GENETIC AND DEVELOPMENTAL ANALYSIS OF THE N-TYPE STRAINS OF THE ROMNEY MARSH BREED OF SHEEP

BY

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SUPPLEMENT

SUPPLEMENTARY PAPERS.

The following papers are presented as a supplement to the main thesis. They involve different aspects of the growth and genetic modification of the coats of sheep, rabbits and mice, but are related in that they are all derived from a general survey of the coats of these animals to find features both of normal and mutant development which could form suitable bases for experimental studies.

A.S.Fraser. " Growth of the mouse coat".

A.S.Fraser and M.F.Hamada. " Comparisons of the birthcoats and skins of some British breeds and crosses".

D.S.Falconer, A.S.Fraser and J.W.B.King. " Crinkled, a new gene in the house mouse with manifold effects on the skin and coat".

A.S.Fraser. " The Rex and Angora coats of the rabbit".

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GROWTH OF THE MOUSE COAT.

by

A. S. Fraser.

The attention which geneticists have given to the mouse had led to the discovery of a large number of genes affecting in some way every aspect of development (Grüneberg 1943). This is very noticeable in the series of genes whose primary score is made from their effects on the coat. Several genes (Ca, Re, wa-1, wa-2, we) affect the shape of the fibres, causing them to be waved to a greater or less degree. Two genes (N and hr) affect the periodic shedding of fibres: the first causing the fibres to break off at skin level when they have completed their growth, the second causing the fibres to be lost from the follicles when they have completed their growth, instead of being retained as is usual for the normal type. Another gene (hr^{rh}) also affects the retention of completed fibres, but in addition causes a fantastic wrinkling of the skin. The fz gene causes a marked waving of the coat and a decrease in its length and density. The cr gene affects the structure of the coat, causing the absence of certain types of fibres. It also modifies the structure of the skin. The ic gene causes a periodic slouging of the whole skin layers (Carter and Phillips 1950). Obviously these genes with their multiplicity of effects constitute a very useful material for study of the normal sequence of development, since the retrograde analysis of their effects (Grüneberg 1943) will show how normal development can be modified. Such analyses can be made specifically for each gene, or more ambitiously an overall average analysis can be planned involving the description of

their effects separately, conjointly in genetic combinations, conjointly in experimental combinations, and in variations of these methods. Dr. D. S. Falconer in preparation for such an approach has built up stocks in which each of these genes has been backcrossed to an inbred line (the Strong A line) for five generations. Such stocks have many advantages over heterogeneous material. The background genetic variation is reduced, allowing comparisons of these genes, particularly the mimic group of waving genes, which will not be invalidated by random variation of modifiers. Since the stocks are reasonably isogenic with the inbred line, the transplantation of skin between animals is possible, and for the same reason, if the genes have secondary effects which are not obviously related to the primary effect, then these will be noticed more clearly in homogeneous material.

As a preliminary to the intensive study of this material, a pre-requisite is the detailed description of the normal development. Such description has been scanty, and apart from the work of Dry (1926) it can be disregarded. Dry (1926) has shown that the mouse coat is made up of several distinct types of fibres. The hair follicles in the mouse form a succession of complete fibres, and several are found in each follicle, in addition to the fibre in the process of formation. He found that successive fibres formed by the same follicle were predominantly of the same or related types, i.e., a follicle is restricted in the range of types which it can form. This suggests that the heterogeneity of the fibre population is coupled with a similar heterogeneity of the follicle population. Before this possibility can be evaluated it is necessary to

examine the classification of fibres in quantitative terms, using genetically defined material. This paper gives the results and implications of such an examination.

Material and Methods.

The Strong A line of inbred mice was used as the principal material, due to its central position in the back-crossing of our stocks. The CBA line, also inbred, was used for comparison, mainly since material was readily available from Dr. T. C. Carter and Miss R. Phillips. Two further strains, which had been selected for body size, were included to give an estimate of the correlation between body size and coat characters. These strains were supplied by Mr. J. King. The large, GL, strain is an extension of that formed by Goodale (1941). The small, MS, strain is, similarly, an extension of that formed by MacArthur (1949). Neither of these strains can be assumed to be genetically homogeneous. The relative sizes of these four strains can be seen from the 6 week body weights given in Table 1.

TABLE 1.

Six-week old weights in grams.

STRAIN	A	CBA	GL	MS
Sex				
♂	19.3	20.6	28.0	14.8
♀	16.8	17.5	24.7	12.3

Skins were taken from a series of mice of each strain, aged between 10 and 30 days, measured from birth. In the GL

strain, no mice older than 25 days were available. The possible error of age estimation is 12-24 hours. The region studied was mid-dorsal, located at 2/5 of the ear-tail base length, measured from the tail base. Samples of fibres were cut off at skin level with a razor, separated on to lantern slides, and projected at x 5.58. The projections were drawn, measured with a map measurer reading to sixteenths of an inch. The original data were used without correction in the computation of the regressions of length on age. The comparisons of these regressions (Table 3) are given in the magnified units. The regression coefficients and the diagrams of length growth rates have been corrected for magnification and are given in centimetres (Table 4, Figures 2, 3 and 4).

The classification of fibres.

Dry (1926) based his classification on three main characters: the occurrence and number of bends which occur along a fibre; the number of rows of medullary septa; the length of the fine unmedullated tip of the fibre.

The bends which occur along a fibre are very obvious, and they allow an easily scored separation into three groups: straight fibres, auchene fibres which have a single bend located at about 2/5 of the completed length (static length) measured from the tip, and zig-zag fibres which have several bends spaced along the fibre at decreasing intervals - the number of bends varies from 3-5, and a clear distinction can be made between auchenes and zig-zags, since the former have only one bend, the latter have three or more. These differences are illustrated in figure 1 which shows a typical fibre separation. The age of the sample was 25 days so growth can be taken

as complete, i.e. the fibres are at their static or completed lengths.

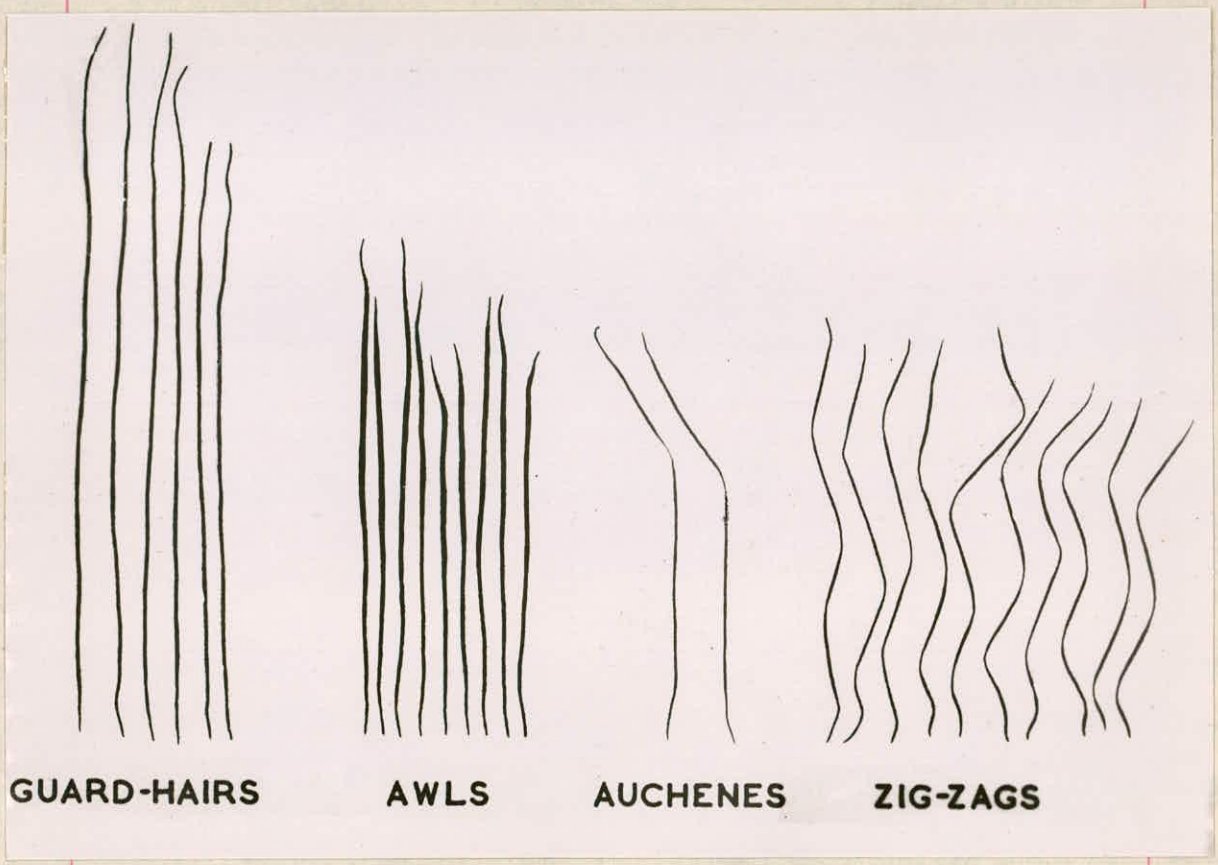

Fig. 1. Typical fibres of the mouse coat, showing the separation into types using shape as the main criterion.

The differences which occur between fibres in the structure of their medulla allow a precise, but not very easily scored separation, since the number of rows of medullary septa can only be determined microscopically. The medulla is separated into units, called septa by Dry (1926), which are arranged along the fibre in rows giving the appearance of a ladder. The number of rows varies from 0-4 between fibres, and usually decreases at the tip and base of fibres; it is

constant along the main shaft of the fibre. Referring to the separation based on shape: auchenes always have at least two rows, very rarely more than two; zig-zags always have only one row; straight fibres have at least two rows, usually three, and infrequently four rows. This character has not been used in the present classification because the difficulty in scoring would reduce the number of fibres which could be measured for the growth rate determinations.

The straight fibres differ in length, being separable into two classes: the long guard-hairs which project above the coat, and the short awls, which are of the same order of length as the rest of the coat. Dry (1926) found that the guard-hairs have a long fine tip, which does not occur in the awls. He defined a length of 0.06 c.m. as the length of tip which divided these types. This character is very difficult to score and therefore it also is not used in the present study. The actual separation is based (a) on the bends along the fibre, and (b) on the length of the fibre, since a reasonably accurate separation of guard-hairs and awls can be made on length alone.

In a few samples the bend which distinguishes the auchene fibres is not very marked, and a continuous sequence of variation can be found between the straight awls and the bent auchenes. This shows that the awls and auchenes are related, and since they are of very similar lengths, the two types can be considered together for the analysis of growth rates, which is, therefore, made in terms of (a) guard-hairs, (b) auchene + awls, (c) zig-zags.

Frequencies of fibre types.

The complete data, both of frequencies and of lengths of

fibres, is very bulky, and, therefore, only the concentrated data are given.

TABLE 2.

Frequencies of different types of fibres in percentages.

	Strain				
	A	CBA	MS	GL	Overall
Guard-hairs	2.29	2.03	2.50	2.52	2.34
Awls	26.21	26.53	19.88	23.70	24.4
Auchenes	.96	2.38	3.17	1.57	1.93
Zig-zags	70.54	69.00	74.45	72.16	71.54

The frequencies of the different types of fibres can be compared relative to the standard errors of estimate. These are .12 (guard-hairs), .56 (awls), .28 (auchenes) and .61 (zig-zags). If the means differ by more than twice these errors, then they can be taken to differ significantly according to statistical criteria of significance. It can be seen that the strain frequencies of guard-hairs do not differ significantly from the overall frequency of guard-hairs except in the CBA line in which the deviation is .31 which is greater than twice the standard error. The strain frequencies of awls, auchenes and zig-zags all differ from the overall frequencies significantly in at least two of the strains. These differences may be true strain differences; on the other hand, they may be due to differences in litter size etc., which occur between the samples of mice used to compare the strains. This question could only be answered by more rigorously controlled comparisons.

There is no correlation between the fibre type frequencies

and the size differences between strains. The overall averages agree with those found by Dry (1926) in his genetically heterogeneous material.

The earliest age at which a fibre sample can be separated into types is 10 days. The frequencies of the different types do not show any variation with age, and therefore it can be concluded that the coat is fully initiated by 10-13 days, i.e. no further fibres are initiated after this age.

The presence of the auchene type is variable: approximately half of the samples lack this type altogether. No differences occur between strains in the proportion of samples which contain auchenes. The variable occurrence of this type is a further argument for it being considered conjointly with the related awl type.

Rate of increase in length of fibres.

As stated above the following analysis of fibre lengths is made in terms of the magnified units measured after projection. The analysis has been further simplified by considering only the mean lengths from each sample, rather than the complete distributions of fibre lengths. The mean lengths are given in figure 2 plotted against age at sampling.

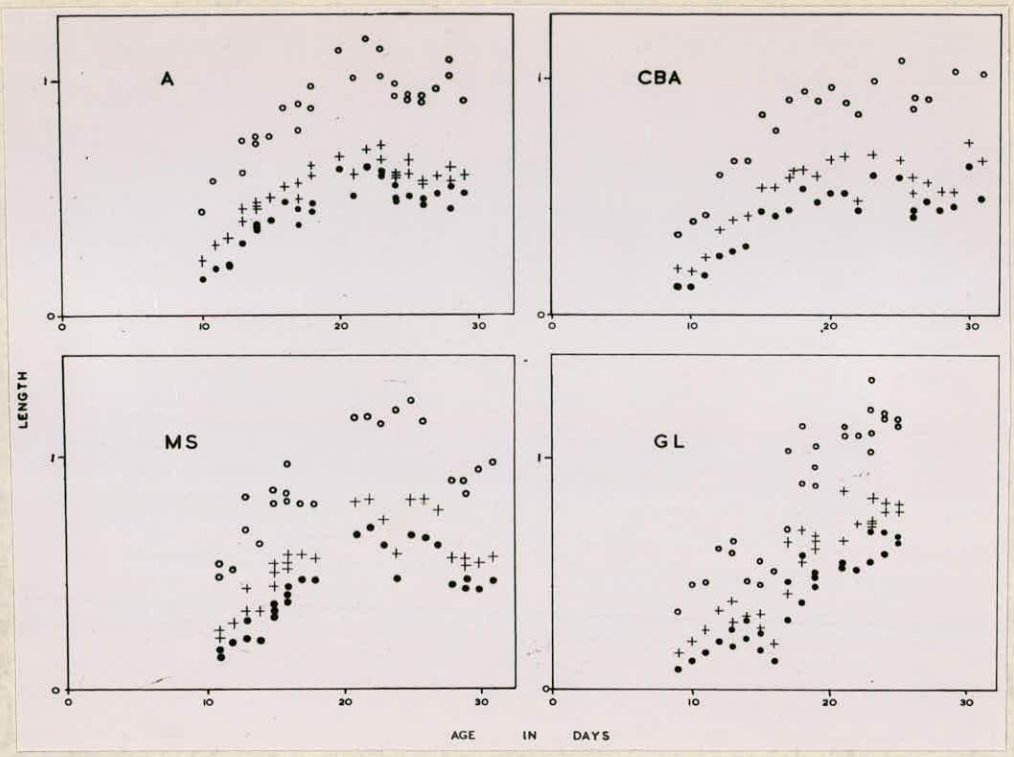

Fig. 2. The average lengths of the guard-hairs (open circles), awls + auchenes (crosses), and zig-zags (closed circles) in the four strains.

