GROWTH OF THE MOUSE COAT

VII. HAIR CYCLES AND SEBACEOUS GLANDS IN HOMOZYGOUS AND HETEROZYGOUS NAKED MICE

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Summary

The action of the Naked gene (N) in homozygous mice has additive effects on keratinization and sebaceous glands but not on the timing of hair cycles.

I. INTRODUCTION

The Naked gene (N) appeared as a spontaneous mutation in an albino stock of normal mice and was first described by Lebedevsky and Dauvert (1927), and subsequently by David (1931, 1932), Snell (1931), Redlichs (1937*a*, 1937*b*), Ebenhorst Tengbergen (1939), F. C. Fraser (1946), Steinberg and F. C. Fraser (1946), and Danneel and Kahls (1947).

A method for recording and measuring the time intervals between consecutive hair cycles in Naked mice has been described in detail in Parts II–V of this series (cf. A. S. Fraser and Nay 1953, 1955; Nay and A. S. Fraser 1954, 1955). In this Part the time intervals between consecutive hair cycles in homozygous (NN) and heterozygous ($N+$) Naked mice is compared and the effect of the N gene on the development of the sebaceous glands in the two genotypes is described.

II. MATERIALS AND METHODS

Three groups of mice were used for the comparison of the time interval between consecutive hair cycles in the two genotypes:

(1) Ten homozygous Naked mice ranging in age between 63 and 370 days and taken from a heterogeneous stock segregating for the Naked gene.

(2) Ten heterozygous Naked mice from the same stock.

(3) Ten heterozygous Naked mice from NA stock, a highly inbred subline of the strong A strain which has been kept segregating for the N gene.

For two months the positions of the hair bands were determined along a line running from head to tail along the side of the animal, and recorded on a series of drawings. The line was divided into 64 units. The data were plotted on graphs against time, and intervals (in days) between consecutive hair cycles for positions 20, 30, 40, and 50 measured.

For the study of sebaceous glands, $++$, $N+$, and NN mice from the heterogeneous stock were used, and both whole mounts and paraffin sections of the sebaceous gland were studied. For durable whole mounts the technique of Quay (1954) was used with some modifications as described below. The mice were skinned

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immediately after killing, the skin pinned to a rectangular cork frame, and fixed for at least 24 hr in 5 per cent. formol-saline. After washing in running water for *c.* 2 hr, pieces of skin 1½–2 cm wide and 3–5 cm long and covering the area between the nape of the neck and the tail root were cut off, shaved, and placed in staining solution in a covered petri dish. The staining solution was prepared from 30 ml 0·5 per cent. oil blue N in *iso*propanol which was diluted with 20 ml distilled water. After 6 hr in staining solution the pieces of skin were taken out and cleaned on both sides with a towel to remove precipitated oil blue particles and other impurities. Subsequently, the pieces of skin were mounted in glycerol, the epidermal surface up, and examined under the microscope. The sebaceous glands and sebum were stained dark blue.

TABLE 1

MEAN VALUES FOR THE TIME INTERVAL (IN DAYS) BETWEEN CONSECUTIVE HAIR CYCLES IN THE TWO GENOTYPES

Position	Time Interval (days)		
	NN	*N*+*	*N*+†
20	19·8	20·8	21·3
30	20·8	22·6	22·6
40	22·9	23·5	23·7
50	23·8	23·9	25·5

* Mice from same stock as homozygous mice.

† Mice from an inbred, unrelated stock.

For the preparation of paraffin sections the skins from freshly killed animals were pinned to rectangular cork frames and fixed in 5 per cent. formol-saline. After washing, the skins, still pinned to the frames, were passed through 70 per cent., 95 per cent., and two changes of absolute alcohol (*c.* 30 min in each) and then cleared in xylol. After clearing, the skins were infiltrated with paraffin (two changes, *c.* 24 hr in each) and rectangular pieces *c.* 1½–2 cm wide and *c.* 3 cm long were cut out and embedded in paraffin–beeswax (5 per cent.) mixture. Serial sections, 10 μ thick, were stained for 30 sec in 0·2 per cent. polychrome methylene blue. After staining and rinsing for a short time in tap water, the stain was fixed in equal parts of 5 per cent. ammonium molybdate and 1 per cent. potassium ferrocyanide for 5 min, washed again in tap water, passed through graded alcohols, cleared in xylol, and mounted in Canada balsam.

