration of mesenchymal cells which are destined to form the papilla. Both these structures, the dermal and epidermal, appear apparently simultaneously when any hair follicle is initiated; which is the primary factor in follicle initiation is a question that has long aroused controversy.

It has been shown during the present work that hair follicle primordia are visible in Ra+ and RaRa mice at ages after they are no longer visible in normal mice, this is further evidence for the view that lack of many functional hair follicles in adult Ra+ mice
Fig. 81. A diagrammatic representation of the initiation of a follicle primordium, showing only the Malpighian layer of the epidermis. Note the fall in cell density on either side of the concentration of cells forming the primordium,
is not due to earlier than normal cessation of follicle initiation. The belated presence of follicle primordia in ragged mice could be caused by: (1) Later than normal initiation of some hair follicles, or (2) an abnormally slow rate of cell division and therefore slower growth of the primordia to form follicles. Furthermore, the slow growth of hair follicles in Ra+ mice is also likely to be the result of an abnormally slow rate of cell division. A low rate of mitosis would allow the formation of follicle primordia in normal numbers (this apparently occurs in Ra+ mice) if these primordia are formed by cell migration and not by increased mitosis rates; but it would retard the growth of hair follicles from primordia (this also occurs in Ra+ mice), since this process depends upon increased cell division.

Most of the 'delayed' or late-initiated Ra+ hair follicles which do not become functional, cease their development just before or just after the time of down-growth into the adipose layers. This is the period of development just preceding hair proliferation and is normally followed by a great increase in the rate of mitosis of cells in the bulb matrix and external sheath of the follicle. The commencement of this very active phase of development could be regarded as a threshold; if the general rate of mitosis of cells derived from the epidermis was low, it is clear that hair follicles would not be able to pass this threshold. Such a situation may exist in ragged mice; at least the assumption of a low rate of cell division could explain many of the abnormalities of ragged mice.

Hair Follicle Growth and Skin Thickness.

It has been observed in this study and it has been shown
by previous authors that the down-growth of hair follicles is always closely accompanied by a great increase in skin thickness, especially in the region of the adipose layer. Furthermore, the extent of the increase in skin thickness seems to be related to the density of down-growing follicles - e.g., in Ra+ and ReRa skin where there are fewer growing follicles, the increase in skin thickness is less than normal. This relationship presents an interesting problem. There must be a direct causal relationship between follicle down-growth and skin expansion unless a third factor causing both follicle growth and skin expansion is postulated. It is considered that there is some evidence for the view that down-growth of the hair follicles causes an expansion in skin thickness, rather than the converse.

(a) Some of the fully grown, early-initiated follicles in Ra+ mice become as long as the largest normal follicles although the skin thickness during the period of maximum follicle elongation is less in Ra+ mice than in normal mice, e.g. At 12½ days after birth:

In Ra+ mice, the maximum follicle length observed was 1080 μ at a skin thickness of 315 μ, whereas in ++ mice, the maximum follicle length observed was 1035 μ at a skin thickness of 450-550 μ.

In Ra+ mice all the hair follicles would be expected to be shorter than normal if increasing skin thickness were the cause of follicle down-growth, since the increase in skin thickness in Ra+ mice is less than normal.

(b) These hair follicles which are shorter and less developed in Ra+ mice than in normal mice usually do not stretch to the full depth of the skin which is available to them even at the
completion of their growth. These follicles therefore cannot have been prevented from growing by the failure of the adipose layer to expand sufficiently. On the other hand, such follicles would be ineffective in causing an increased thickness of the adipose layer and therefore the fact that a considerable proportion of all follicles are of this type could explain the failure of the adipose layer to expand to the normal extent during follicle growth in Ra+ mice.

(c) In RaRa juvenile and adult mice, where the follicles are very short and do not reach the base of the dermis, the adipose layer is correspondingly very thin or non-existent. Since the follicles are shorter than the full dermis thickness they cannot have been prevented from growing by the absence of the adipose layer; but lack of follicle down-growth could be the cause of the failure of the adipose layer to expand in thickness. This evidence suggests that if in Ra+ and RaRa mice there is a direct causal connection between the failure or partial failure of follicle down-growth and the subnormality of skin expansion, then it is of the form: partial failure of follicle down-growth causes a subnormal increase in skin thickness. Conversely, it is not true that the subnormal increase in skin thickness causes a partial failure of follicle down-growth in ragged mice; in fact many follicles in Ra+ mice grow to the normal length despite the subnormal increase in skin thickness. These statements entail the view that in normal mice follicle down-growth causes expansion of the adipose layer; the converse view is not upheld.

(d) There is evidence that the down-growth of some follicles, which does occur in Ra+ mice, is enough to influence the degree of expansion of the adipose layer, e.g. in the neck region of Ra+ mice.
where many follicles fail to complete their development and
down-growth the skin thickness is further below normal than in
the rump region where more Ra+ follicles complete their develop-
ment normally.

Although the possibility that a third factor influences both
follicle growth and skin thickness cannot be conclusively rejected,
it can be concluded from the preceding discussion that:

(i) Follicle development in ragged mice is not prevented by a fail-
ure of skin expansion, but by another cause.

(ii) The failure of some or most follicles to develop fully in Ra+
and RaRa mice probably results in sub-normal skin expansion
in these mice.

It therefore follows that in normal mice:

(a) The down-growth of hair follicles causes expansion in thickness
of the skin, especially of the adipose layer, or is a necessary
pre-condition for such expansion.

(b) There is no evidence that expansion of the skin causes follicle
down-growth.

It is, however, found that in Ra+ mice, the completed growth
of some hair follicles is enough to cause some expansion of skin
thickness although to a sub-normal extent. The skin thickness which
results is therefore insufficient for normal follicle down-growth
in ragged mice, and causes many of the fully grown follicles to be
bent, misorientated, or to grow parallel to the surface of the skin.
These abnormalities persist through the catagen stage and in succeed-
ing hair cycles. During catagen, the misorientation and abnormal
curvature of follicles and hairs seem to become intensified. This
may be caused by a number of factors:
(a) The follicles may merely be showing residual curvature due to there having been insufficient depth of skin available for their growth during the anagen stage. This curvature would become more extreme due to the contraction in length of the follicles during the catagen phase.

(b) The contraction of the skin thickness during the catagen stage may cause bending of the hair follicles since the sub-normal thickness of the skin might cause abnormally high pressure to be exerted upon the hair follicles during the initial stages of contraction.

(c) The lateness of the 'delayed' ragged follicles in entering the catagen phase may have caused them to become out of phase with the contraction of skin thickness which occurs at the commencement of the catagen period. Such follicles while still elongated would be compressed by the contracting adipose layer of the skin.

If one retains the view, previously upheld, that hair follicle down-growth causes an expansion of skin thickness, it may be considered that a widely expanded adipose layer is only forced to expand so long as all the down-growing follicles constrain it. As soon as some follicles lessen the 'pressure' by entering the catagen stage the adipose layer may begin to contract and so distort the retarded follicles which have remained elongated. As described in the sections on adult morphology, these morphological abnormalities of hair follicles are still present in mature ragged mice.

Summing up, the probable causal sequence established by the preceding argument is as follows: In ragged mice, retardation of hair follicle development causes partial failure of follicle
downgrowth, which causes sub-normal skin expansion during follicle growth, which in turn causes morphological abnormality of those follicles which are fully developed.

RaRa mice.

Throughout this discussion, reference has been made largely to the situation in Ra+ mice. However, it is not considered necessary to postulate different causes for the abnormalities in RaRa mice. Apparently an abnormally slow rate of growth of the hair follicles produces a degree of follicle agenesis which is much more severe than in Ra+ mice. Other abnormalities, including sub-normal skin thickness, are correspondingly more severe in RaRa mice.

Additional abnormalities.

(1) Capillary Blood Vessels.

Abnormalities of the superficial blood vessels were seen in skin from RaRa embryos and in skin from new-born RaRa mice of the inviable type showing high-grade oedema. These abnormalities were not seen in Ra+ or viable RaRa mice, and they were always associated with oedema; their general nature was as follows: The superficial blood vessels were larger, more numerous, and generally contained more densely packed red blood corpuscles than in normal mice. The walls of the vessels in some cases seemed thinner than normal, but this could perhaps have been caused by the dilation of the vessels. It is suggested that the occurrence of oedema in RaRa mice may be due to an excessive outflow of plasma fluid from the superficial blood vessels and possibly the lymph vessels. Possible causes of such an occurrence will be discussed elsewhere.
(2) Differentiation of the dermis and adipose layers is slower in RaRa mice than in Ra+ or ++ mice. This could be due to the presence of oedema.

(3) Follicle development is generally further advanced in the rump than in the neck region of RaRa mice. This constitutes a reversal of the normal growth gradient. It may be due to the fact that oedema is always more severe in anterior than in posterior regions. If this is so, it suggests a possible causal relationship between oedema and follicle agenesis.

(4) Oedema of varying severity seems to be found in all or most RaRa mice between the stages of the 14-day embryo and 0-½ a day after birth. Oedema is not usually found in viable RaRa mice which have remained alive longer than 1 day after birth.

(5) Retardation of skin differentiation and follicle development is always greater wherever the occurrence of oedema is more severe, i.e. RaRa new-born mice or embryos showing high-grade oedema are characterised by more obvious skin abnormalities, especially in anterior regions, than animals showing lower grades of oedema. This suggests that oedema may directly influence the retardation of skin differentiation and follicle development in these mice.
(vi) From Whole Skin Mounts.

(a) Introduction.

It has long been known that the hair follicles in most mammals have a tendency to be initiated in groups. The groups are often linear in arrangement; they consist of three or more follicles of which the central follicle is usually the largest. The subject of follicle grouping has been investigated for example, in the guinea pig by Dawson (1930), in the sheep by Carter (1943), in marsupials by Hardy (1946) and in the mouse by Gibbs (1941). Gibbs observed that follicle groups in the mouse were transient. Groups of three (trios) appeared by the fourth day after birth; more follicles were added until the groups were obliterated by the sixth day after birth.

It was thought that the present work might show a difference in the time of development of follicle groups in the neck and rump regions of normal mice. Moreover, if the follicles in ragged mice were initiated at different times than in normal mice, the follicle groups might be altered.

(b) Methods.

Whole mounts of skin were prepared from the neck and rump regions of 17½- and 18½-day embryos and suckling mice. The material was prepared by the method described elsewhere, in the section on follicle density. The groupings and spatial arrangement of the hair follicles were studied by examining the follicles under the microscope in optical cross-section. At first after initiation the follicles are round in 'cross-section', but older follicles develop an oval shape, and later become more elongated in optical section.
due to their growth at an acute angle to the surface of the skin; early and later initiated follicles can thus be easily distinguished.