Three main points can be seen from these data. These are (1) the increase in length over the period 10-20 days is linear, (2) the sequence of initiation of guard-hairs, awls + auchenes, and zig-zags is in that order, and (3) in the A, CBA, and MS strains the coat ceases to grow at about 20 days, whereas the GL strain at the oldest available age (25 days) is still growing. This latter exception emphasises the linearity of increase in length, since in this strain the rate of increase in length is the same over the 20-25 day period, as it is over the 10-20 day period.

The linearity of the rates of increase in length allow these to be analysed by the linear regression method. The

regression coefficients estimated over the 10-20 day period are given in Table 3. The full regression lines are shown in figure 4. These can be compared (a) within strains - between fibre types, (b) between strains - within fibre types. The former comparison is not amenable to rigorous test since the measurements are not independent. However, the regression coefficients are consistently different between types in three of the strains; the only deviation is the awl + auchene type in the MS strain. This consistency argues that the differences are 'real', i.e., that the guard-hairs are formed at the fastest rate, the awl + auchene type at an intermediate rate, and the zig-zags at the slowest rate.

TABLE 3.

Regression coefficients of rate of increase of length per day, in cms.

	Strain				
	A	CBA	MS	GL	Overall
Guard-hairs	.054	.071	.052	.060	.061
Awls + auchenes	.042	.052	.054	.041	.047
Zig-zags	.038	.047	.049	.036	.041

The between strains - within types comparisons were made with the analysis of covariance. The tests are shown in Table 4. These demonstrate that the differences between strains are within the range expected from the sampling deviations, and therefore these differences can be disregarded. Each type of fibre is therefore formed at the same rate in all strains.

TABLE 4.

Differences between regression coefficients, calculated from the magnified units of length.

	Guard-hairs			Awls + auchenes			Zig-zags		
	s.s.	d.f.	MS	s.s.	d.f.	MS	s.s.	d.f.	MS
Deviations from average regression within strains	593.9	45		256.2	47		208.6	47	
Deviations from individual strain regressions	576.1	42	13.717	242.6	44	5.514	194.2	44	4.414
Differences between regressions	17.8	3	5.933	13.6	3	4.533	14.4	3	4.800
Variance ratio	F 1			F 1			F = 1.09		
	Not significant			Not significant			Not significant		

Differences between the means of the regressions are significant and it can therefore be taken that although each type of fibre is formed at a constant rate in all strains, it may be initiated at different times in different strains. Since these differences are small the overall regressions can, for the present purposes, be taken as representative. These are shown in figure 3.

Initiation of growth of the coat.

Estimates of the ages at which the formation of the different types of fibres commences, can be found from the extrapolation of the linear growth rates. Extrapolation is not a rigorous method, and in the present example it cannot be expected to have more than an indicatory significance, since it is possible that fibres do not grow at a linear rate over the whole of the formation period, and since the accuracy of such an extrapolation decreases with increase of the error variance.

The rate of growth of a fibre probably requires a period, which is short compared to the total period of formation, to increase from zero to a constant rate. A similar situation is almost certainly true for the cessation of growth. Since the estimate takes no account of this period, the estimated age of initiation, found from linear extrapolations, will be greater than the actual ages.

In figure 3 are shown the overall regression lines with their fiducial limits: the latter give an idea of the statistical validity of the extrapolations. The ages of initiation, estimated in this way, are 2+, 5+ and 6+ days for the guard-hairs, awls + auchenes and zig-zags respectively.

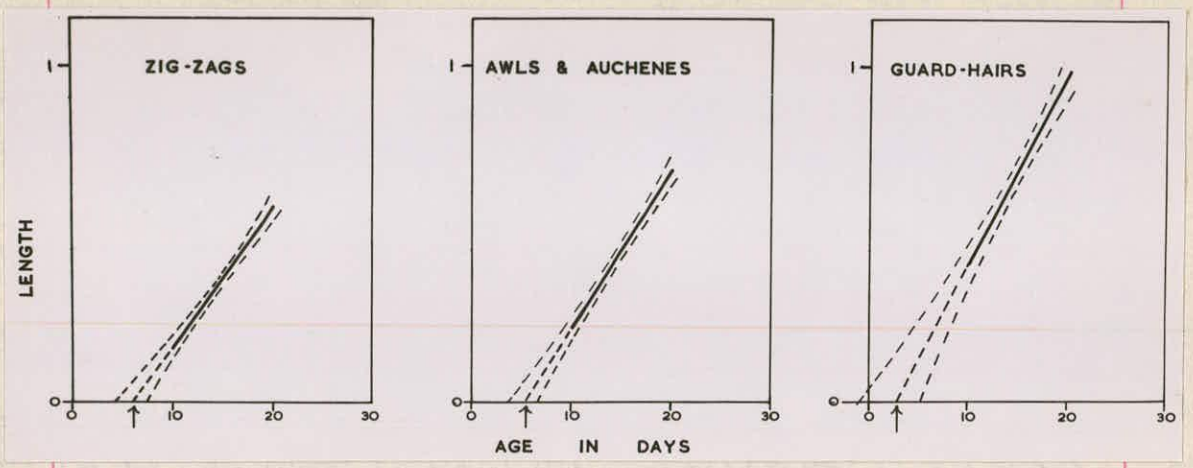

Fig. 3. Overall regressions of the three types of fibres (solid lines) with the extrapolations and fiducial limits (broken lines).

Age of completion of the first coat.

An estimate of the age at which growth of a type of fibre is completed, can be found most accurately from the interaction of its growth rate with its completed length. This is not possible for the GL data which does not include the period of completed growth. In the MS, A and CBA strains, particularly the latter two, inspection of the data given in figure 1 shows that growth is completed after about 20 days. Therefore the lengths of samples after that age can be taken as an estimate of the completed length. In figure 4 are shown the intersections of the regression lines with their respective static lengths.

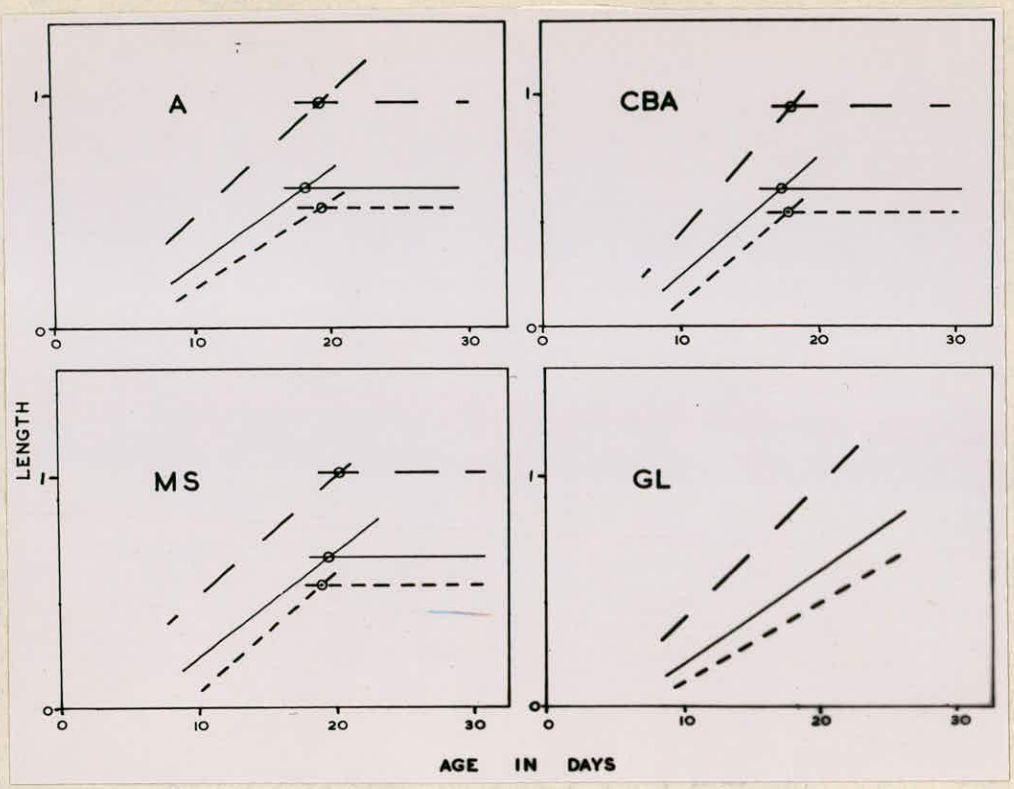

Fig. 4. Analysis of the data shown in figure 2, using linear regressions for the period up to 20 days, and direct average for the period after 20 days. The circles mark the points of intersection and give estimates of the ages of cessation of growth.

The analysis shown in figure 4 is based on the assumption that growth ceases at 20 days. This assumption is not completely accurate, and the inaccuracy will lead to errors, since if this age is earlier than the actual age of cessation, then although the estimate of the regression will not be affected, the estimate of the static length will be lower than the actual value. Conversely if the assumed age of separation is after the actual age, then although the estimate of static length will be unaffected, the estimate of the regression will be lower than the actual value. This will cause the estimated age to be later than the true value. Since the assumed age

(20 days) is close to the values found from the intersections (18-19 days) any errors due to this source will be small compared to errors due to statistical variation. The process could be repeated using 18-19 days as a new dividing line between growing and static periods, but it is unlikely that any great increase in precision would be achieved.

The intersections show that growth of fibres ceases in the different types over a very small time range i.e. the coat ceases to grow as a whole, rather than separately in terms of the different types. A closer examination shows that a small difference occurs between fibre types in the A and CBA strains: the awls + auchenes cease growth before the guard-hairs or zig-zags. This was initially taken to be due to a small error in the method. Dry (1926), however, found from a histological study the same difference, namely that "the largest guard-hairs are prominent among the last hairs to complete growth, as also are the short slim zig-zags". The reason for the earlier cessation of awls + auchenes might be found in their diameter, since they have 3-4 rows of septa, whereas guard-hairs have only 2 rows, and zig-zags only one row. The difference between fibre types in age of completion of growth is a problem, which may be solved by quantitative studies of follicle histology.

Dry (1926) found that the initiation and cessation of growth of the coat, occur as waves moving across the body; these "waves" have clearly defined boundaries, and their rate of movement is fairly fast. The "wave" control of the occurrence of growth has been observed in other mammals, and they have recently been studied in the rat using isoalloxagine as a marker. This substance when fed or injected causes the growing fibres to be coloured yellow; the static fibres are unaffected

(Haddow et al. 1945). The present analysis of growth rates, the histological observations of Dry (1926) and the results from the use of isoalloxagine, all agree in showing that the cessation of growth of the coat is affected by a factor of short duration which affects the coat as a whole. This factor operates in the mid-dorsal regions at 18-19 days in the inbred strains, at 20+ days in the MS strain and after 25 days in the GL strain.

Discussion.

The above description of the growth of the coat raises several problems. The first concerns the occurrence of qualitatively distinct types of fibres. Is this heterogeneity correlated with a heterogeneity of the follicle population, i.e., do fibres differ because they are formed by different types of follicles? The effects on the development of the coat caused by the crinkled gene (Falconer, Fraser and King, in press) indicate an answer to this problem. This gene causes both the guard-hairs and the zig-zags to be absent, the coat consisting only of short, straight fibres which are analogous to the awls of the normal coat. The follicle population also differs from normal; the first wave of follicles, which in normal is initiated at 14-15 days' gestation, is absent in crinkled: the last wave of follicle initiation, which in normal occurs just after birth, is also absent in crinkled. The only follicles to be formed are those whose initiation commences at about 17-18 days' gestation (the second wave of follicles in normal). These effects of the crinkled gene on the fibre and follicle populations suggest a developmental correlation between the two. Namely, that the first wave of follicles forms the guard-hairs, the second wave forms the awls + auchenes and the third wave

forms the zig-zags. This postulate can be examined from the relationship of the present data to similarly quantitative data of the growth of the follicle population. Such a study is in process.

The growth rates of the coats of the different strains raises the second question. Why does no correlation occur between the growth rate of body size and the growth rate of the coat? Most differences in size are correlated, a change in the dimensions of one organ being related to changes in size of other organs. The four strains used differ markedly in size (Table 1), and obviously the total amount of skin is closely related to size. It would seem that the characters of the units which comprise the skin are invariant between strains, only the number of such units varying. No idea can be given of what is a unit of skin, but the concept appears a useful way of concentrating the problem. It is intended to compare the follicle densities etc. of the different strains to determine if differences occur in such characters.