Photomicrographs of both whole mounts and serial sections were taken when necessary.

III. Results

(*a*) *Hair Cycles*

The average values of the time intervals between consecutive hair cycles for each of the four positions for the three groups of mice is given in Table 1. It was shown by *t*-tests that the intervals in $N+$ and NN genotypes do not differ significantly.

(*b*) *Sebaceous Glands*

In the normal mouse the sebaceous glands join the follicles well below the surface of the skin (Plate 1, Fig. 1). The ducts are short and there is no accumulation of sebum in the hair canal above the gland. There might be, in catagen or early telogen, accumulation of sebum below the sebaceous gland (Plate 1, Fig. 4). Unilobal glands could be found occasionally, but they are rather exceptional.

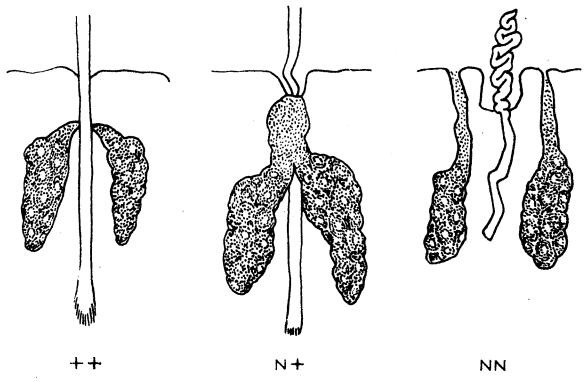

Fig. 1.—Diagrammatic representation of typical sebaceous glands in $++$, $N+$, and NN genotypes. $++$, normal sebaceous gland: the ducts are short and join the hair follicle separately. $N+$, sebaceous gland in $N+$ genotype: the ducts are joined and form an ampulla. NN, sebaceous gland in NN genotype: both ducts are separated from the hair follicle and open independently on the surface of the skin.

The sebaceous glands in the heterozygous Naked mouse differ from those in the normal mouse in many respects. The lobes were much larger, and the ducts were longer and wider, and were fused together, forming an ampulla round the hair fibre. The ampulla and the extended ducts were filled with sebum. Unilobal glands were frequent (Plate 1, Fig. 2).

In homozygous Naked mice the sebaceous gland ducts may fuse and form a wide ampulla immediately under the surface of the skin, or, as happens in most cases, the two ducts do not join but open on the surface of the skin independently, by-passing the hair canal (Plate 1, Fig. 3). The ducts were tortuous, passing below or round the follicles before they reach the surface of the skin. The hair canal itself is extended and contains a crumpled, rudimentary hair. The hair may be a corkscrew-like mass of keratin, which is caked together and extends out above the skin (Plate 1, Fig. 5).

Types of glands, characteristic for each genotype, are presented diagrammatically in Figure 1.

IV. Discussion

There are three most obvious effects of the Naked gene on the hair follicle:

(1) Impaired keratinization.
(2) Abnormal sebaceous glands.
(3) Timing of follicle activity.

According to David (1931), the hairs in heterozygous Naked mice break off because of faulty keratinization. The action of the *N* gene on fibre formation is strongly additive in the homozygous condition: in *N*+ mice the process of keratinization fails shortly before the completion of the hair growth, whereas in the *NN* genotype the same process breaks down to such an extent that the animals never produce a coat.

The differences in sebaceous glands between the two genotypes are but one aspect of the disorganization of the hair follicle by the Naked gene. David (1931) found that the hair follicles were smaller in *N*+ than in ++mice, and still smaller in the *NN* genotype. It seems that the *N* gene causes the deficiency of an agent necessary to produce a functionally efficient hair follicle. In *N*+ animals this deficiency is partly compensated by the presence of the +locus, whereas in the *NN* genotype the gene action is expressed fully.

As shown in Table 1, there is no real difference in the time interval between consecutive hair cycles between the two genotypes (differences are so small as to be statistically insignificant). There are reasons to believe that the regular recurrence of hair cycles in Naked mice is caused by the lack of an inhibitor, which may operate in adult normal mice. Hair cycles in Naked mice become inhibited in conditions such as pregnancy and lactation. The follicles, unchecked, would regrow regularly after a certain period of time, which seems to be constant but differing in length according to the position on the body. Such a view may be supported by the fact that there is no additive action of the *N* gene on the time interval between consecutive hair cycles in homozygous conditions.