(c) Results.

At 17½ days' gestation:

++ neck: Most of the follicles are small and round. The few oval-shaped follicles seen are often flanked by two smaller follicles. Each individual follicle is evenly spaced from all its neighbours so there is no clear arrangement in rows.

Ra+ neck: The follicles are similar to the normal but the further developed oval ones are slightly smaller than normal. The follicles are not arranged clearly in either rows or groups since their spacing is even.

RaRa neck: Most of the follicles are small; the few becoming oval in shape are smaller than those in normal or Ra+ mice.

++ rump: The follicles are mostly small and round, but there are a few of oval shape. They are not arranged in rows or groups since the inter-follicle spacing is even.

Ra+ rump: There are no abnormalities.

RaRa rump: Those follicles becoming oval in shape are rather smaller than normal. Otherwise there are no abnormalities.

At 18½ days' gestation:

++ neck: The follicles are similar to those at 17½ days except that they tend to be arranged in rows, so the spacing between follicles within rows is less than the spacing between rows. A few trio groups are distinguishable.

Ra+ neck: The follicles are normal except that no trio groups are
RaRa neck: The follicles are slightly smaller than normal. Rows of follicles are distinguishable.

++ rump: The follicles are similar to those in the neck region except that there are no trio groups.

Ra+ rump: There is little difference from the normal.

RaRa rump: The follicles are a little smaller than normal. The spacing between follicles is even, so no rows or groups are distinguishable.

At ½ day after birth (Fig. 82).

++ neck: The follicles are mostly small and round-oval in shape. There are a few larger shafted follicles. The follicles are arranged in rows, and in some regions there are clearly defined groups of 3, 5 or 7 follicles. The central follicle of a group is usually larger than the others. In many regions, no follicle groupings are detectable.

Ra+ neck: The follicles appear slightly smaller and rounder and closer packed together than normal. Larger follicles are rarely seen. Follicle groups are much less frequent than in normal skin.

RaRa neck: The follicles are fairly small yet diffusely arranged aggregates of Malpighian cells. No larger follicles are present. The follicle rows are less clear than normal; trio groups only are seen and they are very rare.

++ rump: The follicles are rounder or less oval in shape than in the neck region and there are fewer large follicles. The follicles are close-packed in rows, but there are no groups.

Ra+ rump: The follicles may be closer packed together than is
Whole mount of skin at ½ a day after birth - neck region. (x 98).

Fig. 82. ++; showing numerous follicle groups each containing from 3-7 follicles. The central follicle of a group is usually the largest.

Fig. 83. R+; showing some follicle groups, mainly of 3 follicles, but there are fewer follicle groups than normal.

Fig. 84. R+; showing evenly spaced, non-grouped follicles. The follicles appear diffuse due to their retarded growth and lack of external sheath development.

Note: that the R+ follicles are probably slightly smaller than normal, but their density appears greater.
Whole mounts of skin at 3/4 a day after birth - rump region (x 98).

**Fig. 85.** Follicle groups are much less clearly defined than in the neck region. Follicles are smaller and denser than in the neck region.

**Fig. 86.** Rat. Follicle groups are not clearly distinguishable. The follicles are obviously smaller and denser than in the neck region.

**Fig. 87.** Rana. No follicle groups are visible. The follicles are further developed and denser than in the neck region.
The follicles are the same as in the neck region except that a few larger, shafted follicles are seen.

At 14 days after birth:

**tt neck:** Even the smaller follicles are nearly all oval in optical cross-section. The larger follicles are fairly numerous. The follicles are arranged clearly in rows. Within the rows, groups of 3, 5 and 7 are still visible, but they are becoming obliterated by further follicle formation forming continuous rows. The central follicle of each group is usually the largest.

**Rat neck:** The small follicles are fairly round, they are less oval or elongated in cross-section than normal. Larger follicles are sparse. The follicles are arranged in rows, but only two groups are seen and they are less conspicuous than normal.

**Rat rump:** The small follicles are fairly round but diffuse due to imperfect differentiation of the external sheaths. There are no large follicles. The follicles are arranged in rows but not clearly; only trio groups are seen and they are not common.

**tt rump:** Many of the small follicles are oval, but they are smaller than in the neck region and closer packed together. The larger follicles are moderately sparse. The follicles are clearly arranged in rows but there are no groups.

**Rat rump:** The small follicles are rounder than normal. Larger follicles are fairly sparse. There are rows but not groups of follicles. The rump region is more similar to the neck region than in normal mice.

**RERa rump:** The small follicles are round and slightly diffuse.
A few are larger than in the neck region. There are fairly clear rows but no groups of follicles.

At 2½ days after birth:

++ neck: Most of the small follicles are oval in cross-section. The larger follicles are more numerous than at 1½ days' age. There are clear rows of follicles; the rows are usually continuous but some separate groups of 7 or 9 follicles are seen.

Rap neck: The small follicles are round-oval in cross-section. The follicles are clearly in rows, a few groups of 3, 5, or 7 follicles are seen but they are less frequent than normal.

Rap neck: The small follicles are round and less diffuse than at 1½ days. There are fairly clear rows of follicles and small groups are very occasionally observed.

++ rump: The small follicles are round-oval in cross-section. There are fewer large follicles than in the neck region. There are clear rows of close-packed follicles but no groups.

Rap rump: The small follicles are rounder and smaller than normal. There are fewer large follicles than in the neck region and fewer than in the normal rump region. The follicles are very closely packed in rows; there are no groups.

Rap rump: Most of the follicles are round, some are larger than in the neck region. There are rows but no groups of follicles.

At 3½ days after birth:

++ neck: More of the follicles are now of the larger shafted type. They are arranged in rows, but few groups are visible.

Rap neck: The small follicles are again rounder and less elongated in shape than normal. The follicles are arranged in rows but are not grouped.
Raga neck: The small follicles are round but are no longer diffuse since they show clear external sheath differentiation. The follicles are arranged in rows; some trio groups are still visible.

++ rump: The small follicles are round-oval in shape. The follicles are in rows but not in groups.

Ra+ rump: The small follicles look rounder and smaller than normal. The follicles are very closely packed in rows; there are no groups.

RaRa rump: The small follicles are no longer diffuse in outline. The follicles are arranged in rows; there are no groups.

At 45 days after birth:

++ neck: The small follicles are all oval in shape. The longer follicles are now more numerous; they are uniformly orientated. The rows of follicles are clear; a few large groupings of 7-11 follicles are occasionally observed.

Ra+ neck: The small follicles are round-oval in shape. Larger follicles are quite numerous; they are moderately uniformly orientated. The follicles are closely packed within rows; no groups are visible.

RaRa neck: There are still only small follicles; they are round-oval in shape and not diffuse. The follicles are in rows and there are some not very clearly demarcated trio groups.

++ rump: The small follicles are round-oval in shape and less elongated than in the neck region. The largest follicles are fairly uniformly orientated. All the follicles are fairly clearly arranged in rows; there are no groups.

Ra+ rump: The small follicles are rounder and smaller than
normal. They are closely packed together in rows, but there are no follicle groups.

**RаRa rump:** The small follicles are round. They are fairly closely packed together in clear rows; there are no groups. No larger follicles are usually visible.

Although further skin samples from $4\frac{1}{2}-9\frac{1}{2}$ day old mice were studied, the hair shafts were too dense for the follicle morphology to be clearly visible or for the hair shafts and follicles to be counted in normal and Rα+ mice. Two facts, however, could be clearly established; first, that the hair shafts are less dense in Rα+ than in normal mice, especially in the neck region; and secondly, that the orientation of the hair shafts and therefore probably of the follicles is much less uniform in Rα+ than in normal mice.

In $4\frac{1}{2}-8\frac{1}{2}$ day old Rαα mice, the follicles continue to be arranged in rows which, however, become increasingly indistinct. A few trio groups of follicles can still be observed up to $6\frac{1}{2}$ days, after all groupings have been obliterated in normal and Rα+ mice, at least in the neck region.

From the data presented above, a number of facts emerge:

1. **Follicle groupings occur in normal mice, generally between the times of birth and $3\frac{1}{2}$ days after birth in the neck region; these follicle groups are then obliterated by further follicle initiation.**

2. **Small, relatively little-developed follicles in any area of skin seem associated with a high follicle density, and absence of follicle groupings, viz:**
   a. **In the rump area of normal mice where the follicle density is highest, no follicle groupings were seen.**
Fig. 88. Diagrammatic representation of stages of follicle development, showing the formation of groups.
(b) In Ra+ mice where the follicle density was probably greater than normal through most of the ages examined, there was less clear grouping of follicles than in normal mice.

(3) The follicle groupings in RaRa mice were less clear than normal at all ages studied. Some follicle groups persisted considerably longer than normal, however, suggesting that the rate of follicle initiation was probably slower than normal.

(d) Discussion.

This brief study of the spatial arrangement of follicles in mouse embryos and early suckling mice has made it possible to formulate a simple scheme describing the normal appearance and final obliteration of follicle groups (Fig. 3).

Stage 1. The primary hair follicles have been initiated so that each individual follicle is evenly spaced from all its neighbours. This stage persists until about the 18th day of gestation in skin from the neck region.

Stage 2. The first-initiated follicles now become flanked by secondary follicles, one on either side of the primary follicles, so that trio groups are formed. These groups are orientated uniformly so that successive groups together form rows of follicles. This stage persists until birth.

Stage 3. More lateral follicles are initiated outside the secondary follicles so that groups of five, seven or nine follicles appear. The rows consequently become more clearly defined.

Stage 4. The initiation of more lateral follicles rapidly obliterates the follicle groups; the follicles are now arranged in clearly
defined rows, as in the adult mouse.

If this scheme is considered in conjunction with the results of Falconer, Fraser and King (1951) it seems that the follicles described above as 'primary' would be the guard hair follicles initiated in the embryo before 17½ days' gestation; the 'secondary' follicles forming the trio groups would be awl follicles initiated between 17½ days' gestation and birth; and the later initiated lateral follicles would be zigzag follicles.

The fact that follicle groups are here reported as appearing at much earlier ages than was described by Gibbs (1941) is probably because in the present work the follicle groups were studied mainly in the neck region which is an early follicle developing area.

It is clear from a study of the skin whole mounts that the follicles of less advanced development and smaller 'cross section' than normal, such as are seen in Rs+ skin from birth until 2-3 days after birth, are able to become very closely packed together and therefore attain a greater density than normal because of the smaller area of skin which each follicle occupies, i.e. the size of developing follicles in neonatal mice seems inversely proportional to their density. The possibility that the maximum follicle density attained at any specific time may depend upon the size of the individual follicles is discussed later.
(3) **Juvenile Follicle Density.**

(a) **Introduction.**

In order to understand fully the causes of the abnormalities in hair density which have been described in the adult ragged mice, it was necessary to make a comparison of the changes in hair follicle density during the development of the first pelage in normal and ragged mice.