The cessation of growth of the coat raises the final question. What is the mechanism controlling the cessation and initiation of growth? Dry (1926) has shown that initiation and cessation of growth occur as waves which move along the animal. At any defined stage the coat will be growing on one side of a wave, static on the other. He used dissections and histological methods to establish the existence of this phenomenon. Iljin (1945), using thallium, and Haddow et al. (1945), using iso-alloxagine, have also studied waves of growth. They used these substances because, when fed or injected into an animal, they affect only the growing fibres: thallium causes growing fibres to be shed, iso-alloxagine causes growing

fibres to be coloured yellow. Unfortunately neither of these substances can be used in mice; iso-alloxagine acts only in rats, and in the mouse thallium has no effect on the first coat (Fraser, unpublished). In mice, however, some mutant genes which affect coat characters allow the wave phenomenon to be easily studied. The naked gene causes hairs which have nearly completed their growth to break off near the skin, leaving the region naked. Such regions can therefore be taken as analogous to the static regions in normal mice. The chinchilla gene shows a marked colour difference between growing and static regions, again allowing an easy scoring of the wave phenomenon. It is intended to use these genes to study the autonomy of the waves of growth, which Durward and Rudall (1949) have found, from the use of autografts of skin, to be fully determined.

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OBSERVATIONS ON THE BIRTHCOATS AND SKINS OF SEVERAL BREEDS AND CROSSES OF BRITISH SHEEP.

by

A. S. Fraser and M. K. O. Hamada.

The birthcoats of both coarse and fine fleeced sheep can be separated into different types of fibres. Each type of fibre is formed by a particular type of follicle. The ratios of the different types of follicles are the same in all the types of British sheep studied, and therefore variation of the follicle population can be excluded as a factor in the evolution of the British types of fleeces. Some birthcoats are coarse, others are fine, and some birthcoats are short, others long. The two differences are independent both developmentally and genetically. In the few lambs studied, the coarse type is dominant over the fine type, and the short type is dominant over the long type. It is suggested that the Down shortwool fleece evolved along a different route from that which gave rise to the Merino shortwool fleece.

Dry et al. (Dry 1940; Fraser, Ross and Wright 1951) have in a series of papers described in detail the development of the fleece in N-type and non-N-type Romney sheep. Non-N-type sheep are the normal Romney, and have a long, medium-fine fleece, and a long, fine birthcoat which usually lacks any coarse fibres. N-type sheep are a mutant type which have a coarse, carpet type fleece, and a coarse birthcoat. The two types correspond in the characters of their fleeces to "longwool" and "carpet" breeds, and the difference between them has been considered to be analogous to the difference between such breeds (Dry and Fraser 1950). It is necessary to examine

this suggestion by an analysis of the development of the fleeces of "carpet" breeds. The present paper details the first phases of this analysis.

Several conclusions were reached from the work on the Romney breed. (a) The birthcoat can be separated into distinct groups of fibres. (b) These are related to the different types of follicles which occur in the skin, in that each type of follicle forms one of the groups of fibres. (c) The possibility of assigning fibres to particular kinds of follicles allows one to arrange the fibres in a birthcoat sample in order of their age of initiation. (d) In N-type sheep the first fibres to be initiated grow at a faster rate than the later fibres, whereas in non-N-type sheep the first fibres grow at the same rate or slower than later fibres. (e) This difference of relative growth rates is correlated with, and probably caused by, differences in the depths to which follicles extend into the skin. In N-type the primary follicles are deeper than the secondary follicles, whereas in non-N-type the primary and secondary follicles are at about the same depth. (f) Some factor, called the "pre-natal check" (Dry 1931), causes the primary follicles to be decreased in their size relative to secondary follicles; and it is argued that this is the basic cause of all the changes of development which result in the production of a fine instead of a coarse type of birthcoat, and a "longwool" instead of a "carpet" type of fleece. (g) The "pre-natal check" factor does not affect the constitution of the follicle population.

This analysis of N-type and non-N-type Romneys has therefore led to the description of a factor which causes the development of the fleece to change from the uneven, carpet

type to the even, longwool type. The genetics of this factor have been shown to be simple; either a dominant or a recessive monogenic difference causing the presence or absence of the "pre-natal check".

In the present material several aspects of the birthcoats and follicle populations of some British sheep have been studied. These include the Blackface breed, which has a very coarse carpet type of fleece, and some crosses of shortwool and longwool breeds. The shortwool type of fleece differs from the longwool type in being less than half as long, and in the diameter of the fibres being also much finer. There are two groups of shortwool breeds: the Down shortwools which originated in England, and the Merino breeds which stem from the Spanish Merino. The shortwool breeds involved in the crosses studied were of the former type, Down shortwools. Carter (1942) has suggested that the shortness of the Merino fleece is due to an increase of follicle density causing each follicle to form less fibre. He considers this increase of density to have occurred by an increase in the proportion of later developing secondary follicles. It is of interest to determine whether the shortness of the Down shortwool fleece is due to the same factor.

Material.

The materials used were obtained from lambs of the Boghall Experimental Farm, through the kindness of the authorities of the Edinburgh and East of Scotland College of Agriculture. The Blackface is the only pure breed kept on this farm, other sheep being the results of different crosses which are made as normal husbandry practice. The Cheviot breed is crossed to the Border Leicester breed (shortwool x longwool) to give a

crossbred sheep called the Halfbred. These are then crossed to the Oxford or Suffolk breeds giving triple cross sheep (shortwool x longwool x shortwool). Skin and birthcoat samples were taken within a few days of birth, or somewhat la er, from Blackface, Halfbred, Oxford and Suffolk triple cross lambs. The later samples were not used in the comparisons of the constitution of the follicle population. In addition, three lambs were sampled from a Blackface x Cheviot cross, made by accident in that year. These represent the cross of carpet x shortwool. A few Welsh Mountain lambs were studied for the Department of Agriculture, Bangor.

Methods.

The histological methods differed from standard practice in two respects: dioxan was used for dehydrating, and a wax mixture was used for embedding following the suggesting of Dr. H. Auber, Wool Industries Research Association, Leeds. He advised this mixture to have the constitution of 40 gm. (Paraffin wax, 54.5° MP), 4 gm. (Stearic acid), 2 gm. (Spermacetti) and 1 gm. (Japan wax). A high melting point, 58°, paraffin wax was used in place of Japan wax which we could not obtain. These modifications allowed reasonable sections to be cut without recourse to the celloidin method.

The distinction between primary and secondary follicles was made on the occurrence of a sudoriferous gland, this being present on primary follicles, absent from secondary follicles. Counts were made by drawing sections with a camera lucida.

The following analysis includes many references to the ratios of Primary/Secondary fibres and Primary/Secondary follicles. For simplicity the ratios are given for the frequency of

Primary fibres and follicles being 1.0, i.e., the frequency of S follicles and fibres is given as the variagle.

1. Separation of the birthcoat into groups of fibres.

The types of fibres found in both coarse and fine birthcoats have been described at length by Dry (1934) and summarised by Fraser, Ross and Wright (1951). Therefore the terminology is used without explanation.

In coarse birthcoats (Blackface, Welsh Mountain, N-type Romney, Cheviot x Blackface) the two main groups of fibres are (a) long and coarse, of the halo-hair and hairy-curly-tip types, and (b) short and fine, of the plain-curly-tip and histerotrich types. These two groups correspond respectively to the primary and secondary follicle. The division of the primary fibres into halo-hairs and hairy-curly-tips corresponds to the separation of primary follicles into central and lateral types.

In fine birthcoats (Normal Romney, Halfbreds and crosses) the two main groups are (a) sickle-tips and checked-curly-tips, and (b) plain-curly-tips and histerotrichs. The distinguishing feature between the two groups is the very small size of crimps in the former. These groups correspond to the primary and secondary groups of follicles, but the detailed correspondence between the finer classification of fibres and follicles have not been established so definitely for fine birthcoats as they have for coarse ones. The suggestion (Fraser 1950) that sickle-tip fibres are formed by central primary follicles, and checked-curly-tips by lateral primary follicles, is examined below.

The birthcoats of all the lambs examined can be sorted into the types described by Dry (1934).

Fig. 1. Sequences of fibres from birthcoats representative of each type, to illustrate the independence of the coarse-fine and long-short differences. Each sequence is separated into fibres formed by the different types of follicles as detailed in the text.

The distinction between the groups is not as obvious in birthcoats from shortwool lambs as in birthcoats from longwool lambs, but a classification can still be made. In coarse birthcoats it is clear, and not liable to any major degree of error. The most obvious point from these classifications is that noted by Dry (1934) in the Romney. In coarse birthcoats the primary fibres are much longer than in fine birthcoats, relative to the secondary fibres.

2. Relation of fibres to follicles.

The frequencies of the different types of fibres are given in Tables 1 and 2. All fibres of the halo-hair, super-sickle, sickle series are grouped into the same type.

The ratio of primary/secondary fibres is reckoned, in coarse birthcoats, from the ratio, Halo-hair + hairy-curly-tip/ plain-curly-tip + histerotrichs, and in fine birthcoats from the ratio, sickle-tip + checked-curly-tip/ plain-curly-tip + histerotrichs. These ratios are given in column 4 of Tables 1 and 2.

The ratios of primary/secondary follicles are also given in Tables 1 and 2 (column 7) and in this case are based on actual counts of follicles in samples of skin taken from the same regions, and at the same time, as the birthcoat samples. Only follicles were counted which are actively forming a fibre as judged from the histological picture. However, this will include some secondary follicles which, although they are forming a fibre, have not yet formed one of sufficient length to be included in the birthcoat samples. This error will tend to cause the P/S ratio reckoned from follicle counts to be less than that based on fibre analysis. Another source of error is the loss of small fibres from the birthcoat which, since these are secondary fibres, will cause a deviation in the same direction. Therefore it is expected that the P/S ratio derived from follicle counts will be greater than that from fibre counts.

In figure 2 are shown the two P/S ratios plotted against each other. If they are equal, the regression diagram should show a close scatter around the line bisecting the diagram and passing through the point of origin. It is obvious that there is general agreement between the two estimates of the P/S ratio, but in coarse birthcoats the P/S ratio from follicle counts tends to be greater than that from fibre counts, whereas in fine birthcoats the situation

is reversed. The former tendency can be explained by the loss of small fibres, and misclassification of secondary follicles, mentioned above, but this cannot be the explanation of the latter discrepancy which is in the other direction. The probable explanation in this case is that some primary fibres are misclassified as secondary fibres. However, neither in fine nor in coarse birthcoats are the discrepancies large enough to invalidate the general conclusion that the separations of birthcoat fibres into types is a consequence of the separation of the follicle population into types.

Fig. 2. The P/S ratios found from the birthcoat, and the skin samples, plotted against each other. An obvious general agreement can be seen.

The data also allow one to calculate the ratios of halo-hair/hairy-curly-tip and sickle-tip/checked-curly-tip fibres.

These should equal the ratio of central/lateral follicles, which is about 1/1.9 (Ross 1951). The present data agree with this, the ratio being 1/2.1 in coarse birthcoats and 1/1.94 in fine birthcoats (from average of individual ratios). Therefore it can be stated that these data are not in disagreement with the suggestion of Fraser (1950) that sickle fibres are formed by central, and checked-curly-tips by lateral, follicles.

3. Constitution of the follicle population.

Although many workers have studied the development of the follicle population (see Carter 1943), few have given any data of the ratio of P/S follicles at definite ages. Ross (1950) compared the P/S ratio between N-type and non-N-type lambs of the Romney breed, sampled within a day or two of birth. She found no difference between these two types, the ratio being 1/2.31 in N-type, and 1/2.39 in non-N-type. (These ratios were formed from the ratio of the sum of all the P follicles to the sum of all the S follicles, not from the average of the individual P/S ratios. The overall ratios for British sheep given in this paper have been calculated in the same way.) H er counts included only follicles which were actively forming fibres. Many of the skin samples taken for the present study were obtained within a day or two of birth, and therefore it is possible to compare the P/S ratio between British and New Zealand sheep.

At birth, only P and S follicles can be distinguished, and at this age, although all of the P follicles are actively forming fibres, some of the S follicles are not fully developed. In Tables 1 and 2 the P/S follicle counts given are really

P/S_F, where S_F are secondary follicles actively forming fibres; the counts exclude the S_U follicles, which are secondary follicles not fully developed at birth. Table 3 gives similar P/S_F ratios, from lambs sampled within a day or two of birth. The P/S_F ratios of the N.Z. Romney lambs (Ross 1950) are included to extend the comparisons. Two points can be seen from these data. (1) The Blackface group have a different ratio (1/1.35) from the other groups, and (2) the ratio is constant in all the other groups (1/2.31, 2.39, 2.37, 2.39). The data have been analysed by the contingency table method of χ^2 and the results agree with these conclusions.

Burns (personal communication) has suggested that the difference of the P/S_F ratio between the Blackface and the other groups can be explained by the high frequency of S_U follicles in the Blackface, and the low frequency of S_U follicles in the other groups. In Table 3 are given the $P/S_F + S_U$ ratios found in the Blackface samples. The ratio then approaches that found in the other breeds, and this suggests that the P/S ratio of all follicles, including undeveloped follicles, is constant in all the sheep studied. It appears, then, that in sheep of British ancestry the ratio of P/S follicles is, when measured at birth, invariant. Since the material included samples from lambs of all the main types of birthcoat, except the Merino, it follows that variation of P/S ratio is not a determinant of birthcoat type in these sheep.

The high proportion of undeveloped secondary follicles in the Blackface breed may be a feature characteristic of this breed alone, or it may be an effect of the extreme coarseness of the Blackface birthcoat. That it is not a determinant of the coarse type of birthcoat can be seen from the Blackface x

Cheviot and N-type Romney groups. These both have coarse birthcoats yet their P/S_p ratios do not differ from those of lambs with fine birthcoats, and they both have a low proportion of incompletely developed secondary follicles.

4. Differences between birthcoats.

In the material included in this study there have been two main differences of birthcoats. Some are coarse, others are fine, and some are long, others are short. The Blackface, Welsh and N-type birthcoats are all coarse and long. The birthcoat of normal Romneys (non-N-type) is fine and long. The birthcoats of the Halfbred and triple crosses are all fine and short. The Blackface x Cheviot cross lambs have a coarse and short birthcoat. Although there are only 3 of the latter lambs available, their birthcoats are all similar, and differ from the other coarse birthcoats of the Blackface, Welsh and N-type lambs. These three sheep are proof that it is possible to have a short birthcoat which is coarse. It would be of interest to repeat this cross to verify this conclusion, and to determine the type of fleece which develops from such a birthcoat.