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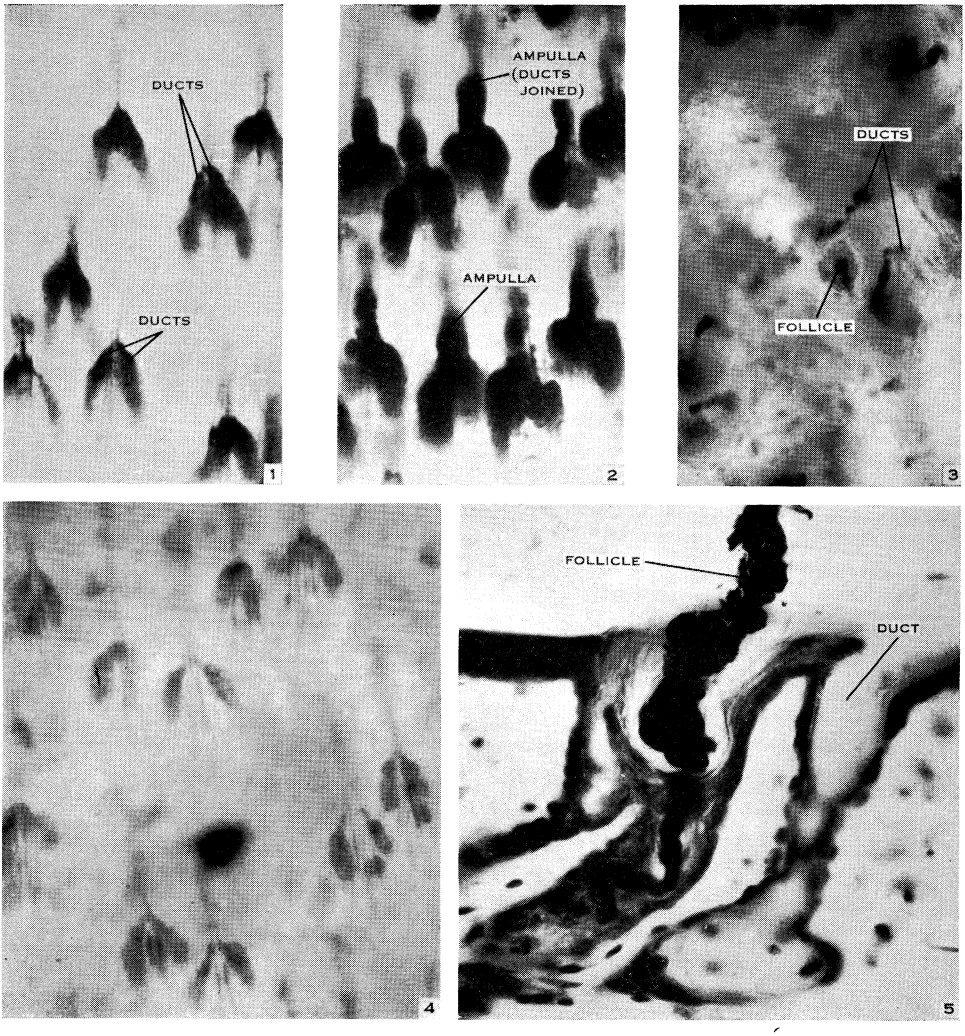

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EXPLANATION OF PLATE 1

Photomicrographs of sebaceous glands in $++$, $N+$, and NN genotypes. Figures 1–4: whole mounts, stained in oil blue N

Fig. 1.—Normal sebaceous gland: the ducts are short and join the hair follicle separately.

Fig. 2.—Sebaceous gland in $N+$ genotype: the ducts fuse and form an ampulla.

Fig. 3.—Sebaceous gland in NN genotype: both ducts are separated from the hair follicle and open wide apart on the surface of the skin.

Fig. 4.—Normal sebaceous glands: sebum in hair canal below the sebaceous glands.

Fig. 5.—Sebaceous gland in NN genotype in side view. Paraffin section, stained in polychrome methylene blue.