The density of follicles at any specific time within a unit area of skin upon a growing animal may depend upon a number of factors:

1. **The rate of initiation of follicles up to the time of density measurement.**

2. **The amount of stretching of the skin produced by the growth of the animal after follicle initiation, and before density measurement.** (Stretching of the skin after follicle initiation will reduce follicle density).

3. **The rate of growth of the follicles up to the time of measurement may affect the follicle density, since a large follicle will take up a greater area of skin than a small one, and also may inhibit further follicle initiation in a proportionately greater area around itself.**

4. **The rate of progression of follicle initiation within a given period of time may affect the density of the follicles at the end of that time; e.g. the sudden initiation of the maximum possible number of follicle primordia at a certain time may result in a greater total density of follicles than if there had been a gradual or progressive initiation of follicles up to that time, since in the latter case the earlier initiated...**
follicles would have grown appreciably and may have exercised an inhibiting effect upon the initiation of later follicles (as in para. 3).

If a specific period of follicle initiation is to occur upon a growing animal and if the density of follicles so produced is to be measured at a later time "X" upon the same animal, then it is clear that the later the period of follicle initiation occurs, i.e. the nearer it occurs to the time "X", then the greater will be the follicle density measured. This is because there will have been less growth of the animal and consequently less stretching of the skin and lessening of follicle density between the times of follicle initiation and density measurement. This theory can be clarified by the following example.

The three main types of pelage hair in the mouse: the guard hairs, awls and auchenes, and zigzags, occur respectively in the following proportions in the adult coat; 2%, 26% and 72%.

It is generally agreed from the work of many authors, e.g. Dry (1926), Gibbs (1941) and Falconer, Fraser and King (1951), that the guard hairs arise from the hair follicles first initiated in the mouse embryo, the awls from later initiated follicles, and the zigzags from the last follicles to be initiated after birth. If a specific density of the guard hair follicles is initiated upon a relatively small 14-16 day mouse embryo, these follicles will be found to occur very sparsely in the adult mouse due to the considerable intervening growth of the animal and the growth or stretching of the skin between the follicles. A similar density of follicles initiated upon a large 17-19 day embryo (i.e. the awl follicles) will be denser in the adult, and a similar density of follicles initiated after
birth will occur even more densely in the adult, since there will be correspondingly less stretching of the skin between the times of follicle initiation and the measurement of follicle density. It is considered by the present writer that such a mechanism might partially account for the fact that the density of these different types of hair in the adult are vastly different, although the periods of time over which their respective types of follicles are initiated are not very different, e.g. Zigzag follicles are probably not initiated over a period longer than 6 days, and guard hair and awl follicles not for a lesser period than 3-4 days; and yet the zigzags are about thirty five times denser in the adult than the guard hairs. It is certain that the density of the guard hair follicles at the time of their initiation is not thirty five times less or even ten times less than the density of a zigzag follicles at the time of their initiation (cf. Tables 9 and 19).

The points outlined above assume the correctness of a concept that has been suggested by previous authors, e.g. Colin (1943) and Hardy (1949). This concept attempts to explain the fact that hair follicles in mammals are initiated only up to a certain maximum density and with an even spatial arrangement so that there is orderly spacing between individual follicles or groups of follicles. The concept assumes a process of follicle interference so that the initiation of any hair follicle at a specific point inhibits the initiation of any other follicles within a certain surrounding area. To the knowledge of the present author, no mechanism for such a process of follicle initiation has ever been demonstrated, but it
is suggested elsewhere in this thesis that the number of available Malpighian cells is a factor which could limit the density of initiation of follicle primordia and cause their arrangement to be orderly.

(b) Methods.

The technique of cutting tangential sections of skin was not used in this study since follicle counts on such material would probably not have included the small follicle primordia which are only slight thickenings of the epidermis. The following methods were used:

1. To estimate follicle density in oedematous and non-oedematous embryos of 14-16 days' gestation: transverse sections of these embryos were cut as described earlier. Hair follicles per transverse section were counted and averaged for each slide. These averages for slides from the same regions of oedematous and non-oedematous embryos were then compared (Table 18).

2. To estimate the density of hair follicles and hair shafts in ++, Ra+ and RaRa mice between the ages of 17½ days' gestation and 4½ days post-partum:

(a) whole mounts of skin from the neck and rump were made, as described in the section on adult morphology. The skin was shaved when necessary, removed, scraped free of sub-cutaneous muscle, fixed in Bouin's fluid, washed, stained in Delafield's haematoxylin and differentiated in acid alcohol. The densities of hair follicles and hair shafts were estimated under the microscope by the
use of an ocular graticule.

(b) Sagittal sections of skin were cut as described earlier. Comparisons of follicle density were made by estimating the average number of follicles per microscope field at a standard magnification (204 x). These comparisons of follicle density between ++, Ra+ and RaRa mice were used to check the differences found by the counts on the whole mounts of skin up to the age of 4½ days post-partum. After this age, the hair shafts become very closely packed together in the skin and it was not possible to estimate follicle density in ++ or Ra+ mice from the whole skin mounts. Therefore from 4½ until 21½ days after birth only the comparisons of follicle density made from sagittal sections were available.

For 17½ and 18½ day embryos, comparisons were always between sibs; post-partum ++ and Ra+ comparisons were between sibs but no sibs or sometimes Ra+ sibs only were available to compare with RaRa mice since viable RaRa mice were only obtainable from backcross matings: Ra+ x RaRa.

The quantitative data are presented below in tabular or graphical form; the morphological data which were obtained during this study are presented elsewhere in the appropriate section.

In all cases the incompletely formed follicles found in Ra+ and RaRa mice were scored as follicles since in the early stages they could not be separated from follicles which were destined to develop normally.

(c) Results.

For the sake of clarity some results are shown in the form
Table 18.

Embryo Follicle Density. The average number of follicles per section on each slide.

<table>
<thead>
<tr>
<th>Age - 14.5 days</th>
<th>Age - 15.5 days</th>
<th>Age - 16.5 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not oedematous</td>
<td>Oedematous</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>16.00</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td></td>
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<tr>
<td>11</td>
<td>10</td>
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<tr>
<td>12</td>
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<td>11</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.61</td>
<td></td>
</tr>
<tr>
<td>P &lt; 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each pair of observations represents the average number of follicles per section (of 10 μm) in equivalent regions of an oedematous and non-oedematous embryo. The observations are given in order from anterior to successively posterior regions of the embryos. In each case, the follicles are significantly more dense in the non-oedematous embryo (putative ++ or Ra+) than in the oedematous embryos (putative RaRa).
Table 19.

Follicle Density. Measured on whole skin mounts. Number of follicles/unit area of skin (0.432 sq. mm.)

<table>
<thead>
<tr>
<th>Age</th>
<th>Neck</th>
<th>Rump</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>++</td>
<td>Ra⁺</td>
</tr>
<tr>
<td>17½ day embryos</td>
<td>65 ± 1.8</td>
<td>48 ± 1.1</td>
</tr>
<tr>
<td>18½ day embryos</td>
<td>106 ± 2.7</td>
<td>102 ± 3.8</td>
</tr>
<tr>
<td>After birth:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>·½ day</td>
<td>129 ± 3.9</td>
<td>138 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>97 ± 4.1</td>
<td>111 ± 4.3</td>
</tr>
<tr>
<td>1½ days</td>
<td>93 ± 3.4</td>
<td>106 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>94 ± 2.4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>99 ± 4.3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>103 ± 6.1</td>
<td>139 ± 3.8</td>
</tr>
<tr>
<td>2½ days</td>
<td>90 ± 0.8</td>
<td>109 ± 2.1</td>
</tr>
<tr>
<td>3½ days</td>
<td>100 ± 3.6</td>
<td>113 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>111 ± 2.9</td>
</tr>
<tr>
<td>4½ days</td>
<td>98 ± 2.3</td>
<td>111 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>103 ± 3.8</td>
</tr>
<tr>
<td>5½ days</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6½ days</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

For each mouse, two figures are quoted – for the average follicle density on the neck and on the rump.
Table 20.

Hair shaft density per unit area of skin (0.432 sq. mm.)

<table>
<thead>
<tr>
<th>Age</th>
<th>Neck</th>
<th>Rat</th>
<th>Neck</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>After birth</td>
<td>++</td>
<td>Rat</td>
<td>++</td>
<td>Rat</td>
</tr>
<tr>
<td>1/2 day</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1 1/2 days</td>
<td>11 ± 2.6</td>
<td>2 ± 0.4</td>
<td>4 ± 1.6</td>
<td>2 ± 0.8</td>
</tr>
<tr>
<td>2 1/2 days</td>
<td>19 ± 1.3</td>
<td>12 ± 1.3</td>
<td>14 ± 0.5</td>
<td>6 ± 0.3</td>
</tr>
<tr>
<td>3 1/2 days</td>
<td>44 ± 1.9</td>
<td>14 ± 4.0</td>
<td>58 ± 2.8</td>
<td>9 ± 0.6</td>
</tr>
<tr>
<td>4 1/2 days</td>
<td>52 ± 3.0</td>
<td>7 ± 0.8</td>
<td>72 ± 6.2</td>
<td>5 ± 0.6</td>
</tr>
<tr>
<td>5 1/2 days</td>
<td>52 ± 1.5</td>
<td>17 ± 1.7</td>
<td>36 ± 3.4</td>
<td>23 ± 1.8</td>
</tr>
</tbody>
</table>

(The results are shown for 9 pairs of mice)
These graphs show follicle density trends measured from whole skin mounts. The results are from typical sample measurements (from Table 19) off 1 mouse of each genotype at each age.

 Follicle density measurements show the average number of follicles per unit area of skin (0.432 sq. mm.).
Fig. 91 and Fig. 92

These graphs show follicle density trends measured from sagittal sections of skin. Note that:

(i) It is possible that the follicle density in Ra+ and RaRa mice may be underestimated compared with the normal up to 9½ days after birth, because the ragged follicles are smaller than normal and each will be less likely to appear on any specific sagittal section. At later ages the density of ragged follicles may be slightly overestimated since their abnormal curvature will make them more likely to be cut in section.

(ii) The follicle density counts include the "retarded follicles" in ragged mice, some of which remain non-functional.