The independence of the two pairs of differences shows that the developmental systems which determine these differences are similarly independent, although interaction may occur. Since Dry et al. (see Fraser, Ross and Wright 1951) have shown that the difference between coarse and fine is due to the "pre-natal check", which determines the relative depths to which early and late follicles grow into the skin, it follows that this factor does not cause the long-short difference. The developmental basis of the difference in length of coat must be looked for in some other aspect of skin and follicle development.

We have seen that the P/S ratio is invariant, and therefore can be excluded as a possible cause. If the follicle density were high in the short and low in the long type, one might attempt to explain the difference on the basis of competition between follicles (Fraser 1951). However, Burns (1933) has shown that the density of fibres per square inch is 9,000 in the Hampshire (a Down shortwool type), and 25,000 in the Rambouillet (a Merino shortwool type) showing that the Merino type has a much higher density than the Down shortwool type. Further, the density of the longwool type of fleece is of the order of 6,000-8,000, and therefore although it is necessary to verify this, it appears unlikely that variation of follicle density is the explanation. Bergen and von Mouersberger (1948) give data of the average body weights and fleece weights of Down shortwool and longwool breeds. If the regressions of fleece weight on body weight are calculated (see Figure 3) it is found that the Down breeds have a lower fleece weight per unit body weight than the longwool breeds, i.e., the Down breeds have a lower production of wool per unit area of skin than the longwool breeds. This could be due to two possible causes: (1) the amount of fibre substrate may be lower in shortwools than in longwools, and/or (2) the follicles of shortwool breeds may have a lower efficiency of utilising fibre substrate than those of longwool breeds. It seems likely that one or other or both of these factors is the underlying cause of the difference between the longwool and shortwool types. Galpin (1948) has measured the rate of wool production per unit area of skin in English Romney sheep and the present study emphasises the necessity of extending her methods to include different breeds of the main fleece types.

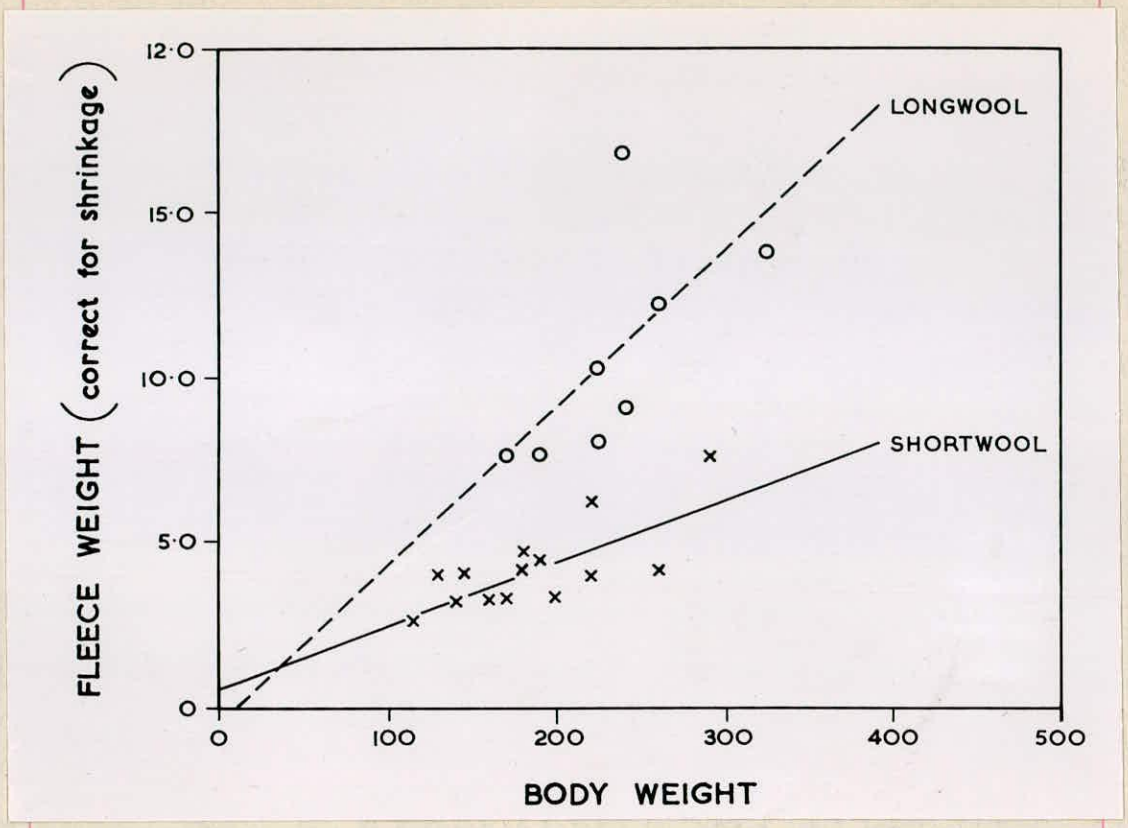

Fig. 3. Regressions of fleece weight (corrected for shrinkage) on fleece weight taken from data given by Bergen and von Mauersberger (1948).

5. Genetics.

The present material included several different types of crosses, and it is possible from these to reach some conclusions on the genetics of birthcoat type. The various crosses are shown in Figure 4 to illustrate the relationships between them and the five parent breeds: Blackface, Border Leicester, Cheviot, Oxford and Suffolk. Only lambs of the Blackface breed have been examined, but the descriptions of the other breeds as long or short are derived from observations of people who have examined the birthcoats of these breeds, and it is unlikely that these descriptions are incorrect.

The cross, Blackface x Cheviot, is between a coarse-long

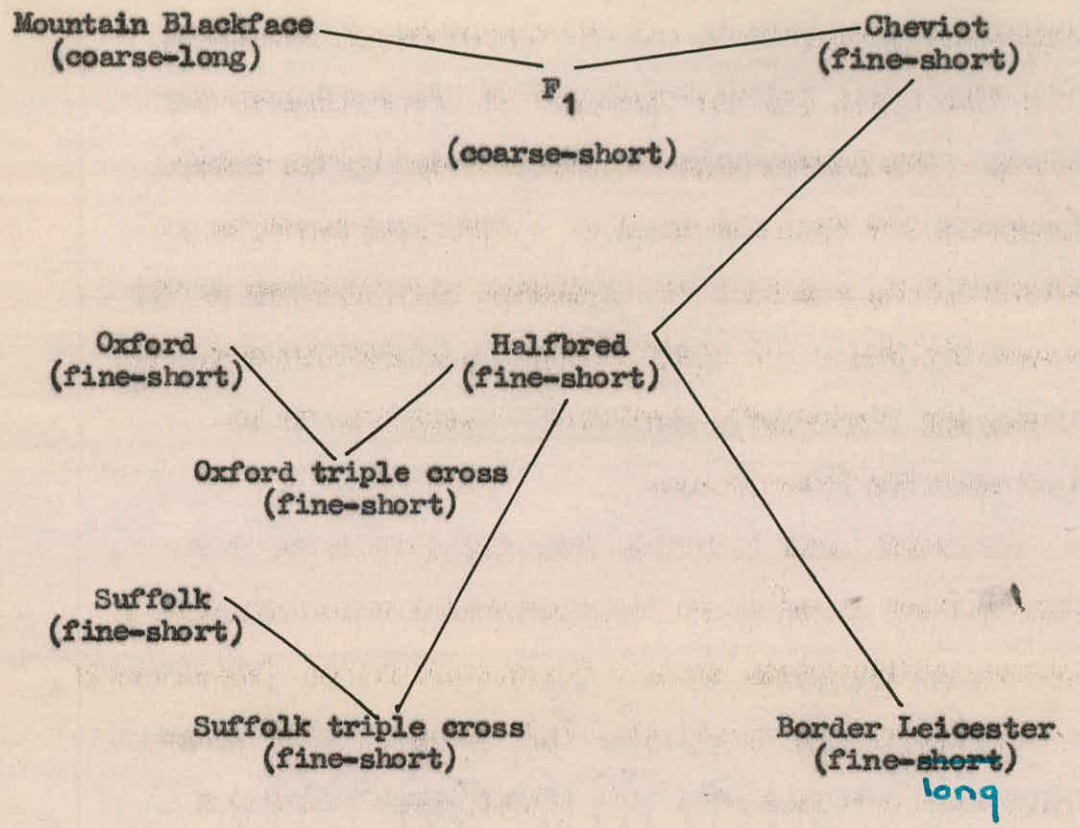

Fig. 4. The relationships of the various crosses used in the present study.

breed and a fine-short breed. Although only 3 of these were available they were uniformly and distinctly of the coarse-short type, showing (a) the independence of the coarse-fine and long-short differences, (b) the dominance of the coarse over the fine type, (c) the dominance of the short over the long type. The latter point is corroborated by the other crosses which are from the cross of a fine-long breed to a fine-short breed, and from the backcross of this cross to two fine-short breeds. All the cross-lambs, both first cross and backcross, are fine-short, showing the short type to be dominant over the long type.

It would be of interest to make the necessary crosses to determine the genetics of the coarse-fine and long-short differences of birthcoat type. These would be (a) (Blackface x Cheviot) x Blackface, to determine the segregation of long-short, in coarse birthcoats, (b) (Blackface x Cheviot) x Cheviot, to determine the segregation of coarse-fine in short birthcoats, (c) (Cheviot x Border Leicester) x Border Leicester, to determine the segregation of the long-short difference in fine birthcoats.

Discussion.

There are many breeds of sheep in existence, but these can be grouped according to their fleeces into five main types. These are (a) wild type, (b) carpet type, (c) longwool type, (d) English shortwool type, and (e) Merino shortwool type. In Britain the sequence of evolution appears to have been wild type - carpet - longwool - shortwool. The present results, and those of Dry et al. (see Fraser 1951) indicate that it may be possible to determine the most probable sequence of evolution

from the analysis of the epigenetic systems which cause the differences of fleece type.

Dry (see Dry and Fraser 1950) has shown that in the Romney breed the difference between a carpet and a longwool type of fleece is inherited as a unit, and Fraser (1950) has suggested that the primary difference is in the depth to which the primary follicles grow into the skin. In carpet fleeces the primary follicles extend deeper than secondary follicles, whereas in longwool fleeces the primary and secondary follicles extend approximately equal distances into the skin. The direction of the evolutionary change is almost certainly from carpet to longwool type, and therefore Dry (1934) applied the term "pre-natal check" to a factor which opposes the greater extension of the primary follicles; there is thus a weak check in the more primitive carpet type, a stronger check in the longwool type. It was found that the coarseness of the birthcoat could be taken as an inverse measure of the intensity of the pre-natal check, this being low in coarse birthcoats, high in fine birthcoats. It can be concluded that one of the epigenetic systems which has been altered during the evolution of the fleece is the pre-natal check.

The present study has shown that another epigenetic system, independent of the pre-natal check, is that determining the difference between long and short birthcoats. This difference of the length of birthcoats is almost certainly the precursor of the difference between long and short fleeces. Therefore it can be concluded that the difference between the longwool and shortwool types of fleece is independent of the pre-natal check mechanism. For convenience the basis of the

long-short difference is called the length mechanism.

The suggested evolutionary sequence of British breeds is wild - carpet - longwool - shortwool. If this is correct, and if the change from carpet - longwool is due to the occurrence of the pre-natal check, the shortwool breeds should also have the pre-natal check, i.e., fine birthcoats. This is so. Further, since the change of the length mechanism is supposed to be the basis of the longwool-shortwool difference, all the breeds previous to that step should be of the long type. In agreement with this, all the carpet type breeds examined have had long birthcoats analogous to those of N-type Romneys. Therefore it is reasonable to conclude that in Britain the evolution of the shortwool type of fleece occurred as a two-step change from the carpet type; the first step being the occurrence of the pre-natal check, the second the change of the length mechanism.

Two aspects of the evolution of the fleece have not been discussed: the wild type - carpet type change, and the evolution of the Australian Merino type. No data exist which suggest any mechanism which can explain the wild-carpet change. Carter (1942) has given data on the follicle populations of the Australian Merino, which suggest the basis of the evolution of that type. He found that in the Merino the ratio of P/S follicles is, in adults, of the order of 1/30, whereas in longwool breeds the ratio is of the order of 1/6. He considers that this increase of the proportion of secondary follicles results in an increase of follicle density, i.e., the new S follicles are interspersed amongst existing follicles rather than the new S follicles causing a lower density of P follicles. This is reasonable and agrees with the increased follicle

density of the Merino fleece; the density of longwool fleeces is of the order of 6,000 fibres per square inch whereas that of the Merino is of the order of 30-60,000 fibres per square inch (see Berger and von Mauersberger 1948). An increase of the P/S ratio from 1/6 to 1/30 will on this basis cause the follicle density to increase from 6,000 to 27,000. Carter's hypothesis is therefore adequate to account for the increase of fibre density which has been noted, and the increase of follicle density is sufficient to account for the shortness of the Merino fleece. The evolution of the Merino shortwool type can be taken as due predominantly to an increase in the proportion of secondary follicles, and therefore to be distinct from the evolution of the British breeds in which this change does not appear to have had any major importance.

The increase of secondary follicles is not the only factor in the evolution of the Merino type, since Merino lambs may or may not have a coarse birthcoat. This has been noted by Schinckel (personal communication) in the Australian Merino, and by an anonymous author in Merino lambs introduced into England from Spain (A Practical Treatise on the Merino and Anglo-Merino Breeds of Sheep. London, 1809). The latter author states, "There is occasionally a peculiarity in the wool of the lamb when first dropped, differing from any breed in this country (if it indeed may be termed wool) many of them appearing entirely covered with hair; which I do not find mentioned by any author I have met with except Dr. Parry (Communications to Board of Agriculture, v. 5, p. 346) who observed that the wool of Merino lambs, in general, is evidently coarser and harder than that of the sheep. It seems, however, that different flocks vary in this respect. The lambs of the

Infantado and Paular races are covered with a coarse sort of hair, which afterwards changes into very fine wool. The same appearance is sometimes to be found among the lambs of the Negrete breed in England". This shows that in the original Merino population from Spain strains differend in the occurrence of the pre-natal check. Therefore it must be concluded that in the evolution of the Merino two lines can be distinguished: one in which the pre-natal check is incorporated, the other in which it is not.