(iii) The counts of RaRa follicles include many small primordia at most ages—these may not be visible in skin whole mounts and so the density estimates shown here for RaRa mice are relatively higher than those obtained from whole skin mounts.

\[
\pm 2 \text{ Standard Errors, one standard error on each side of the mean.}
\]

Follicle density measurements show the average number of follicles per 4 microscope fields (x 204 magnification).
FOLLICLE DENSITY — NECK REGION

FOLLICLE DENSITY — RUMP REGION

FOLLCILE DENSITY — NECK REGION

AGE IN DAYS

FOLLCILE DENSITY — RUMP REGION

AGE IN DAYS
of graphs. The graphs showing the follicle density data from whole mounts of skin give the measurements from only one mouse of each genotype at each age studied, but the trends shown are typical. It is not practicable to show the full data graphically, but they are presented in a table (Table 19). All the follicle density measurements made from the sagittal skin sections are shown graphically (Figs. 27 to 92).

The most important results shown in the data from the skin whole mounts and the skin sections are:

1. In all three classes of mice (++, Ra+, RaRa), follicle density is relatively low before birth; it rises to a maximum some time after birth and before 4½ days, and thereafter declines steadily.

2. In all three classes of mice the follicle density is consistently higher in the rump than in the neck region after birth.

3. In RaRa mice the follicle density is consistently less than in normal or in Ra+ mice in both the neck and rump regions, at most of the ages studied.

4. In both regions of Ra+ mice the follicle density is generally below normal before birth, slightly above normal from birth to 3½ days, and thereafter little different from normal.

The stage of advancement of follicle development can be precisely expressed as the number of follicles producing fully formed hair shafts within a specific area of skin. Results of such an analysis are given in Table 20. The variation between mice of the same age and genotype is considerable, but certain general conclusions can be drawn:

1. In ++ and Ra+ mice follicle development is slower in the rump
of follicle initiation appears to be relatively high.

Low follicle density in RaRa mice is probably due to slow initiation and slow growth of follicle primordia after initiation. (Small follicle primordia may not always be clearly visible and may not be scored in follicle density counts).

In Ra+ mice, the abnormally low follicle density before birth must be due to a delay in the time of initiation of some follicles and possibly also to the previously observed slow growth of follicles which would raise the proportion of unscored primordia. Both these factors could conceivably produce the abnormally high follicle density which seems to arise after birth. A delay in the time of follicle initiation would result in less growth and stretching of the skin between follicle initiation and density measurement, and the smaller follicles produced by a later initiation might exercise less of an inhibiting effect upon the initiation of neighbours. A higher final density of follicles would therefore result. The slower growth of Ra+ follicles would similarly allow a higher density of follicles to arise, if it be accepted that a small and slender hair follicle, by occupying a smaller physical area of skin, and by incorporating fewer basal Malpighian cells, would less successfully inhibit the initiation of neighbours than would a large follicle. That the follicle densities of normal and Ra+ mice become similar again about 4½ days after birth, probably indicates that the normal mouse, having a lower density of follicles from 0-3½ days, has then been able to initiate new follicles at a greater rate than the Ra+ mouse whose denser follicle arrangement will exercise a greater inhibiting effect.

Finally, it is suggested that the higher post-birth follicle
density on the rump than in the neck region of all the three classes of mice could be similarly due to later initiation and/or slower growth of some of the follicles in the rump region compared to those in the neck region. That both later initiation (exemplified by the continued appearance of follicle primordia in older mice) and slower growth do occur is indicated by qualitative histological observations on sagittal skin sections (section (2)(ii)). It has been described above how slower follicle development and later follicle initiation could both produce a higher follicle density.

It is suggested from this discussion that:

(i) The abnormally low pre-natal follicle density in Ra+ mice is caused by delayed initiation of some follicles.

(ii) The abnormally high post-birth follicle density in Ra+ mice is caused by: (a) later initiation of those follicles normally formed between $17\frac{1}{2}$ and $18\frac{1}{2}$ days' gestation (this is demonstrated by the sub-normal follicle density in Ra+ mice before birth); and (b) slower growth of follicles. (It is clear from the studies of skin whole mounts and sagittal sections that many of the follicles in Ra+ mice are smaller and slimmer than corresponding normal follicles).

(iii) The higher post-birth follicle density in the rump than in the neck region of all three classes of mice is caused by later initiation and/or slower growth of some of the hair follicles in the rump area. It is found in normal mice that when the follicle densities of the neck and rump areas are least different, i.e. before birth, then there is least difference in the stage of follicle development between the two regions. After birth, when the rump follicle density is
is clearly higher than that in the neck region, the follicle development is clearly less advanced in the areas of the rump than of the neck. This is suggestive of a causal relationship such that slower follicle growth causes a higher follicle density. The converse relationship is not permissible, since before birth the rump follicle density is always lower than the neck follicle density; slower follicle development is apparent in the rump region before the higher follicle density in that region.

Both the causal explanations given for the last two observed phenomena which were (i) abnormally high post-birth follicle density in Ra+ mice, (ii) higher post-birth follicle density on the rump than on the neck in ++, Ra+ and RaRa mice, make use of a new concept. This concept can be stated as follows: At any specific time, the hair follicle density in any area of skin tends to attain its maximum possible level, in the absence of any gross abnormality preventing follicle initiation; this level will depend upon the size of the individual follicles, such that larger hair follicles by occupying a greater area of skin will inhibit the initiation of neighbouring follicles over a wider area than will smaller follicles. Diminished follicle size therefore permits increased follicle density.

Probably, after the earlier stages of follicle initiation in the 14-18 day embryo the maximum follicle density which is physically possible is usually maintained until the epidermis begins to lose competence to initiate new follicles, as in the 5-8 day old mouse. Although at any one time the maximum follicle density will depend on the size of the follicles, it will vary from time to time if the growth and stretching of the skin occurs periodically and
not continuously; if this is the case, a time lag would be expected after the follicle density has been diminished by a period of skin stretching before the initiation of new follicles again produces the maximum follicle density. Possible implications of this process are discussed below in paragraph (v).

(iv) The low follicle density in RaRa mice is caused by slower initiation of the hair follicles. The proliferative activity of the epidermal cells which is required to produce follicle initiation seems grossly below normal so that follicle initiation is slowed too much to produce a following abnormal rise in follicle density. The maximum possible follicle density is therefore not attained.

(v) The apparent waves of increase and decrease in follicle density (Table 19, Figs. 39-42) could be caused by the interaction of two main processes:— (a) Follicle initiation—tending to increase follicle density; (b) Lateral expansion of the skin by growth or stretching—tending to diminish follicle density and reduce follicle interference. The stretching of the skin probably does not occur at a uniform rate, but periodically. A phase of skin stretching will reduce follicle interference and so permit a further wave of follicle initiation. Schinkel (1953) has observed that in the sheep, the rate of development of secondary follicles in early post-natal life is closely associated with the growth rate of the animal and particularly with the rate of expansion of the skin. In the 0-10 day old mouse, Gibbs (1941) has described alternate periods of epidermal thickening, and diminishing thickness due to stretching and sideways migration of epidermal cells. Periods of stretching of the epidermis were closely followed by waves of follicle initiation. Such periodic waves of follicle initiation
in the mouse may allow a discrete separation of follicles into groups, each group containing follicles of a characteristic size, since it is probable that at the completion of follicle growth early-initiated follicles are larger or stouter than late-initiated follicles.

It is now argued that the type of hair fibre produced by any individual follicle may depend upon the size of that follicle at the commencement of hair proliferation, such that large hair fibres are produced by large follicles and finer fibres by smaller follicles. So long as the rates of growth of all hair follicles are fairly similar, the relative sizes of any hair follicles will depend upon their times of initiation; early-initiated follicles will be stouter (although probably not longer) than late-initiated follicles. If it be accepted that the relatively long and coarse guard hair and awl fibres are produced by early-initiated follicles, and the finer zigzag fibres by late-initiated follicles, then the above scheme provides an explanation for the separation of the mouse hair fibre population into discrete fibre types with few transitional hair fibres.

Thus, a discrete heterogeneity of the hair follicle population can, once established, account for the observed heterogeneity of the adult hair fibre population in normal mice.
PART III - INTERACTION OF THE GENES RA-RAGGED AND N-NAKED

(a) Introduction.

The gene N-naked causes cyclic periods of hair loss and regeneration in the mouse. The histological and morphological abnormalities caused by this gene have been described by a number of authors, notably; David (1932a) and Steinberg and Fraser (1946).

The hairs of heterozygous naked mice are imperfectly keratinised, so that they break off close to the skin at or near the completion of their growth. The baby coats of young N+ mice are less sleek and rougher than those of normal sibs. The hair begins to break off under the lower jaw and behind the eyes at 10-14 days after birth. The area of epilation spreads caudally in the form of a wave leaving only a stubble. Epilation occurs later in posterior areas because hair growth is completed later in these regions.

Hairs on the nose, feet, tail and in the genital area appear to be normal and do not break off. The breaking off of the hairs in other regions is complete in about a week. From 18-30 days after birth the animals are almost naked except in the areas indicated above.

Successive periods of regeneration and subsequent breaking off of hair occur according to the normal cycles of hair growth in the mouse. Hair growth always occurs in a clearly demarcated region on the body area of the mouse and hair loss likewise produces clearly demarcated zones of baldness. Hair regeneration as well as hair loss commences in anterior regions of the animal (usually behind the eyes and under the jaw) and spreads posteriorly.

After the first two or three hair cycles, periods of hair
regeneration and hair loss overlap, so that new hair growth may be occurring anteriorly whilst epilation is still occurring posteriorly on the same animal.

It seems probable that individual hairs in heterozygous naked mice each break off at similar stages of growth; the fact that the zones of hair regeneration and epilation are clearly demarcated and show respectively a dense covering of hair or almost complete baldness, indicates that neighbouring hair follicles within a specific zone are in the same phase of growth. This conclusion is in agreement with that reached by the studies on sagittal sections of skin from normal juvenile and adult mice during the present work. The examination of comparable Ra+ skin sections, however, indicated that neighbouring hair follicles were out of phase with each other in their stages of growth. Follicles in the anagen or active growth phase were often flanked by follicles in resting phase.

In order to confirm or deny this interpretation, it was decided to study the interaction of the genes Ra and N. On the assumption that in Ra+ mice the neighbouring hair follicles were out of phase in their growth cycles, it was predicted that in N+Ra+ mice there would be no clearly demarcated areas of complete baldness during epilation; it was expected instead that some hair shafts would have broken off whilst their neighbours were continuing normal growth, so that a wide area of uniformly partial baldness would result from the commencement of epilation. Similarly, instead of the gradually spreading regions of clearly defined dense hair growth which appear during regeneration in N+ ++Ra mice, it was expected that new hair growth would appear diffusely over a wide area of the body with a density less than that in either ++N Ra+ mice or N+ ++Ra...
mice.

(b) Methods.