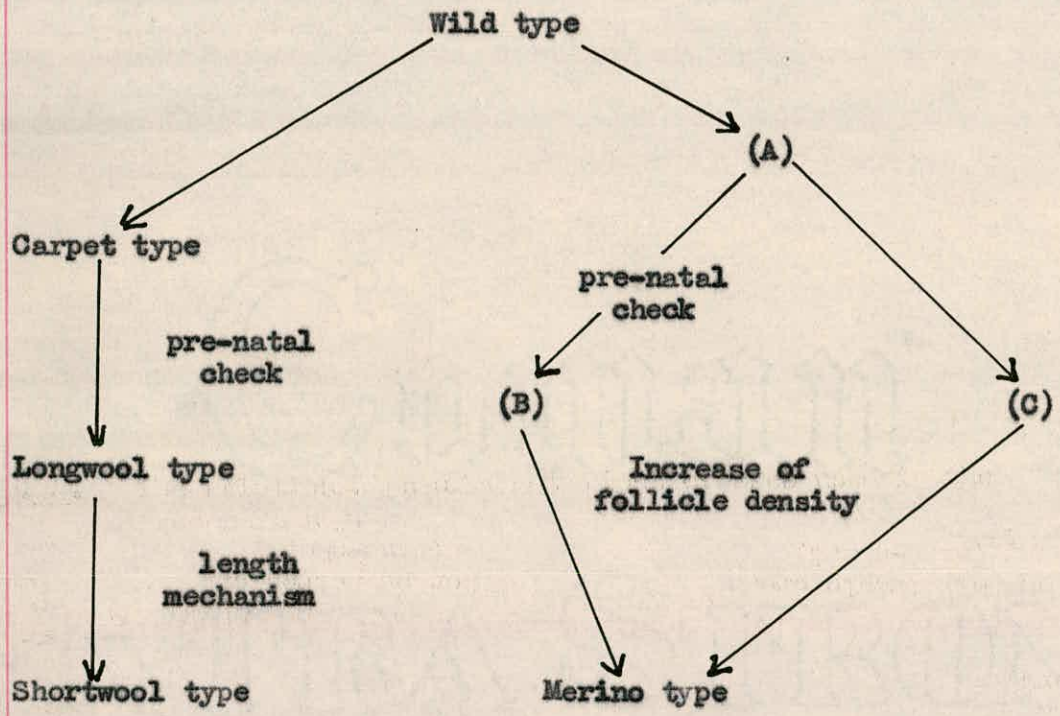

Fig. 5. A probable pattern of the evolution of the main types of fleeces. The unknown A type in the Merino evolution cannot be identified at present. The B line will have coarse birthcoats, and C line will have fine birthcoats.

The chart of the evolution of the main fleece types shown in Figure 5 is given as the sequence which appears probable from the analysis of the factors underlying the main characters of the fleece. Obviously information is required

on the follicle populations of all the main types before the effect of its variation can be fully described. In a later paper the senior author intends to analyse, on the basis of the competition theory of determination of wool growth (see Fraser 1950), the effects of variation of the three main determining systems: the pre-natal check, the length mechanism and the constitution of the follicle population.

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(Fine)

Sheep No.	Fibre counts P SK	Fibre counts P Ch-CT	Fibre counts S PCT+Hi	Ratio	Follicle counts P	Follicle counts S	Ratio	Type
1	6	13	49	1/2.57	21	48	1/2.28	Oxford-cross
15	5	11	49	/3.06	63	145	/2.30	" "
3	23	44	135	/2.01	62	122	/1.97	" "
7	10	31	115	/2.80	49	128	/2.61	" "
2	57	84	346	/2.45	27	62	/2.30	Suffolk-cross
4	41	85	300	/2.38	36	82	/2.28	" "
13	21	36	164	/2.80	20	49	/2.45	" "
14	6	12	37	/2.05	35	87	/2.48	" "
5	12	18	82	/2.73	16	44	/2.68	Halfbred
6	23	38	120	/1.96	27	68	/2.52	"
194	8	13	61	/2.90	25	63	/2.52	Romney (English)
195	14	33	167	/3.55	20	70	/3.50	Romney (English)

The counts of the different types of fibres into which fine birthcoats can be separated, and the ratio of primary-Secondary fibres derived from these counts; also the counts of P and S follicles, and their ratio (P/S), derived from samples of skin taken at the same time, and from the same region as the birthcoat samples (various ages).

TABLE 2.

(Coarse)

Sheep No.	Fibre counts P H-H	Fibre counts P HCT	Fibre counts S PCT+Hi	Ratio	Follicle counts P	Follicle counts S	Ratio	Type
12	35	71	210	1/1.98	48	106	1/2.20	Blackface x Cheviot
11	50	102	291	/1.91	55	123	/2.23	" " "
B 1	48		73	/1.67	22	41	/1.86	Blackface
B 3	31	77	78	/0.72	106	138	/1.30	"
B 5	11	26	49	/1.32	33	60	/1.81	"
B 6	9	14	44	/1.91	77	198	/2.57	"
B 8	32	65	125	/1.28	70	75	/1.07	"
B 9	37	77	151	/1.32	67	87	/1.30	"
B 10	20	43	70	/1.11	62	79	/1.27	"
B 12	14	31	56	/1.24	80	99	/1.24	"
B 14	140		149	/1.06	46	67	/1.46	"
B 15	41		73	/1.73	73	136	/1.86	"
W 1	17	39	153	/2.73	29	69	/2.37	Welsh

Table 2. As for Table 1, but for coarse birthcoats (various ages).

TABLE 3.

ROMNEY MARSH		SUFFOLK CROSS	OXFORD CROSS	BLACKFACE	
Non-N-type	N-type			(1)	(2)
1/3.83	1/3.39	1/2.30	1/2.28	1/1.30	1/2.10
/3.87	/1.78	/2.28	/1.97	/1.07	/2.35
/2.81	/2.63	/2.45	/2.61	/1.30	/1.97
/1.91	/2.38	/2.49	/2.78	/1.27	/1.86
/1.88	/2.60		/2.30	/1.24	/1.94
/1.91	/1.86		/2.50	/1.36	/2.01
/2.62	/2.62			/1.46	/1.96
/2.05	/2.61			/1.86	/2.04
	/1.77				
	/1.93				
	/3.69				
Av. 1/2.31	1/2.39	1/2.37	1/2.39	1/1.35	1/2.02

Ratios of primary to secondary follicles in samples taken from lambs within a few days of birth. The Blackface ratios are given in two series. (1) Excludes undeveloped follicles, and (2) Includes undeveloped follicles. The overall ratios were found not from the average of the individual ratios, but from the ratio of the sum of P follicles to the sum of S follicles.

THE GENETICS AND DEVELOPMENT OF 'CRINKLED', A NEW MUTANT IN THE HOUSE MOUSE.

by

D.S. Falconer, A.S. Fraser & J.W.B. King.

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1. INTRODUCTION.

The new mutant of the house mouse (Mus musculus L.) that forms the subject of this paper appeared in 1948 in the progeny of a male treated with nitrogen mustard. The discovery of the mutant has been briefly reported by Auerbach and Falconer (1949), and a short description of the mutant has been given by Falconer (1949). The question of whether the mutation was induced by the treatment or was spontaneous cannot be further discussed here. The purpose of this paper is, first, to give an account of the genetics of the new mutant, and then to describe the morphology and development of the various abnormalities produced by the gene. The chief interest of the new gene lies in the variety of the effects that it produces. The most striking of these are an abnormal texture of the coat, a bald patch on the neck behind each ear, some small kinks at the tip of the tail, reduced aperture of the eyelids, ulceration of the cornea in later life, and a respiratory disorder. The seeming diversity of these manifold effects of the gene present an intriguing problem for analysis, and because most of the abnormalities are relatively easy to study both morphologically and developmentally this new mutant forms an admirable subject for the study of gene action

at the morphological level.

The principle of the unity of gene action which as been developed by Grüneberg (see especially 1938, 1943b and 1948), leads us to expect that all the final effects of a gene are the direct or indirect results of a single abnormality of development, and that the observed pleiotropy is therefore spurious, and can be represented by a pedigree of causes originating from a single effect of the gene on the development of the organism. One of the chief objects of the present work was to obtain more evidence about the truth of this principle, since the number of genes that have been sufficiently well studied is still too small to give it any general validity. We have sought, therefore, to make a wide survey of the whole complex of the abnormalities produced by this new gene, rather than to make a complete analysis of any one of them. The results of this survey, so far as they go, are not in conflict with the principle of the unity of gene action.

Abnormal development caused by mutant genes is of interest also for the light that it may throw on the developmental processes of the normal animal. The study described here has been particularly useful in elucidating certain processes in the development of the coat of the normal mouse, and further study, if undertaken, would undoubtedly yield additional information of value in this respect.

TABLE 1.

Segregation of crinkled.

Type of mating	No. of matings	Progeny +	Progeny cr	Progeny Total	χ^2	P
cr cr ♀ x +cr ♂	5	33	39	72	0.50	0.3
+cr ♀ x cr cr ♂	4	52	50	102	0.04	0.8
+cr ♀ x +cr ♂	12	444	127	571	2.32	0.1
Combined					1.54	0.2

2. GENETICS.

Before the investigation of the pleiotropy is described it is necessary to show that the whole complex of abnormalities is produced by a single gene. The evidence is as follows. First, the whole complex arose simultaneously by mutation. Secondly, the different abnormalities never became dissociated by segregation. Thirdly, the segregation of the complex as a whole agreed with Mendelian expectation for a single recessive gene, as will be shown below. The name crinkled, with the symbol cr, was adopted for the mutant on account partly of the peculiarly folded ears and partly of the kinked tail that are characteristic of young mutant mice at the time when classification is usually made. The criteria for classification will be described in detail in the next section (see Table 2). Here it need only be said that classification could be made with certainty at the age of 10 days.

That the gene is recessive was shown by the facts that only normal offspring were produced when crinkled mice were mated to unrelated normals, and that only crinkled offspring were produced when crinkled mice were mated together. The segregations from backcross and intercross matings are given in Table 1. The backcrosses gave ratios close to the expected 1:1. The intercrosses gave fewer crinkled segregants than were expected, but the deficiency is well within the limits of sampling error. The single-factor segregations from the two types of mating have been combined by the method described by Mather (1937), and they show no significant overall deviation from expectation and no significant heterogeneity between the types. The hypothesis

of a single recessive gene was thus supported by the segregation which showed in addition that penetrance was complete and viability was not noticeably reduced before the age of classification.

The full penetrance and viability of the new gene up to the time of classification fit it for use as a potential chromosome marker. It was therefore tested for linkage with as many other markers as was practicable, and no evidence of linkage was found. The loci against which crinkled was tested were $\underline{c^{ch}}$, $\underline{p}$, $\underline{d}$, $\underline{s}$, $\underline{lx}$, $\underline{a}$, $\underline{bt}$, $\underline{Re}$, $\underline{b}$, $\underline{T}$, $\underline{fz}$ and sex. Additional tests are now in progress, and the detailed results of all the linkage tests will be published together in another paper.

During the course of the linkage tests the combination of crinkled with each of the other genes was, of course, observed. No epistatic interaction was found, crinkled being readily classifiable in the presence of each of the other mutants listed above, and each of these was classifiable in the presence of crinkled.

3. MORPHOLOGY AND DEVELOPMENT.

Description of abnormalities.

In this section the principal abnormalities exhibited by crinkled mice will be described in as much detail as can be observed without microscopic examination, and those that are suitable for the classification of segregating litters will be pointed out. In the succeeding sections some of the abnormalities will be described in greater detail and the possible relationship of all to a common cause in development will be discussed. Except where other wise stated, the abnormalities described here have been regularly observed in all the crinkled mice that have been examined.

Though, as will be described later, crinkled embryos can be recognised by their external appearance as early as the 13th day of gestation, most of the abnormalities are not visible until about 10 days after birth, when the coat has begun to appear. Four abnormalities are, however, externally visible before this. These are:

(1) The skin is noticeably thinner than that of normal mice. This can be observed soon after birth and persists throughout life. It is particularly noticeable in the tail which is recognisably thinner than the normal from 6 days onwards. Classification can then be made on this basis unless the litter contains mice that are much under-developed.

(2) About 3 or 4 days after birth the pigmentation of the skin is much less intense than that of normal mice, the

darkening of the skin of crinkled mice being delayed by about 2 days. This character can be used as a basis of classification in litters of about 4 days, except when other genes affecting the density of pigmentation are also present.

(3) A transient abnormality, from which classification can usually be made at the age of 6 days, is a smooth and shiny appearance of the skin.

(4) Between the ages of about 10 and 15 days the ears are folded in a peculiar manner, and the pinnae appear to be drawn up toward the mid-dorsal line (see plate 1B). A longitudinal fold persists for a short time afterwards, and the thinness of the skin is readily seen in the pinna of the ear.

With the growth of the coat the more important and characteristic abnormalities appear. Some of these are illustrated in the photograph of an adult crinkled mouse in plate 1A.

(5) A relatively trivial character is the absence of two or three of the five sensory hairs on each side of the face. In normal mice two sensory hairs grow from adjacent follicles immediately above the upper eyelid, one from a follicle situated behind the eye, and two from fused follicles near the angle of the jaw. The follicles from which these hairs arise have been described and figured by Grüneberg (1943b). Crinkled mice regularly lack the hair situated behind the eye and one of the two near the angle of the jaw. They usually also lack one of the two above the eye. (Among 18 crinkled

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mice examined 2 possessed both of the hairs above the eye on each side and 3 possessed both on one side only. The remaining 13 had only one on each side.) It is possible to detect the presence or absence of these hairs with the naked eye in adult mice, but they are more easily seen with a binocular microscope soon after birth. Segregating litters can be classified with certainty at birth from the presence or absence of the hair behind the eye, which is readily detectable with the aid of a small magnification. (This hair was absent from every crinkled mouse examined, amounting to at least 50, and was never absent from normals of which at least an equal number was examined.)

(6) The coat is thin and has an abnormal texture which makes it look ungroomed. The guard-hairs, which can be seen in normal mice projecting beyond the level of the rest of the coat, cannot be seen in crinkled mice. The structure of the coat is the most important abnormality of crinkled mice, and it will be fully described in the next section.

(7) A small area behind each ear is completely bald. This and the preceding character are apparent at the age of 10 days, and classification can be made then with ease and certainty.

(8) The agouti pattern is usually modified in crinkled mice, which tend to be darker on the back and yellower on the sides than normal mice. The top of the head between the ears is the region most darkened and is often completely without yellow pigment. This modification of the colour pattern was

very variable and was most marked in crinkled mice that were genetically related, by previous crossing, to the Strong-CBA pure line. It is therefore probably controlled by genetic modifiers.

(9) The tail is usually completely bald, though occasionally there are a few scattered hairs. This character alone is adequate for classification from the age of 10 days onwards.

(10) Tail rings are usually absent. These are the transverse folds in the skin which contribute to the characteristic appearance of the tail of normal mice. Irregular tail rings may sometimes be seen in crinkled mice, especially when there are some hairs on the tail.

(11) The tails of adult crinkled mice usually have one or more small sharp kinks near the tip. When young - that is, from about 5 to 14 days - the tail invariably has a number of kinks or undulations often distributed along more than half of its length. The kinks straighten outas the mouse grows up, and when ossification is complete they are confined to the extreme tip or are sometimes lost altogether, at least to outward appearance. The skeletons of the tails of some adult crinkled mice are illustration in Fig. 1. The kinking of the tail is an additional aid to classification at 6 days of age.

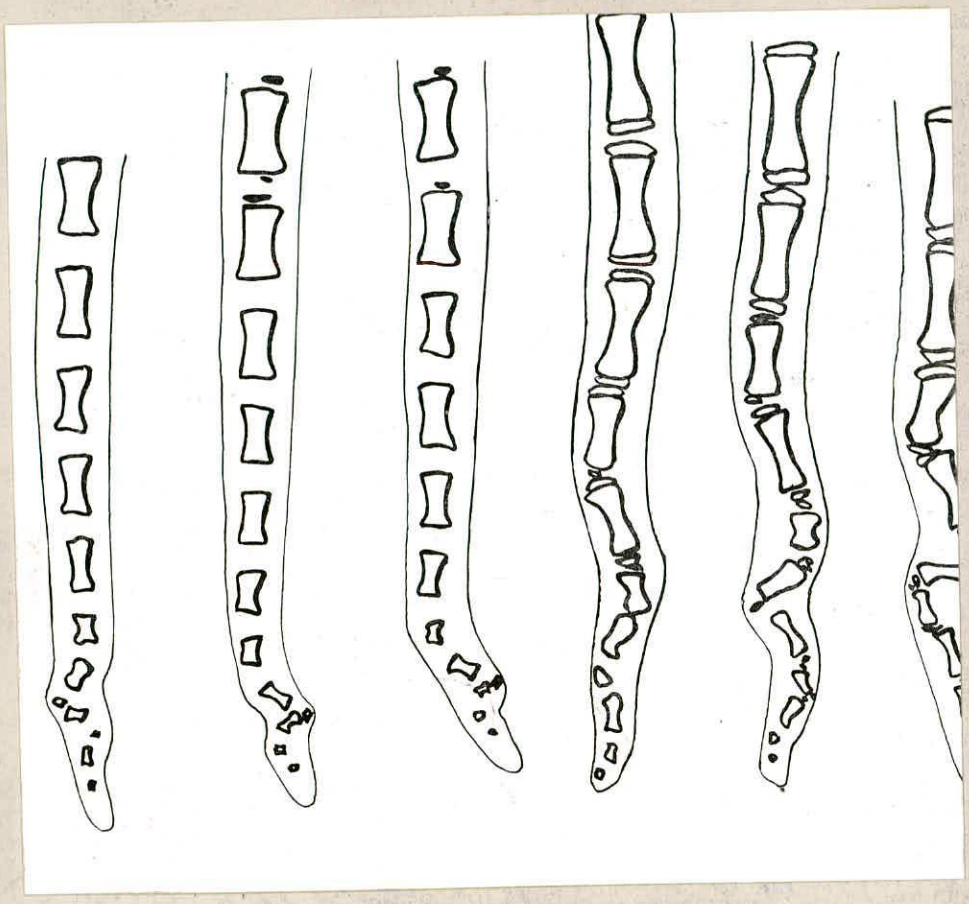

Fig. 1. Tails of crinkled mice. Camera lucida drawings of alizarin-stained transparencies. About half of each tail is shown. The three on the left are from 3-week-old mice and the three on the right from adults.

(12) From the time the eyelids open at about 14 days the aperture made by the lids is smaller than in normal mice. The diameter of the opening is often little more than half that of the normal, and though the reduction of the diameter varies it can always be easily recognised.

(13) In later life the cornea becomes opaque and often ulcerates. It cannot be said that all crinkled mice acquire corneal opacity, because this character was not carefully observed, andmany mice died or were killed before it had developed. But it is probable that most acquire it before the age of about 6 months and possibly all before 1 year.

(14) Nearly all adults exhibit a respiratory disorder best described as 'snuffling'. The sound made by the breathing of these crinkled mice resembles the infectious 'snuffling' that occurs in most mouse stocks. But the health of the crinkled mice does not seem to suffer, and the complaint does not infect normal mice inhabiting the same cage.

(15) Finally, growth is slower, mortality is higher, and fertility of females is lower in crinkled than in normal mice. Already at birth crinkled mice are smaller than their normal litter-mates. Twelve crinkled and twenty normal mice were weighed individually between birth and 3 weeks. At birth the crinkled mice were on the average about 5% lighter than their normal litter-mates, and the difference increased steadily to about 20-30% at 3 weeks.

Mortality was shown by the genetic evidence to be normal up to the age of classification at 10 days. Thereafter it increased slightly up to the time of weaning at 3 weeks, and in the week after weaning it was very high. A graph of the survival rate of crinkled mice after 10 days of age is shown in Fig. 2. The corresponding graph for normal mice, shown in the same

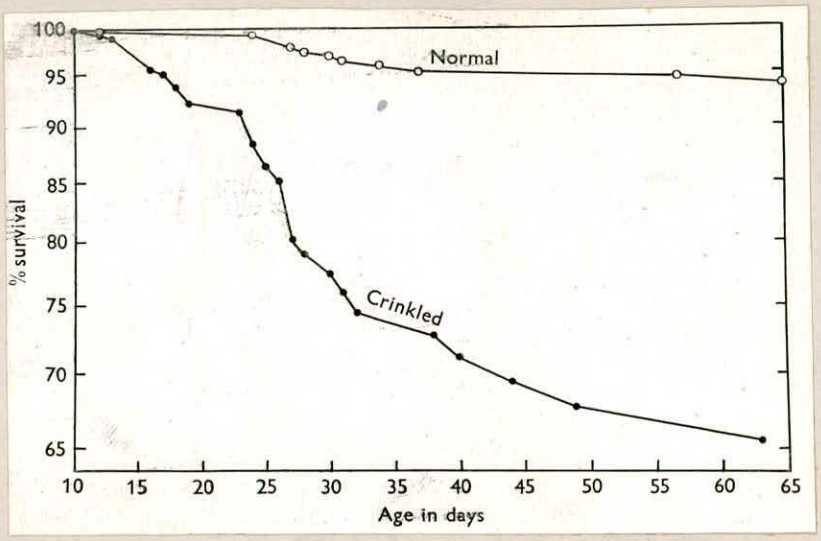

Fig. 2. **Survival of crinkled and normal mice, showing the proportion of those alive at 10 days that survived at different ages. The percentage survival is plotted on a logarithmic scale. There were 185 crinkled mice alive at 10 days, but 121 of these were later killed intentionally, most of them between 15 and 25 days. These were, of course, excluded from the estimates of survival rates at subsequent ages. The points on the graph are therefore based on successively fewer individuals, the last point, at 63 days, being based on 31. The graph for normals, which refers to the C57-black inbred strain, is based on 376 individuals alive at 10 days, of which 253 were later killed intentionally.**

figure, refers to mice of the C57-black inbred strain which was maintained under the same conditions. It will be seen from the figure that about 60% of crinkled mice alive at 10 days survived to be adults, whereas about 95% of the C57 mice survived.

The male crinkled mice that survived to be adult bred well, and the females usually did. But the litter size of crinkled females was a little below that of normals and their mothering ability was also somewhat reduced. The average number born per

litter to crinkled mothers was 7.0, while to normal mothers of the same stock it was 8.2; the average number weaned by crinkled mothers was 5.7 compared with 7.9 by normal mothers. Neither of these differences is significant.

The criteria for the classification of segregating litters are summarised in Table 2.

TABLE 2.

Summary of criteria for the classification of segregating litters.

Age of litter	Characters	Reliability
Birth	Absence of hair behind eye	Certain
4 days	Lack of skin pigmentation	Not always possible
6 days	(i) Thin and shiny skin (ii) Thin and kinked tail	)Nearly always)certain
10 days	(i) Coat texture (ii) Bald patch behind ears (iii) Bald tail	))Certain)

Structure of the coat.

The coat of the normal mouse has been very fully described by Dry (1926), and only a short description will be given here. Three main types of hair can be distinguished in the normal coat, guard-hairs, awls, and zigzags. The guard-hairs, which form only 2% of the coat, are long straight hairs which can be seen with the naked eye projecting beyond the level of the rest of the coat. The awls, which form about 28% of the coat, are also straight but are only about one-half to two-thirds as long as the guard-hairs. The zigzags, which

form the remaining 70% of the coat, are as long as, or shorter than, the awls, but as their name implies they are not straight. They have two or more sharp bends and their diameter is constricted at each bend. In addition to these three main types, another called auchenes is found in the coats of some normal mice. This differs from the awls only in having a single constriction and bend about midway along the hair. The auchenes are very variable in frequency, and they often grade into the awls through a transitional series of intermediates. For these reasons they have not been considered here as a type distinct from awls. The internal structure of the three main types differs in the number of longitudinal rows of air cells. Guard-hairs have two rows; some awls have two and some three; zigzags have one row in the straight parts, but at the constrictions the air-space is obliterated and the hair is solid. The general appearance of the three types is illustrated in Fig. 3, and their internal structure in Fig. 4. The foregoing description refers to the back of the animal. In other regions of thebody, such as the tail, feet and ears, the structure of the coat and of the hairs themselves is more or less profoundly modified.

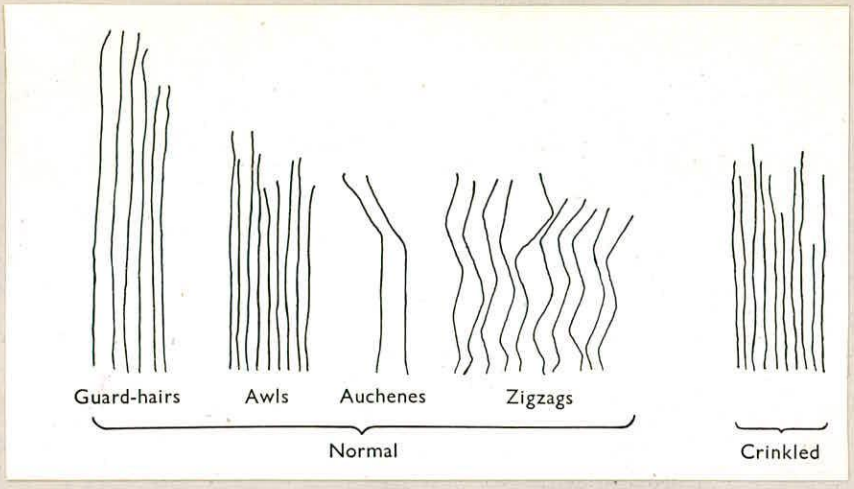

Fig. 3. Camera lucida drawings of hairs from the coats of normal and crinkled mice, to show the general appearance of the different types.

The coat of crinkled mice differs strikingly from the normal by containing only one type of hair. The hairs are all short and straight and resemble in general appearance the awls of normal mice. The internal structure, however, differs from all the hairs of normal mice, since the number of rows of air-cells is not constant throughout the length of each hair, and the diameter of the hair varies correspondingly. Regions with two rows of cells alternate with narrower regions having only one row and with broader regions having three rows. The general appearance of crinkled hairs is illustrated in Fig. 3, and their internal structure in Fig. 4.

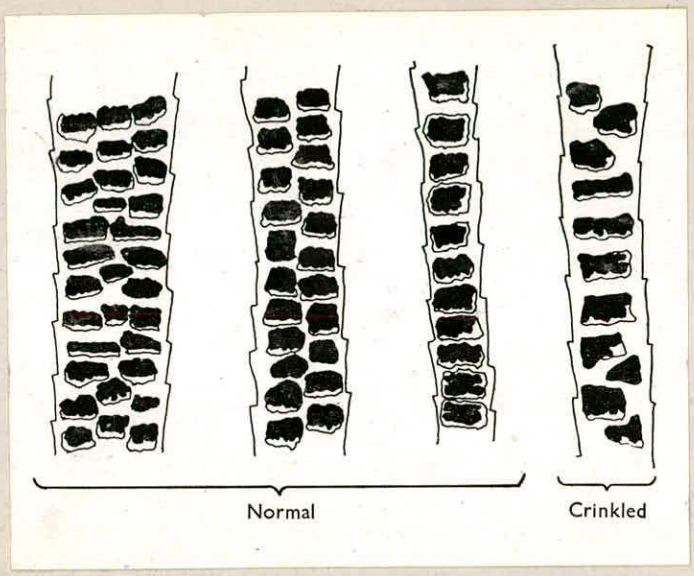

Fig. 4. Camera lucida drawings of hairs from the coats of normal and crinkled mice, to show the internal structure. On the left is a hair with three rows of air-cells, as in some awls. Next to it is one with two rows as in some awls and all guard-hairs. Next to this is a hair with one row as in all zigzags.

The abnormal structure of the coat, superficially visible as an abnormal texture, is the most significant of the complex of abnormalities produced by the crinkled gene, and its correct interpretation is fundamental to the understanding of the whole complex. Two alternative interpretations of the abnormal structure are possible. First, the crinkled gene may suppress the differentiation of the hairs into distinct types without materially affecting the total number of hairs present. Or, secondly, it may suppress the development of two of the types of hair - guard-hairs and zigzags - leaving a coat with a reduced number of hairs all homologous with the awls of normal mice. Attempts to compare the densities of the coats quantitatively were unsuccessful, but the obvious thinness of the crinkled coat

made the latter interpretation seem the more probable, and the study of the development of the coat now to be described proved conclusively that it is the correct one.