The external pelage development of litter mates from N+ ++Ra x ++N ++Ra matings was studied daily from birth until 22 days after birth when the growth of the first coat was complete. This was done so that the abnormalities due to the naked gene alone could be studied, and to enable N+ ++Ra mice to be identified from ++N ++Ra mice at as early an age as possible.

Further observations were then made upon litter mates from N+, Ra+ x ++N, ++Ra− matings or N+, ++Ra x ++N, Ra+−matings. These mice were observed daily or every other day from birth until up to 3 months after birth. Mice of genotypes N+ ++Ra, N+ Ra+, ++N Ra− were compared with normal sibs, although the four genotypes were rarely obtained in one litter.

Observations were made by eye, or by a low-power binocular microscope.

(c) Results. External Comparative Morphology.

(1) ++N ++Ra and N+ ++Ra mice.

1½-1¼ days after birth:

At birth and during the first day after birth, no differences between N+ and normal mice are seen.

1½-2½ days:

N+ mice can be identified from normal with almost complete certainty at 2½ days after birth. The dorsal pelage hairs of N+ mice seem sparser and possibly shorter than normal and they are less uniformly orientated.
4½-5½ days:

The dorsal pelage hairs of N+ mice are still sparser than normal and they are often twisted or bent. The hairs do not point uniformly upwards from the dorsum as they do in normal mice. The ventral down is shorter and sparser than normal in N+ mice.

6½ days:

The dorsal down of N+ mice is sparser, more curly and may be shorter than normal. The ventral down is sparser than normal.

9½-11½ days:

The coats of N+ mice are shorter and more stubbly than normal on the dorsum and venter.

12½ days:

Bald patches begin to appear behind the eyes of N+ mice due to the breaking off of hair.

15½ days:

The bald patches are larger and in most cases have spread to the dorsal head area. The rest of the coat in N+ mice is rougher, duller and less sleek than normal and is stubbly just posterior to the bald areas.

16½ days:

The head, neck and anterior regions of the dorsum are now at least partially bald.

19½ days:

In N+ mice, the entire venter and the anterior two-thirds of the dorsum (except for the midline) are now almost naked. A few sparse, long hairs remain in these areas and a faint stubble which is hardly visible above the surface of the skin.

22½ days:

There is little change from the 19½-day stage; the few
remaining hairs are generally more numerous in the region of the rump.

(iii) ++N++Ra, ++N Ra+, N+ ++Ra, and N+ Ra+ mice (from birth to 3 weeks).

2½-4½ days after birth:

The dorsal pelage hairs of both N+ ++Ra and N+ Ra+ mice are sparser and possibly shorter than those of ++N Ra+ and ++N++Ra mice at this age. N+ ++Ra and N+ Ra+ mice cannot however be distinguished from each other.

6½-8½ days:

The dorsal down of N+ Ra+ mice is clearly shorter than normal and may be slightly shorter than that of N+ ++Ra mice in some cases.

9½ days:

N+ Ra+ and N+ ++Ra mice have a more irregular and curly dorsal fur than ++Ra+ ++N ++Ra mice; but N+ Ra+ and N+ ++Ra mice still cannot be separated with certainty.

10½-12½ days:

The coats of N+ ++Ra mice appear stubbly in anterior areas and complete epilation has commenced in the region posterior to the eyes. In N+ Ra+ mice the coat is more widely stubbled and it is sparser and shorter; definite epilation has not yet commenced. N+ ++Ra and N+ Ra+ mice can now be definitely distinguished from each other.

12½-14½ days:

Clear epilation has now commenced posterior to the eyes and on the heads of N+ Ra+ mice, i.e. 1-3 days later than in N+ ++Ra mice. The bald patches appearing behind the eyes produce a goggle-
like appearance. The fur is rough, ill-aligned and fairly thin behind the ears and on the shoulders. The region of stubbled hair is larger than in N⁺ ++Ra mice; probably this is because the area of epilation is less clearly demarcated.

15½-16½ days:

In N⁺ Ra+ mice the zone of baldness has spread from behind the ears to the shoulders. Posterior to the scapulae the coat is becoming less dense. The nose and the dorsal midline are still fairly well haired. The ventral coat is rough and sparser than in ++N Ra+ mice, showing that hair loss has begun but is spreading diffusely over the area.

17½-18½ days:

The dorsum is now fairly bald anterior to the waist in N⁺ Ra+ mice; posterior to the waist the coat is becoming sparse. In N⁺ ++Ra mice the baldness is more complete in the anterior epilated zones, but the coat is generally dense in non-epilated areas.

19½-21½ days:

In N⁺ ++Ra mice the head and anterior part of the dorsum are bald. The rest of the dorsum is stubbled except for the rump where the coat is almost normal in density and texture. In N⁺ Ra+ mice the head and anterior dorsal areas still have sparse hair but the posterior dorsal area is becoming sparse and appears ragged; i.e. the region of epilation is more widespread but less complete within any specific area than in N⁺ ++Ra mice.

21½-23½ days:

Both N⁺ ++Ra and N⁺ Ra+ mice are now quasi-naked. A few long sparse hairs remain on the venter and rump, and on the rest of the dorsum especially in N⁺ Ra+ mice.
These mice are sibs at an age of 40 days, showing the commencing loss of the second hair coat in the $N+$ mice. Note the even dense coat of the $++N$ $++Ra$ mouse, the sparser coat of the $++N$ $Ra+$ mouse, the clearly demarcated zones of baldness and hairiness in the $N+$ $++Ra$ mouse, and the widespread and diffuse thinning of the coat in the $N+$ $Ra+$ mouse.
(iii) N+ ++Ra and N+ Ra+ mice. (After 3 weeks' age).

Further detailed comparisons were made between N+ ++Ra and N+ Ra+ mice until three months after birth. The first three cycles of hair growth and epilation were thus compared. This process will not be described in detail, but a few of the more important features can be mentioned.

At the age of about 28 days the growth of the second coat begins on the snout, anterior flanks and behind the eyes of N+ ++Ra mice. New hair growth does not begin until 30-34 days in N+ Ra+ mice. The new growth of ventral and dorsal hair in these mice is less dense than in either N+ ++Ra mice or ++N Ra+ mice and its growth commences over a wider area than in N+ ++Ra mice. By 38-42 days, clearly marked areas of baldness on the venter and anterior dorsum appear in N+ ++Ra mice. In N+ Ra+ mice no clearly demarcated zones of baldness appear, but the hair is lost by a diffuse increase in sparseness. (Fig. 96).

Similar differences between N+ ++Ra and N+ Ra+ mice occur in the third hair cycle but later cycles are less clearly defined even in N+ ++Ra mice and it is possible that neighbouring hair follicles are less closely in phase in old mice.

(iv) Summary.

Those observations which are most important for the purpose of clarifying the mode of action of the ragged gene can be summarised as follows:

(1) Cycles of hair growth do occur in Ra+ mice.
(2) The coat is 1-2 days later in commencing to break off or grow in N+ Ra+ mice than in N+ ++Ra mice.
(3) Normally in naked mice, the presence of the Ra gene has less
effect on the first coat than on the second and third coats. Even for the first coat, however, the breaking off process is more generally spread over the body area in N+ Ra+ mice, and does not spread posteriorly by the expansion of a clearly delimited zone as in N+ ++Ra mice.

(4) When the coat is regenerating, N+ Ra+ mice have a less clear demarcation of the zone of regeneration than have N+ ++Ra mice, and the density of the new growing hairs in N+ Ra+ mice is less than in either N+ ++Ra or ++N Ra+ mice.

(5) When epilation is occurring, demarcation of the affected zone is always less clear in N+ Ra+ mice than in N+ ++Ra mice; instead of an all or none delimitation of bald and haired zones the coat becomes generally sparser over a wide area in N+ Ra+ mice.

(6) Cases occur of new hair growing up between the sparse remaining hairs of the previous generation more frequently and more conspicuously in N+ Ra+ mice than in N+ ++Ra mice.

(7) The density of the coat in N+ Ra+ mice always appears less than the coats of N+ ++Ra or ++N Ra+ mice.

(8) Three mice which had been classified as N+ ++Ra, and five which had been classified as N+ Ra+ were tested genetically; the classifications were found to be correct.

An incidental observation was made upon a single N+ Ra+ female mouse. This mouse became pregnant and the process of hair regeneration was completely halted for a period of 20 days commencing 6 days before the birth of the litter. Hair regeneration is usually a continuous process and was in fact proceeding throughout this period in the non-pregnant sibs of the mouse.
(d) **Discussion.**

A number of conclusions can be drawn from the results of the interaction of the ragged and naked genes:

(i) Cycles of hair growth do occur in Ra+ mice, and as in ++N +Ra mice, the cycles appear to be always more advanced in the anterior regions of the animals. Neighbouring individual hair follicles cannot therefore be entirely out of phase with each other.

(ii) The hair cycles in Ra+ mice seem to be 1-2 days later than normal in entering any specific phase.

(iii) The evidence that large zones of partial baldness or sparse new hair growth occur in Ra+ mice instead of the restricted and clearly delimited zones of epilation or regeneration in ++Ra mice is in agreement with the predictions made before this experiment was carried out.

It was suggested in the introduction to this section that such evidence would prove that neighbouring hair follicles were out of phase in Ra+ mice. That they are not entirely out of phase, however, has been shown by the fact that cycles of hair growth do occur in Ra+ mice. It must be concluded therefore that individual neighbouring hair follicles in Ra+ mice are less closely in phase with each other than in normal mice; and that the hair cycles in Ra+ mice seem later in commencing or completing any given stage than in normal mice.

Evidence is presented elsewhere suggesting that these two facets of abnormality are not unrelated.
PART IV - BODY WEIGHT AND SIZE

(a) Introduction.

It might be expected that ragged mice would be smaller than their normal sibs at any specific age. Any mutant showing hypotrichosis may require an abnormally high metabolic rate to maintain the normal body temperature, and therefore might be unable to attain the normal body size.

(b) Methods.

The body weights of Ra+ mice and their normal sibs were compared. The mice (males and females) were weighed daily (correct to 0.1 gm) from the age of 12 days to 35 days after birth. A total of 16 ++ mice and 16 Ra+ mice from 6 different litters were weighed. The litters were from backcross matings: Ra+ x ++.

The body lengths of RaRa mice and their Ra+ sibs, and other normal mice of similar age and sex were compared by placing the mice upon an ordinary 12-inch ruler and measuring them from nose-tip to tail root. About 6 mice of each genotype were measured at weaning and about 10 mice of each genotype at maturity. It was not considered necessary to weigh Ra Ra mice since they seemed grossly smaller in size than normal or Ra+ mice of similar age.

(c) Results.