Development of the coat.

It was known that in the normal embryo hair follicles are externally visible on the body at the age of 14 days and that the follicles of the sinus hairs are visible 2 days earlier (Grüneberg, 1943a). Litters containing both crinkled and normal embryos were therefore obtained at ages from 12 days of gestation by approximately daily intervals up to 10 days after birth, when most of the hairs have probably pierced the skin. The ages were measured from the time of observation of the vaginal plug in females which were not suckling, or from the time of birth (which normally occurs 19 or 20 days after copulation), or from the external appearance of the normal embryos according to the descriptions and figures given by Grüneberg (1943a). The ages of embryos measured from the vaginal plug were always checked by this latter method and are unlikely to be more than half a day in error. The state of development of the follicles was examined in sagittal sections of the mid-dorsal region or in whole mounts of skin from the same region.

In normal embryos the development of the follicles of the back was found to take place in the following manner. The follicle primordia were first visible as thickenings of the Malpighian layer of the epidermis at 14 days of gestation.

They were then visible both in sections and in stained whole mounts, and they were also readily seen externally in fixed unstained embryos (see Plate 2A). One day later, at 15 days, these first formed follicles had grown down obliquely into the dermis, and their lower ends had expanded into a bulb below which a cluster of dermal cells formed the rudiment of the hair papilla. Newer follicles, which had not grown so far into the dermis, were then also present, some of which were no more than thickenings of the Malpighian layer. The primordia of new follicles were seen in the succeeding daily stages up to about 3 days after birth, and the follicles visible at any stage consequently differed much in size and in the depth to which they had sunk into the dermis. The largest ones were always at the greatest depth and the smallest ones nearest the surface. The difference in size persisted up to the latest stage examined, 10 days after birth, but all were then nearly at the same depth, at the bottom of the dermis. The comparison of successive stages made it clear that the size and depth of the follicles was directly related to their age, the larger ones being the older. It was clear also that the follicles formed at the later stages were more numerous than those formed earlier.

The hairs produced by the largest follicles had already pierced the surface of the skin by 1 day after birth. The more numerous smaller follicles then present had only just started to form hairs, and these did not pierce the surface till between 3

and 4 days after birth. The hairs produced by the smallest follicles of all, which were formed after birth, probably did not perce the skin till about 8 or 10 days after birth.

It seemed probable from the general impression given by the relative sizes and frequencies of the follicles and from the ages at which their hairs erupted that the type of hair produced by a follicle may be related to the age at which the follicle forms. On this hypothesis the guard-hairs would be produced by the first formed and largest follicles, the awls by the intermediate ones, and the zigzags by the smallest follicles which form principally after birth. Since it is known (Dry, 1926) that follicles nearly always produce the same type of hair in successive hair generations, a morphological and developmental differentiation of follicle types is to be expected, and the age at which the follicles form seems a likely basis for this differentiation. The three supposed types of follicle were, however, not clearly distinguishable from each other, and no marked discontinuity in the rate at which follicles were added to the population was observed. Critical evidence for establishing the connection between type of hair and time of follicle formation is therefore not available from the study of the normal development, though with more refined methods it could probably be obtained. But the development of the follicles in crinkled mice, now to be described, is strongly in favour of the hypothesis.

The development of the hair follicles of crinkled embryos

differed from that of normals in two important respects. First, instead of starting to form follicles at 14 days and continuing to form them until 3 days after birth, crinkled embryos started only at 17 days and stopped at about the time of birth. Secondly, the growth of the follicles was slower than in normals, and the appearance of the hair rudiments in corresponding follicles was delayed by about 2 days. Between 14 and 17 days crinkled embryos were readily distinguishable externally from normals by the total absence of follicles on the body (see Plate 2A). At 17 days the first rudiments of follicles were seen in sections, but they were not visible externally. These first formed crinkled follicles were therefore differentfrom the first formed normal follicles, and it must be concluded that the follicles normally formed at 14 days were absent from crinkled embryos, and not merely delayed in their appearance. At 18 days the first formed follicles had grown some way down into the dermis and the early stages of new follicles were present. New follicles were probably again present at 19 days, but after birth no rudiments of new follicles were detected in the preparations. The follicles formed by crinkled mice resembled in size and general appearance those formed by normals at the same age, except that their growth into the dermis was slower. The development of hair follicles in crinkled and normal mice is illustrated diagramatically in Fig. 5, and photographs of sections of 18-day embryos are shown in Plate 1C.

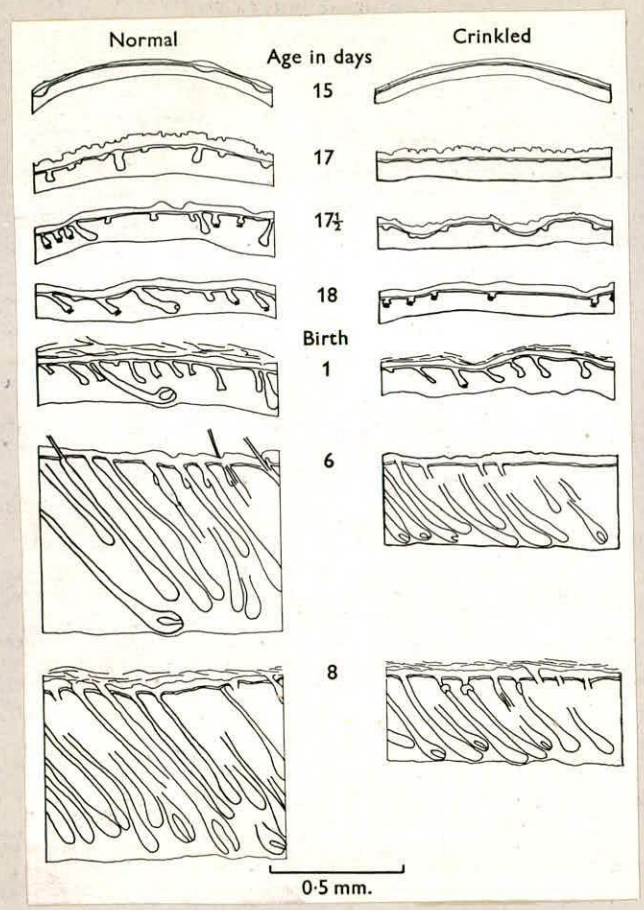

Fig. 5. Diagram of the development and growth of the hair follicles on the back of normal and crinkled mice. Camera lucida drawings of stained sections.

In consequence of the restricted period of their formation the follicles of crinkled mice are much more uniform in both size and depth than those of normal mice. Thus, from 18 days of gestation onwards, crinkled skin in both sections and whole mounts can be distinguished at a glance by the uniformity of the follicles.

The slower growth of the crinkled follicles was best seen 1 day after birth. The follicles were then clearly shorter than most of those of normal mice, and had not yet formed the hair rudiment which was already clearly visible in the corresponding

normal follicles. The hair rudiments in the crinkled follicles did not reach the stage of 1-day normals till 3 days after birth, and the hairs did not pierce the surface till between 5 and 6 days instead of between 3 and 4 days.

The embryological study described above leads to the conclusion that the hair follicles of crinkled mice correspond in their development and morphology with those formed by normal mice at the same time, that is, between 17 days of gestation and birth, and that the follicles normally formed before 17 days and after birth are absent from crinkled mice. The absence of guard-hairs and of zigzags from the crinkled coat and the resemblance of the crinkled hairs to the awls of normal mice constitutes strong evidence in support of the hypothesis that guard-hairs are produced by follicles that form before 17 days, awls by those that form between 17 days and about the time of birth, and zigzags by those that form chiefly after birth. Finally, the uniformity of type in the hairs of crinkled mice is seen to be due not to a lack of differentiation but to the absence of the follicles that normally produce two of the three types of hair.

The cause of the anomalous internal structure of the crinkled hairs was not revealed by the embryological study. It may perhaps be connected with the slower growth of the follicles. The histology of the skin and hair follicles of crinkled mice was not studied in detail. No gross differences of histological structure were, however, observed apart from

the dermis being thinner in consequence, probably, of the absence of the large deep-seated follicles. In particular, the sebaceous glands associated with the existing follicles were present, a fact that is relevant to a later part of the discussion.

Development of the sensory hairs.

The abnormality of the sensory hairs, though a trivial character in the adult mouse, is of great importance to the study of the action of the crinkled gene in the embryo, since the development of these hairs enables us to determine with great precision the age at which the first effects of the gene become manifest. The follicles from which the sensory hairs grow differ from those of the body hairs in the possession of a blood sinus surrounding the hair root, from which they are called sinus follicles. They develop two days earlier than the body follicles when the epidermis is still only one cell thick, and the mesoderm plays a more conspicuous role in their formation. The body follicles start their development with a thickening of the lower layer of the epidermis, and this causes the externally visible hump. The mesoderm forms the rudiment of the hair papilla only after the epidermal structure has started to grow down into the dermis. The sinus follicles, on the other hand, start their development with only a slight thickening of the epidermis, while at the same time the underlying mesoderm cells proliferate to form a compact mass which pushes the epidermis upwards and so forms the externally

visible tubercle. The first stages of the development of two sinus follicles are illustrated in Fig. 6.

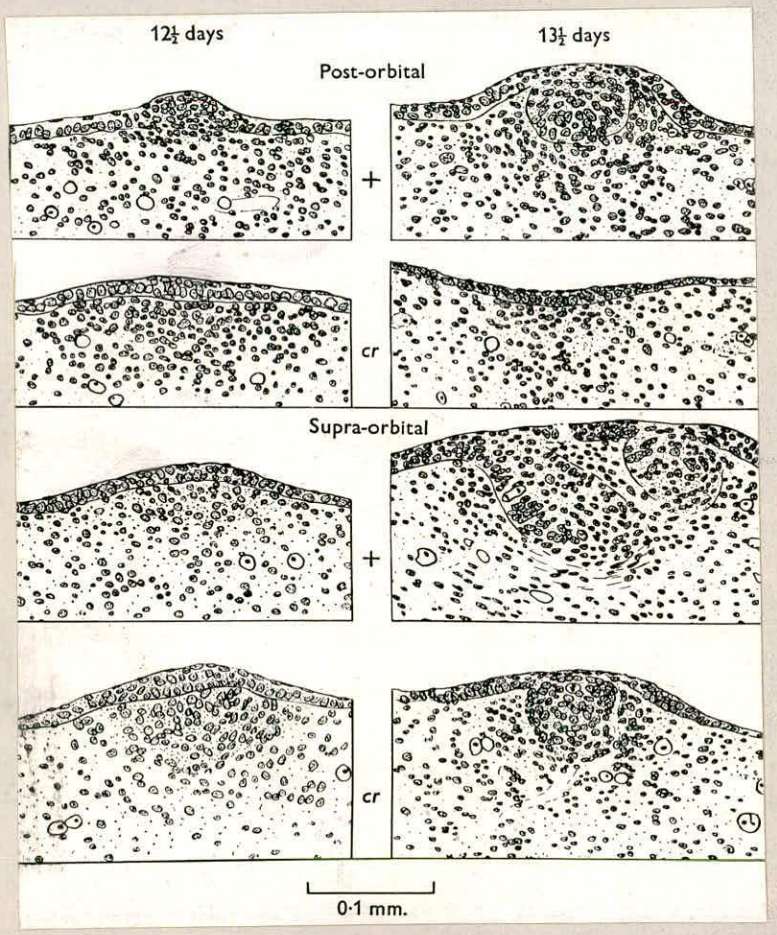

Fig. 6. Development of the post- and supra-orbital sinus follicles in normal and crinkled mice. Camera lucida drawings. The two supra-orbital follicles of the 13½-day normal were drawn from different sections, but the relative positions shown are approximately correct.

We are chiefly concerned with the five sinus follicles on each side of the face. Four of these are grouped in two pairs so that only three tubercles are externally visible.

These have been called supra-orbital, post-orbital and post-oral respectively by Grüneberg (1943b), and the ages at which they appear have been described (Grüneberg, 1943a,b). This information is summarised in Table 3 together with the number of sensory hairs that grow from each tubercle in normal and in crinkled mice. The difference in the development of these

TABLE 3.

Sensory hairs on each side of the face.

Position of tubercle	Approx. age of first appearance of tubercle	No. of sensory hairs produced in Normal	Crinkled
Supra-orbital	12	2	(1 in 80%) (2 in 20%)
Post-orbital	12½	1	0
Post-oral	13	2	1

follicles in normal and crinkled embryos was studied in two litters of 12½ days foetal age and two litters of 13½ days. It was known from the external examination of older embryos that from 14 days onwards crinkled mice lack the post-orbital tubercle. This tubercle was, however, present in all the embryos in the 12½ day litters. There were 10 embryos in each of these litters. In each litter half the embryos were expected to be crinkled, and the probability that by chance no crinkled embryo was present was therefore negligibly small. It must be concluded therefore that the post-orbital tubercle is present in crinkled embryos of 12½ days and that it subsequently disappears. In order to see whether any difference between normal and

crinkled embryos could be detected histologically frontal sections of the ten embryos of one of the 12½-day litters were examined. The follicle was clearly recognisable in all, but the state of its development varied. In five embryos it was poorly developed and in three it was well developed, while in two its state of development was intermediate. An unequivocal classification into crinkled and normal was therefore impossible. Poorly developed and well-developed follicles are illustrated in Fig. 6. The crinkled embryos in the two 13½-day litters were externally recognisable by the post-orbital tubercle being less conspicuous than in the normals. Frontal sections of two normal and two crinkled embryos were examined, and the difference in the state of development of the post-orbital tubercle was much more marked than it was in external view. In the normals the tubercle formed a conspicuous prominence with a well-differentiated follicle below it. In the crinkled embryos there was no prominence but, in contrast, the site of what appeared externally to be a poorly developed tubercle was marked by a slight depression. This was seen on both sides of both embryos, and it is illustrated in Fig. 6. The epidermis of the depression was slightly thickened and the underlying mesodermal cells were slightly concentrated. There is little doubt therefore that the first stage of the development of the post-orbital sinus follicle in crinkled embryos is normal, and that the action of the crinkled gene intervenes at 12½ days and prevents the further development of the follicle, which then regresses.