(i) No difference in body weight was found between ++ and Ra+ littermates between the ages of 12 and 35 days after birth. It can be assumed that there is no difference in the weights of these mice between birth and 12 days after birth.

(ii) RaRa mice were found to be about 30% shorter than ++ or Ra+
mice at weaning and 5-10% shorter at maturity. Cursory observations suggest that there is little difference in size between +++, Ra+ and RaRa mice in the first few days after birth; RaRa mice begin to look smaller about 5 days after birth.
found on the ramp, posteriorly, under the chin and on

head-neck junction of the nuchal crest above the occiput.

Barotrauma in the norma column of the head have on the dorsum

neck and base region of the head.

a lesser than normal tension of the anterior and sternal surfaces in the

the neck and base region of the head.

a discrepancy greater than normal tension of the cervical plates in the

base.

The region in the neck region and posteriorly.

a discrepancy greater than normal density of the head base and

interscapular density of the neck.

uncovered in the neck region but in an anterior-posterior gradient or

an abnormality of density of the neck region in the region.

The abnormality observed in the neck region.

use of the few non-penetrating postures as possible.

use the area selected below as the reason for the presented.

Medicines are also selected in the head and juvenile cases.

The heart and heart positions of the head neck.

are also discussed.

Any implications relevant to General theories on heart growth

normal juvenile mode and embryos

are interpreted in terms of the discrepancy between head and

in adult mode so that abnormalities in adult mode

designed to suggest a causal system existing at the abnormalities

ed in death in the appropriate section. This relevant discussion

Many of the results of this work have already been discussed.
Structural abnormalities of some hairs in Ra+ and RaRa mice. (Transitional hairs, morphologically intermediate between the usual hair fibre types are abnormally frequent).

No reduction in hair follicle density in Ra+ mice, despite the abnormally low hair density due to the lack of many zigzag fibres. (There are many follicles which do not produce hairs in Ra+ mice, and a smaller number in RaRa mice).

Numerous morphological abnormalities of the skin and hair follicles in Ra+ and RaRa mice. (The causes of these abnormalities have already been discussed in the concluding part of the section on Adult Morphology).

In juvenile ragged mice the most important abnormalities are:

(i) Abnormally short whiskers and other sinus hairs in Ra+ and RaRa late embryos and neo-natal mice.

(ii) Abnormally late emergence of dorsal hairs in neo-natal Ra+ mice; and consequently delayed dorsal pigmentation in Ra+ mice with pigmented hair.

(iii) The non-appearance of hairs in RaRa mice; and consequently no pigmentation of RaRa mice except of the ears and genital region.

(iv) Abnormally small pelage follicles in RaRa embryos.

(v) Abnormally delayed differentiation of the skin layers in RaRa mice.

(vi) Abnormal follicle density which is:

(a) Consistently lower than normal in RaRa mice.

(b) Abnormally low before birth in Ra+ mice.
(a) Slightly higher than normal from birth to 3½ days in Ra+ mice.

(d) Normal or higher than normal after 3½ days in Ra+ mice.

(vii) Abnormally retarded growth and development of follicles, especially the later initiated follicles, in Ra+ and RaR Ra mice.

(viii) Abnormally out-of-phase development of follicles in young Ra+ mice, due to excessive retardation of the later initiated follicles.

(ix) Abnormal morphology and orientation of follicles in Ra+ and RaRa mice, due probably to the subnormal increase in skin thickness during the anagen phase, and to the out-of-phase development of follicles. This has been discussed previously.

The abnormal follicle density in ragged mice has already been fully discussed and it was suggested that the cause was later-than-normal initiation and slower growth of some ragged follicles; it was shown that these two abnormalities could cause the low prenatal follicle density in ragged embryos, and the higher than normal post-birth follicle density in Ra+ mice.

It can therefore be seen that each of the first seven abnormalities enumerated above as found in juvenile ragged mice demonstrate a single important fact, namely that there is a general delay in follicle development and hair growth in ragged mice. From this principal fact it is proposed to interpret the entire range of hair abnormalities found in adult ragged mice.

The abnormal retardation of follicle growth in Ra+ mice, coupled with the fact that follicle initiation and follicle growth
cease almost at the normal time, results in the incomplete development of many of the later initiated follicles in Ra+ mice. These follicles, which normally produce zigzag hairs, cannot do so, and therefore there is a reduced density of zigzag fibres in the adult. The presence of the non-functional follicles, however, ensures that there is no reduction of follicle density in the adult Ra+ mice; the follicle density may in fact be higher than normal. The absence of many zigzag fibres, which in agouti mice are invariably yellow-banded, causes a darkening of the dorsal fur colouration in adult agouti Ra+ mice.

If it is assumed that some of the earlier initiated zigzag and awl follicles which are only slightly retarded in development, produce hair fibres in Ra+ mice, then it can be inferred that such follicles would be likely to produce fibres of less than the normal thickness or length; hence the abnormally low average length of zigzag and awl fibres in Ra+ mice. The greater than normal average length of the guard hairs in Ra+ mice is discussed later; it may be due to a reduced competition between follicles due to the presence of many non-functional zigzag follicles in Ra+ mice. The higher than normal density of guard hairs and awls in adult Ra+ mice is obviously a result of the abnormally high follicle density at and after birth and it has been shown that this in turn is possibly due to a slow commencing rate of follicle initiation and slower than normal follicle growth in Ra+ mice. The situation is, however, complicated by the fact that Falconer, Fraser and King (1951) have postulated that the hair follicles initiated in the 14-17 day old mouse embryo produce guard hairs, the follicles initiated from 17 days until birth produce awl fibres, and those initiated
after birth produce zigzag fibres. Since in the 17-day Ra+ embryo the follicle density is apparently sub-normal, and the density probably does not become normal until about the 19th day of gestation, and yet since the density of guard hairs and awls in adult Ra+ mice is greater than normal it must be concluded that some of the follicles initiated after 17 days of gestation produce guard hairs, and possibly some of those initiated after birth produce awl fibres in Ra+ mice. It therefore seems possible that, first, the time of initiation of a hair follicle does not finally control the type of fibre it will produce (since follicles initiated at different times in ++ and Ra+ mice may produce the same types of fibres); and secondly, that guard hair fibres can be produced in Ra+ mice by follicles initiated at a later time than guard hair follicles are initiated in normal mice.

A possible explanation is that the type of fibre produced by any hair follicle depends upon the size which the follicle has attained by the time hair proliferation commences. In fact, it has been observed in many different mammals that the larger types of hair fibres are probably produced by the larger follicles. Now the effective size attained by a hair follicle will depend upon its time of initiation, its speed of growth and the time of commencing hair proliferation.

Between ++ and Ra+ mice the relative effective sizes of hair follicles may be altered by all three factors. Therefore, on the above theory, if some of the guard hair follicles in Ra+ mice are initiated later than any of the guard hair follicles in normal mice, then either hair proliferation commences later than normal in those Ra+ follicles, or else they develop at a faster rate than
normal. There is some evidence for the first possibility in the later than normal emergence of the first dorsal hairs in Ra+ neonatal mice; the second possibility seems the least likely since generally the growth rate of most follicles is slower than normal in Ra+ mice.

It is assumed in the preceding discussion that certain discrete periods of follicle initiation are each associated with the production of a certain type of fibre in the normal mouse, and it is also clear that this process has been disorganised in Ra+ mice. Since the sequence of follicle initiation and growth, and the production of a heterogeneous fibre population are obviously complicated developmental processes, it is not surprising that their disruption should cause certain abnormalities. It is suggested that these abnormalities are, in adult Ra+ mice, the production of hair structure defects, and numerous transitional hair types, e.g. Guard hair/awls and Aw3/zigzags. For instance in Ra+ mice, awl follicles which have been initiated later than normal may change over from awl to zigzag production near the end of the hair proliferation period. (The "zigzag portion" of these transitional hairs is invariably at the proximal end of the hairs).

A further important feature is the fact that the density of zigzag hairs in adult Ra+ mice varies widely between different body areas in the same mouse, although the density of zigzags in the same body areas of different mice is fairly constant. The zigzag density in any Ra+ mouse increases dorsally along an anterior-posterior gradient, from about 0.5 fibres per unit area in the neck region to 30.2 fibres per unit area in the rump area; there is probably a similar anterior-posterior gradient along the venter of
all Ra+ mice. There is a high density of zigzag fibres in a few other regions of Ra+ mice, notably: in a small area closely posterior and mesial to the ears, and in the ventral tail root and genital region.

It may be significant that the regions of high zigzag density in Ra+ mice are the regions which, according to Dry (1926), Wolbach (1951) and other authors, cease hair growth later than all other areas during the growth of the first pelage in normal mice, e.g. The head region where there is 0-1% of zigzags in Ra+ mice, normally ceases hair growth at 17 days after birth, but on the rump where the zigzags comprise 50-60% of the total fibres in Ra+ mice, hair growth normally ceases at 19 days after birth. Similarly, the cessation of hair growth is very late in the regions posterior and mesial to the ear.

Histological data from the present study suggest that follicle initiation may be slightly slower in the rump region than in the neck region in normal and possibly in Ra+ mice before birth, and the whole process of follicle growth from just before birth until its completion is slower in the rump region than in the neck region in normal mice, and to a lesser degree in Ra+ mice. Furthermore it is confirmed that hair growth is completed later in the rump than in the neck region of normal mice; the same tendency is shown less clearly in Ra+ mice. It seems therefore that follicle development and hair growth, in normal and Ra+ mice, commence at similar times in the neck and rump regions, but proceed slower and takes place over a longer period of time in the rump region.

It has been seen that the main effect of the ragged gene in Ra+ mice is to slow down the development of the later initiated
follicles so that many of them have not completed their development by the time follicle growth ceases soon after the normal time. It is possible that the mechanism by which the ragged gene achieves this result is physiologically less effective against the rump follicles, because they have a longer period in which to complete their development than the follicles on the neck and in other anterior areas. The longer growth period of follicles developing in posterior or other late hair-growing areas of the mice could in this way act as a buffer against the effect of the ragged gene, which is to prevent the complete development of some follicles by retarding their growth. Therefore, a greater proportion of abnormal, non-functional follicles would be expected in the neck region than on the rump and in other posterior or late hair-growing areas.

Alternatively, it is conceivable that the antero-posterior gradient of abnormality in Ra+ mice could be interpreted in another way, if it were assumed that superficial oedema was the direct cause of follicle growth retardation. Oedema was observed in a few Ra+ embryos, and wherever observed in Ra+ or RaRa embryos it was seen to be more marked in anterior than in posterior regions. Thus, it is possible that low-grade oedema, in most cases below the threshold of recognition, could cause an antero-posterior gradient of decreasing follicle retardation in Ra+ mice. There are three main arguments against this second hypothesis:

(i) Oedema has been seen in few Ra+ embryos, and yet the coat abnormalities are similar in all Ra+ adults.

(ii) Oedema has not been seen (by macroscopic or microscopic examination) in any post-natal Ra+ mice, although it is after birth that the retardation of follicle growth is
most obvious.

(iii) Oedema, whether studied in Ra+ or RaRa embryos, never appears prominently on the anterior part of the head, yet in this region the density of zigzag hairs in the Ra+ adult is at a very low level.

Whatever the cause, the retardation of follicle growth produced by the ragged gene decreases along an antero-posterior gradient. Since in normal mice there is an antero-posterior time gradient such that follicle and hair growth in anterior regions precede that in posterior regions, an additional effect of the ragged gene is to make this time gradient less distinct in Ra+ mice by retarding the progress of developmentally advanced regions more than that of the later developing areas.

So far, the discussion has mainly been concerned with the abnormalities of Ra+ mice, and these have been interpreted in terms of a general retardation of follicle and hair development. In RaRa mice, it has been shown that there is evidence for a much greater degree of retardation of follicle and hair development, than in Ra+ mice. This has resulted in correspondingly greater abnormalities of a qualitatively similar nature in the adult RaRa mice. Follicle growth is so much retarded that only a few follicles are able to complete their development and produce hairs. As in Ra+ mice, the abnormalities are least and therefore the hair growth most apparent in the late hair-growing areas of the posterior rump and venter. Even in these areas, however, the hair is extremely sparse and late in appearing. No hairs are found posterior to the ears, since the hairs in this area are predominantly zigzags in normal mice and the late-growing zigzags should be the hair type
most completely removed in RaRa mice.

It has now been established that it is possible to interpret all the coat abnormalities of adult Ra+ and RaRa mice in terms of the retardation of follicle growth. The next step is to discuss the cause of follicle growth retardation. Such a cause could be of two main kinds; first, mechanical interference with follicle growth and second, a physiological process which diminished the mitosis rate of the hair follicle cells. The first possible cause can be dismissed for two reasons: (i) Interference with follicle development in RaRa mice is such that a few follicles do complete their development. If the interference was of a mechanical nature, it would be expected that completely developed follicles would occur in isolated groups at points of local failure of the mechanical interference. In fact, the functional follicles occur singly and are fairly evenly spaced from each other. (ii) There is no observational evidence from the histological studies of any mechanical prevention of follicle development.

It seems probable, therefore, that the slow growth rate of hair follicles in ragged mice is due to an abnormally slow rate of division amongst the cells whose proliferation normally produces follicle down-growth. These cells are the follicle external sheath cells, which are derived from the Malpighian layer of the epidermis.
2. The Cause and Effects of Oedema

(a) The effects of oedema

(i) Superficial generalised oedema is a characteristic possibly of all RaRa embryos from 13.5 days gestation until birth, and probably of a few Ra+ embryos between the same ages. Certain abnormalities in post-15.5 day RaRa embryos, such as slow initial development of follicles and slow differentiation of the epidermis and other skin layers, are always more extreme in the most oedematous regions of those embryos showing high-grade oedema. Probably these abnormalities are caused by the presence of oedematous fluid, and they could be the result of an abnormally low rate of mitosis amongst the cells of the epidermis. It has been shown above that the slow rate of follicle growth which probably causes most of the hair abnormalities in adult ragged mice could be the result of a decreased rate of mitosis amongst the Malpighian cells of the epidermis.

It is possible that the occurrence of superficial oedema could result in a diminished rate of cell division amongst the epidermal cells. Bullough (1952) has shown that the epidermis has low tissue priority for nutrients and that mitosis in the epidermis ceases early if unfavourable conditions are experienced by the host animal. Such conditions are listed as: excessive exercise, cold, shock; or lack of cell nutrients, which may be the result of other abnormalities. There is no reason to expect the occurrence of any of these abnormal conditions in ragged mice except the last. It is known that cells require nutrients in order to divide, and it is also known that cells obtain their
nutrients from the fluids in the tissue spaces. The presence of oedema, i.e. superfluous extra-cellular fluid, would be expected to lower the concentration of cell nutrients in the tissue fluids and thus inhibit cell division.

However, to explain the retarded post-birth follicle growth in all ragged mice, an abnormally slow rate of cell division after birth must be postulated.

Therefore, the weakness of this hypothesis is the fact that oedema has been observed only in RaRa embryos and newborn mice, and in a few Rat embryos; but not in Rat suckling mice or in most viable RaRa suckling mice. It therefore becomes necessary to assume, first, that at least low grades of oedema, below the threshold of recognition, must occur in all ragged mice and, second, that either such oedema must persist after birth or its inhibitory effects upon cell division must continue after birth.

(ii) It has been established that in the original unselected stock used in this work, all RaRa mice die at or before birth. It has also been seen that all RaRa embryos in the unselected stock show oedema, and that the embryos showing high grades of oedema are probably the most likely to die before birth. Oedema is probably therefore related to the cause of death in RaRa embryos.

Newborn RaRa mice were observed in some cases to emit fluid from the mouth or nostrils before death; this may be an indication of oedema of the lungs, which could be the cause of death. No evidence of oedema of the lungs was obtained from the sections of 13½-16½ day oedematous embryos, but until birth the blood supply
to the lungs is small and the hydrostatic pressure in the lung capillaries is probably low. Oedema of the lungs probably could occur only as a result of the sudden alteration of these conditions which takes place at birth.

(b) The cause of oedema.

Many possible causes of pulmonary and peripheral oedema have been discussed in the medical literature, e.g. Howell (1946), Elkinton (1950), Sarnoff and Berglund (1952) and Haddy, Richards, Alden and Visscher (1954). Some of the important causes are listed below:

(i) Abnormally great hydrostatic pressure in the capillary blood vessels, causing an outflow of blood plasma to the tissue spaces. This can be caused by:

(a) Partial "backward" failure of the heart, which produces an excess of blood on the venous side of the circulation so that blood accumulates in the veins and lung capillaries. Further pressure may then produce pulmonary oedema, and if the right ventricle cannot transfer blood from the veins through the pulmonary vessels, then congestion of the neck, liver, and spleen veins and peripheral oedema will result. In extreme cases, there may be extra-cellular fluid in the pleural cavity (hydrothorax) and in the peritoneal cavity (ascites).

(b) Mechanical obstruction of the veins, by tumours, etc.

(ii) Abnormally low concentration of plasma proteins - e.g. serum albumin, or any other factor which diminishes the
osmotic pressure of the blood plasma and causes its outflow from the capillary vessels.

(iii) Abnormally increased capillary permeability. This can be caused by:
(a) Heat.
(b) Cold.
(c) Capillary poisons, e.g. heavy metal salts, bacterial toxins, histamine.
(d) Oestrogens and other steroids in certain circumstances (Hechter, Krohn and Harris 1941 and 1942).
(e) Vitamin C deficiency.

(iv) Obstruction of the lymphatic vessels, which causes the non-removal of proteins from the tissue fluids.

(v) Retention of salt in the body in excess of water. This produces hypertonic body fluids and therefore excessive extra-cellular fluids.

(vi) Abnormally low tissue pressure.

(vii) Vitamin B₁ deficiency.

In the present investigation, no evidence has been found for the occurrence or non-occurrence of any of these abnormalities except (i) a. Some symptoms indicating heart failure have been observed in Rana mice showing high-grade oedema. In transverse sections of 15½ and 16½ day oedematous embryos, peripheral and other blood vessels were seen to be dilated, and yet they often contained a high density of red blood corpuscles. The rupture of blood vessels walls apparently resulted in localised regions of haemorrhage in some oedematous embryos. In sagittal skin sections
from $17\frac{1}{2}-18\frac{1}{2}$ day oedematous embryos and oedematous newborn RaRa mice, the superficial blood vessels were seen to be abnormally dilated, packed with blood corpuscles, and in some cases ruptured. This evidence suggests that there is abnormally high hydrostatic pressure in the peripheral blood vessels of RaRa embryos and neonatal mice and that this results in an excessive outflow of blood plasma to the surrounding tissue.

Evidence for the occurrence of pulmonary oedema just prior to death in newborn RaRa mice has already been cited.

Finally, a number of newborn oedematous RaRa mice, produced from intercross matings, have been dissected immediately after death and each compared with a similarly dissected ++ or Ra+ sib. About twelve such pairs of mice have been examined; in about five of the twelve cases the heart ventricles from the RaRa mouse were clearly smaller than those from the ++ or Ra+ sib. No gross pulmonary abnormalities were observed in any of these RaRa mice.

The evidence considered as a whole is inconclusive, but justifies the tentative suggestions first, that some abnormality of the heart could be the cause of oedema in RaRa mice by raising the hydrostatic pressure in the superficial blood vessels and producing excessive sub-epidermal fluid by the outflow of blood plasma; and second that the cause of death in RaRa embryos and neonatal mice may be heart failure or pulmonary oedema.
3. **Hair Density and its Relation to Hair Length and Thickness.**

From a series of studies on wool growth, Fraser (1951b and 1952a, b, c) developed a theory that individual hair follicles in a specific area of skin compete with each other for a limited quantity of fibre-forming substrate, so that a reduced density of hair follicles may produce coarser fibres or a faster rate of growth of the hair shafts.

The present work provides a means of testing this theory, since areas showing widely different hair densities have been studied in ++ and Ra+ mice. The data on hair thickness in some cases support Fraser's theory, e.g. in ++ mice all hair fibre types tend to be slightly finer on the rump than in the neck region where the total hair density is less. In Ra+ mice the awls are similarly finer on the rump than the neck as expected, but on the other hand, the zigzags and guard hairs seem slightly finer in the neck region where the hair density is markedly low.

Moreover, in Ra+ mice the hair density is everywhere abnormally low and so the average fibre thickness would be expected to be greater than normal. This is not so, except possibly in the case of the awls in the neck region.

Finally, contrary to expectation, the Ra+ zigzag fibres tend, if anything, to be finer than normal especially in the neck region where they would be expected to be most abnormally thick because of the extremely low hair density in this region. It seems then that Fraser's theory operates only under certain conditions. The thickness of Ra+ guard hairs is not apparently affected, possibly because being the earliest developing hair type their thickness
will be determined before differences in follicle density become effective. The fineness of some zigzag fibres in Ra+ mice is probably due to the retarded growth of their follicles and the consequent abnormal conditions for fibre development.

During the present work, no direct measurements of hair growth rate have been made, but estimates can be obtained by comparing the apparent relative fibre lengths of ++ and Ra+ suckling mice with the fibre lengths obtained by measurement in adult ++ and Ra+ mice. It is probably correct to assume that the first pelage hairs to emerge in ++ and Ra+ suckling mice are the guard hairs. These hairs are slower to emerge and thereafter shorter in young Ra+ mice than in their normal sibs. Likewise, the sinus hairs, especially the whiskers, are retarded in development in Ra+ embryos and young mice. It is therefore interesting that measurements have shown that the whiskers of adult Ra+ mice are mostly as long as those of normal mice and that the guard hairs of adult Ra+ mice are on the average longer than those of normal mice. The measurements of adult pelage hairs must have included hairs from pelages other than the first, but it may be correct to argue that if Ra+ guard hairs are shorter than normal near the commencement of the growth of the first pelage, they will be similarly shorter at the commencement of later pelages; or alternatively, that if Ra+ guard hairs are longer than normal in later pelages than the first, they will be similarly longer than normal at the end of their growth in the first pelage. It therefore seems probable that the guard hairs and possibly the whiskers in Ra+ mice must
grow more slowly than normal at first, and then later at a faster rate than normal or for a longer period than normal. The evidence from the histological study of the growth of the first pelage and from the observations on the interaction of the \( Ra \) and \( N \) genes, suggests that hair growth in \( Ra^+ \) hair cycles starts and finishes later than normal, but does not necessarily continue for longer than normal in any individual follicles. The slow commencing rate of growth of the guard hairs and whiskers in \( Ra^+ \) mice may be due to the slow rate of follicle development which has been demonstrated. The abnormally fast rate of growth which may occur later can be explained, for the guard hairs at least, on Fraser's competition theory; the lack of functional zigzag follicles in \( Ra^+ \) mice, which becomes obvious at 6-8 days after birth, might result in a diminution of competition for a fibre-forming substrate. This could cause a faster than normal rate of growth of fibres in those follicles which are functional and which were initiated early. (According to Fraser, early-initiated follicles are the most efficient at utilising the fibre-forming substance).

4. **The Periodic Hair Growth Cycle.**

It has already been described how the development, particularly of late-initiated follicles is abnormally retarded in \( Ra^+ \) mice. Many of the follicles whose growth is most severely retarded, do not complete their development in the first pelage; they remain as non-functional clumps or peg-shaped aggregations of Malpighian cells and can be seen in the dermis throughout later adult hair.
cycles. Other follicles whose growth is less severely retarded, complete their development and produce hairs; their development has however lagged behind and is therefore out of phase with that of early-initiated follicles. These retarded hair follicles enter the catagen and telogen phases of the first hair cycle later than the other neighbouring follicles. Such retarded, but functional, follicles apparently continue their progress through subsequent hair cycles autonomously; they remain out of phase with neighbouring follicles, and enter each stage of successive hair cycles later than the neighbouring follicles. It is the out of phase behaviour of these follicles which causes the cycles of hair loss and regeneration in N+ Ra+ mice to be characteristically diffuse rather than clearly demarcated into zones as in N+ +Ra mice.

In the rump region of Ra+ mice, follicle development is less severely retarded than in the neck region. Therefore, in the rump region, a smaller proportion of follicles becomes non-functional, but it is probable that as high a proportion becomes out of phase whilst remaining functional, as in the neck region.

During the development of the first pelage in normal mice, the growth of the late-initiated follicles is rapid and by 5½-6½ days after birth in, for example, the neck region, or by some later time in the rump region, all the follicles have reached a similar stage of growth. They are all almost equally developed, elongated, and stretch to the base of the adipose layer. All neighbouring follicles are in fact closely in phase in their development. From this time onwards the follicles remain in phase with each
other for the remainder of the first hair cycle and also throughout a number of subsequent hair cycles. In mice older than about four months the hair cycles become less distinct, probably because the hair follicles in any specific region are becoming less closely in phase with each other.

In contrast, by \(5\frac{1}{2}-6\frac{1}{2}\) days after birth in the neck region of \(Rc^+\) mice, neighbouring hair follicles are clearly out of phase with each other in their length and stage of differentiation, due to the slow development of the late-initiated follicles. As has been shown, this out of phase characteristic of \(Rc^+\) follicles persists in subsequent hair cycles in the adult mouse.

These observations suggest that the normal ability of neighbouring hair follicles to be in the same phase of growth in adult mice is to some extent an autonomous characteristic of each hair follicle, depending upon the fact that during their original development in the juvenile mouse, the follicles reach a specific stage of growth simultaneously at a certain time after birth. Where this does not happen, as in \(Rc^+\) mice, individual follicles pass through later growth cycles to some extent independently of their neighbours.

Although it is clear that neighbouring hair follicles normally behave synchronously in their progress through successive growth cycles, the study of ragged mice reveals that a considerable degree of autonomy of individual follicles underlies the normal behaviour. This fact has also been demonstrated experimentally by David (1934a) in the house mouse and Collins (1918) in the deer mouse, who showed that plucking the fibres from hair follicles
initiates a new cycle of hair growth in those follicles and that the follicles so treated become and remain grossly out of phase with their neighbours in subsequent hair cycles.

The nature of the hair growth cycle in mice is clarified in two important respects by the above discussion. First, a cause of the synchronous behaviour of neighbouring follicles is suggested, and secondly, the possible degree of autonomous behaviour by individual hair follicles in their times of commencement or cessation of growth is emphasised. The postulated cause of synchrony, which depends upon neighbouring follicles reaching a specific stage of growth at the same time during the development of the first pelage, provides an explanation for the wave-like progression of cycles of hair growth in the mouse. All the follicles in any specific body area will be in the same phase of the growth cycle, since they developed synchronously during the growth of the first pelage; in any different body area, however, all the follicles will have developed earlier or later in the first pelage and so although in phase with each other in their development they will be at an earlier or later stage of the growth cycle than the follicles in the first body area. The follicles of different body areas therefore enter synchronously within each area, but successively between areas, upon any particular stage of the growth cycle. The order in which the follicles of different areas progress through the growth cycles should largely depend upon the order in which they completed development in the first pelage.
It has been suggested by many authors that the initiation of any hair growth cycle depends upon the release or inhibition throughout the body of a substance or series of substances e.g. hormones. The autonomy of individual hair follicles which has been shown to exist, makes it possible for follicles in different body areas to respond at different times to a general growth stimulus which probably occurs simultaneously in all body areas. This is the process which causes the hair growth waves. The pattern of these growth waves depends upon the order in which the hair follicles in different areas respond to the stimulus, and this in turn is determined by the order in which the follicles developed during the growth of the first pelage. On this theory therefore, the wave-like progression of hair growth in the mouse, and possibly also in the rat, depends upon the synchronous development of neighbouring hair follicles reacting with a certain degree of autonomy of individual follicles.

The cyclic nature of rodent hair growth thus seems to depend upon the periodic release of a growth-stimulating substance reacting with a certain "inertia" of the hair follicles, so that follicles in some body areas react later to the growth stimulus than those in other areas. It has been shown that this inertia is due to the autonomy of individual hair follicles, which causes them each to commence a new cycle of growth only after a certain time-interval has elapsed since their completion of the previous cycle, i.e. only after their own internal development has reached a certain stage. The concept of follicle "inertia" was introduced by Durward and Rudall (1949) for an interpretation of
hair growth cycles in the rat, but they did not explain the cause of the phenomenon.

As in all discussions about cyclic forms of hair growth, it has been necessary to postulate the periodic general release of a growth stimulating substance or the suppression of a growth inhibitor which could initiate each hair growth cycle. The nature of this initiating mechanism presents an interesting problem. Haddow et al. (1945) and Durward and Rudall (1949) have shown the existence of increased vascularisation of the skin during hair growth cycles. Butcher (1937 and 1940), Baker (1951), Baker and Whitaker (1948) and Houssay (1954) have shown the importance of hormones in controlling cycles of hair growth. Bullough (1949a and 1952) has demonstrated the importance of carbohydrate as a cell nutrient necessary for relatively high rates of mitosis in the epidermis. It seems most likely that the cause of initiation of a hair growth cycle is the release or suppression of a hormone or an alteration of the balance of hormones in the bloodstream. The increased vascularisation of the skin probably indicates the increased nutrient supply required for the excessive cell division which occurs during a period of hair growth, but it may be the direct result of a hormone release; Bullough (1949b, c) has shown that oestrogenic hormones both stimulate mitosis and cause dilation of the capillary blood vessels.

The present work has thrown little light upon the cause of the initiation of hair growth cycles. It was however shown earlier that the commencement of hair growth cycles in the mouse was apparently characterised by increased proliferation amongst
all cells of the germinative epithelium, whether these cells were located in the Malpighian layer of the epidermis or in the external sheaths of hair follicles. The realisation that response to the growth stimulus may be peculiar to a definitive cell type rather than a distinct morphological structure - the hair follicle - may assist any attempts to discover the nature of the growth stimulus.

It is clear that any information on mechanisms initiating or controlling cell division will be extremely useful in such diverse and important fields as the study of normal growth, tissue regeneration, and abnormal or malignant growth.
SUMMARY

Ragged is a semi-dominant mutation causing hypotrichosis in the mouse. Many of the homozygotes die at birth or in embryo with a generalised form of oedema; the heterozygotes are normally viable.

The homozygous adults are almost naked; the heterozygotes have an abnormally low density of under-fur, i.e. the zigzag fibres, and a slightly higher than normal density of the other hair fibres, i.e. the guard hairs and awls.

In the adult homozygotes, the follicle density is less than normal and not all the follicles which are present produce hairs. In the heterozygotes, the follicle density is at least as high as normal, but many of the follicles do not produce hairs.

The failure of many follicles to produce hairs in heterozygous ragged mice and the absence of many follicles in the homozygotes are caused by a retardation of the rate of growth of many of the follicles in juvenile ragged mice. Even some of the follicles which do produce hairs develop more slowly than normal and as a result some of them appear to become and remain out of phase with their neighbours in the later periodic hair growth cycles. The retardation of follicle development also seems to cause a sub-normal expansion of skin thickness in ragged mice during the post-natal growth of follicles for the first pelage. It is suggested that sub-normal skin expansion in both juvenile and adult ragged mice is the cause of certain morphological abnormalities of many of the functional hair follicles.

The slightly higher than normal density of guard hairs and awls in adult ragged heterozygotes is related to an abnormally high
follicle density in neo-natal ragged heterozygotes; the pre-natal follicle density of the heterozygotes is, however, less than normal. In juvenile ragged heterozygotes, the follicle density is below normal at almost every age examined, both before and after birth.

A brief study of the interaction of the Ra-ragged and n-naked genes indicated that cycles of hair growth did occur in ragged heterozygote mice, but seemed to confirm that adjacent hair follicles were abnormally out of phase in their development.

The relevance of the present results to general theories on the control of hair growth in rodents is discussed.
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