The supra-orbital tubercle in all of the ten 12½-day

embryos that were sectioned consisted of only a single follicle rudiment. But in the 13½-day embryos it contained two well-formed follicles in the normals and only one in the crinkled (see Fig. 6). It is uncertain whether the second follicle of the normals forms from a separate rudiment which could not be recognised in the 12½-day embryos, or whether it forms by the splitting of a single rudiment. However that may be it is clear that the second follicle fails to form in the crinkled embryo, and the time at which the gene acts on this follicle is therefore the same as for the post-orbital follicle. It must be supposed that when the crinkled gene starts to have an effect the first supra-orbital follicle has passed a critical stage and become autonomous in the sense that it does not require the action of the normal allele for its continued differentiation, whereas the second supra-orbital and the post-orbital follicles have not reached this critical stage and consequently fail to continue their differentiation.

The post-oral follicles were difficult to observe and less attention was paid to them. They were not detected with certainty in any of the 12½-day embryos. In the 13½-day embryos they were single in the crinkled and probably double in the normal ones. It seems probable therefore that the development of this pair of follicles is similar to that of the supra-orbital pair.

There are many other sinus follicles on the face, those on the snout which form the whiskers being the most conspicuous.

Their development was not studied in detail because no obvious abnormality was detected in the crinkled embryos, which seemed to possess the full complement of whisker follicles.

The later development of the sinus follicles of crinkled embryos was marked by the same retardation as was observed in the ordinary follicles of the body; the follicles grew more slowly and their hairs erupted later than in the normals.

It is interesting to note that the post-orbital sinus follicle is usually lacking in mice with congenital hydrocephalus (Grüneberg, 1943b). This was shown by Grüneberg to be a secondary effect of the gene, caused probably by the extra tension in the skin of the face resulting from the swelling of the cranium. There is no evidence that an abnormal skin tension plays any part in the suppression of follicle formation in crinkled mice.

In connection with the study of the pleiotropy it is important to decide whether the failure of the skin to form follicles originates in the epidermis or the dermis. When an ordinary body follicle first becomes visible the only differentiation is in the epidermis, and the dermal contribution to the follicle appears only after the epidermal part has differentiated. Therefore, unless there is an unknown and invisible centre of induction in the dermis, the initiation of the follicle occurs in the epidermis. Since the missing follicles of crinkled mice do not even form the first epidermal differentiation the defect probably lies in the epidermis.

Though the mesodermal contribution to the sinus follicles is more massive and appears earlier, it is probable that here too the initiation of the follicle comes from the epidermis, since the mesoderm is then not yet differentiated into dermis. The failure to form the missing sinus follicles is therefore probably due also to a defect of the epidermis. It is clear, furthermore, that the gene's action is in the epidermis as a whole, and not in the follicles themselves, because apparently identical follicles are affected differently according to their time of formation.

Analysis of the pleiotropy.

In the embryological study described above, the cause of the abnormal coat structure of crinkled mice was traced to the failure of the epidermis to form follicles during certain periods. In addition, the growth of the follicles that did form was found to be slower. It seems probable that these two errors may be causally related to each other through a more fundamental error not yet discovered, but a deeper investigation of this point was not attempted. We must now examine the other externally visible abnormalities of crinkled mice in order to see whether they can be shown to result directly or indirectly from one or other of these two developmental errors, or whether they reveal any additional action of the gene unrelated to the two errors already discovered. It was not possible to investigate the various abnormalities thoroughly enough to lead to a complete understanding of their causation, and several of them would certainly repay

further study. Enough was done, however, to show that all could be regarded as probable, or at least possible, results of the abnormal development of the coat already discovered. But at the same time the study of some of the abnormalities showed that the action of the gene on the development of the coat is not as simple as was at first supposed.

The abnormalities discussed below are numbered in accordance with the list on p. , though they are taken in a different order. The abnormal texture of the coat (No. 6) and the reduced number of sensory hairs (No. 5) have already been sufficiently discussed.

(2) Pigmentation of the skin. The pigmentation seen in the skin of normal young mice before the hairs have grown is not, strictly speaking, in the skin itself but in the hairs developing in it. The reduced pigmentation of crinkled mice at this age is therefore simply the result of the slower development of the hairs. The delay of 2 days in the formation of the hairs, which was found by the microscopic examination of the skins, agrees well with the observed delay in pigmentation.

(3) The shiny appearance of skin when compared with normal mice at 6 days of age is also due to the delayed development of the hairs, which have then erupted in the normal but not in the crinkled mice.

(8) Agouti pattern. The agouti pattern of normal mice is produced by a band of yellow pigment near the tip of most of the hairs. The width of the band differs in different regions of

the body, and thus the gradation of tone between back and belly is produced. The width of the band differs also in different individuals, thus giving rise to the considerable variation of shade found in agouti mice. Not all the hairs possess an agouti band. According to Dry (1928) no guard-hairs but all zigzags possess it, while some awls possess it and some do not. Therefore the absence of the zigzags in crinkled mice would be expected to cause a general darkening of the colour, since the proportion of hairs with the agouti band would be reduced. This general darkening, however, does not occur, probably for the following reason. The presence of an agouti band is probably controlled, to some extent at least, by the time at which the hair is formed, the first formed tending to lack the band and the last formed to have it. The delay of about 2 days in the formation of the crinkled hairs would therefore increase the proportion of those that have the agouti band, and the expected tendency to darkening would thus be counteracted.

The abnormal agouti pattern is not a general change affecting the whole body in the same way, but a change in the differentiation of back and sides. Its cause is to be sought in a comparison of the distribution of the agouti band among the hairs of the back and of the sides. Samples of hairs were therefore taken from the head between the ears and from the flank, and the proportion of hairs without an agouti band was determined. Eleven normal and five crinkled mice were thus

sampled. The guard-hairs and zigzags of the normals were irrelevant to the problem since they are not present in crinkled mice, but they confirmed Dry's statement mentioned above; all of 28 guard-hairs were non-agouti and only 5 of 1865 zigzags were non-agouti. The frequency of non-agouti awls varied much from mouse to mouse. The mean frequency of the 11 normal mice was 53% non-agouti on the head and 19% on the flank. Normal mice therefore show a considerable difference between head and flank in the frequency of non-agouti awls. This difference was found to be greatly exaggerated in the crinkled mice, though there was much variation between mice. The mean frequency was about 90% non-agouti on the head and about 2% on the flank.

The reason for this exaggerated differentiation between back and sides is not known, but it is possibly connected with the retarded development of the hairs.

(1) Thin skin. The thinness of the skin, which is most conspicuous soon after birth, may be simply explained by the lack of the largest follicles that form between 14 and 17 days in normal mice, and by the slower growth of the follicles present. The skin's growth in thickness was seen in the sections to keep pace with the downward growth of the follicles in both normal and crinkled mice (see Fig. 5). It seems probable, therefore, that the thickness of the skin is determined by the growth of the follicles.

(7) Bald patch behind ears. The hair immediately behind the ears of normal mice is thin and fine, and an examination showed it to consist entirely of zigzag hairs. Farther away

from the ears awls appeared and became more numerous as the distance from the ear increased. The baldness of this region in crinkled mice is thus the consequence of their failure to form zigzag hairs. Furthermore, normal mice were found to form hair follicles in this region only after birth, since no follicles were present there in sections of new-born mice. The absence from this region of guard-hairs and awls in normal mice and of all hairs in crinkled mice is thus in accordance with the hypothesis that the follicles formed after birth are mainly those which produce zigzag hairs.

(4) Pinna of the ear. The hairs on the pinna of the ear introduce a complication, because the follicles on the pinna of normal mice were found to form only after birth, as in the region behind the ear, and yet crinkled mice have hairs on the pinna. Sections of new-born crinkled mice resembled those of normals in the absence of follicles on the pinna. Therefore in this region crinkled mice are able to form follicles after birth. The hairs on the pinna are probably not much less dense than in normal mice, but they are very much shorter and thinner, and are thus not entirely normal. It seems necessary therefore to postulate a general delay in follicle formation in this region of both normal and crinkled mice, so that the follicles would be homologous with the awl type on the body, and would thus be formed by crinkled mice.

The peculiar folding of the pinna in young crinkled mice

has not been carefully studied. It is probably caused partly by an abnormal tension in the skin of the head resulting from the lack of follicles there, and partly by the thinness of the pinna due to the small size of the follicles in it, the smallness of the follicles being inferred from the abnormally small size of the hairs that they produce.

(9) Baldness of tha tail. Sections of the tails of crinkled mice showed a complete absence of follicles, as was to be expected from the absence of hairs. The study of the development of hair follicles in the tail of normal mice disclosed the surprising fact that no follicles are formed between 17 days of gestation and 2 days after birth. One-third of the follicles were already well developed at 17 days and probably formed at 16 days of gestation. The remainder formed between 2 and 3 days after birth, and were arranged regularly one on each side of the existing follicles. Thus no follicles form in the normal tail during just that period during which crinkled mice are able to form follicles. This fact is clearly the explanation of the baldness of the crinkled tail. The presence of a few hairs on the tails of some crinkled mice probably means that there is sometimes a slight overlap of the end of the first period of follicle formation on the normal tail with the beginning of the period in which crinkled mice form follicles.

(11) Kinking of the tail. In order to study the kinking of the tail of crinkled mice longitudinal sections of the distal half of the tails of normal and crinkled mice from birth to 6 days

were examined. Up to 2 days the skeleton was entirely normal. At 3 days, however, it was bent in a sinuous curve, which later became corkscrew-shaped and deformed the outward shape of the tail where the bends pressed closely against it. Plate 2B illustrates the tails of normal and crinkled mice 2 and 3 days after birth. The cause of the kinking of the skeleton is probably as follows. The kinking appears to be simply a mechanical result of the sheath of skin elongating more slowly than the skeleton which it surrounds. To accommodate its extra length the skeleton therefore twists within the sheath of skin which is not sufficiently compact to keep it in its place. A comparable kinking of the medullary tube within its mesodermal surroundings occurs in the embryos of pseudencephalic mice and was attributed by Bonnevie to the same cause (quoted by Grüneberg, 1943, p. 101). Since there is no reason to suppose that the skeleton of the tail grows faster in crinkled than in normal mice, the difference of growth rate is more probably due to a retardation in the growth of the skin. This in turn is probably due simply to the absence of follicles which in the normal tail constitute a large fraction of the bulk of the skin. This may be seen in Plate 2B, particularly at the top of the picture of the 2-day tail where the section has become partly tangential. The absence of the follicles would therefore materially reduce the length of the skin sheath, and the kinking might follow when the force of compression on the skeleton exceeded a certain limit. The subsequent straightening of the

skeleton which leaves the adult tail with kinks only at the tip may possibly be the result partly of the elongation of the vertebrae which would then be less easily accommodated when lying obliquely within the tube of skin, and partly to a compensatory growth of the skin in response to the abnormal tension. Though the foregoing explanation of the kinking of the crinkled tail is little more than conjectural, it seems sufficiently credible to remove the need for postulating an additional action of the crinkled gene.

(10) Tail rings. Tail rings form in normal mice 3 days after birth when the hairs of the tail have pierced the skin. They can be seen in whole mounts of skin as transverse folds through which the hairs pass. Their absence from most crinkled mice is probably the result of the absence of hairs, though the tension in the skin discussed in the previous paragraph may be a contributory cause.

(12) Eyelids. Detailed examination of the eyelids of crinkled and normal mice revealed two important defects in the crinkled lids. First, the number of eyelashes was much reduced, both the longest and the shortest lashes being absent. Secondly, structures interpreted as Meibomian glands were absent. In the normal eyelids these form conspicuous masses resembling sebaceous glands in histological structure. Their absence from crinkled eyelids was particularly striking. Since the Meibomian glands are specialised sebaceous glands their development is presumably dependent on the presence of certain hair follicles

or at least of the follicle primordia. Since the ordinary sebaceous glands associated with hair follicles were present in crinkled mice, the cause of the absence of the Meibomian glands must be sought in the suppression of follicle formation. It was necessary, therefore, to find out whether the primordia from which the Meibomian glands develop in the normal are formed during either of the periods of follicle suppression. Sections through the eyelids of new-born mice were examined. Little difference between the crinkled and normal eyelids was found. There were a few recently formed follicles along the margins of the lids, but no trace of anything resembling sebaceous or Meibomian glands even in the normals. This shows that the Meibomian glands of the normal are formed after birth, when crinkled mice are unable to form follicle primordia, and the failure of crinkled mice to form the glands is thus a consequence of the general suppression of follicle formation after birth. The reduced number of eyelashes is seen also to result from the same cause, since the majority of follicles in the normal eyelids form after birth.

The reduction in the size of the opening between the eyelids of crinkled mice is probably the direct result of the absence of many eyelash follicles, which occupy a considerable volume in the edges of the normal eyelids, and perhaps also of the lack of Meibomian glands.

It is interesting to note that an absence of Meibomian glands is occasionally found in man, and that they are then replaced by an extra row of malformed eyelashes (Wolff, 1948).

It seems probable that the primordia form more or less normal follicles instead of specialising as Meibomian glands. The function of the secretion of the Meibomian glands in man is said to be to prevent the tears from wetting the edges of the eyelids and so escaping.

(13) Cornea. The opacity of the cornea which develops in later life is probably due to the formation of scar tissue following mechanical injury, which may sometimes result also in ulceration. It was thought at first that mechanical injury would result from the incomplete protection afforded by the deficient eyelashes of crinkled mice. This hypothesis is disproved, however, by the fact that hairless mice (hr/hr) which lose all their eyelashes at about the age of weaning retain healthy corneas. The eyes of some hairless mice were examined at the age of about 4 weeks, and it was found that all the eyelashes had been shed and some of these were found underneath the eyelids where they formed a matted ring surrounding the eyeball; yet the cornea was quite undamaged. The corneal defect of crinkled mice is therefore probably due to the absence of the Meibomian glands, whose secretion has presumably a protective function.

(14) Respiratory disorder. It was thought at first that the snuffling of crinkled mice might be due to an infectious disease to which their deficient covering of hair might render them more susceptible. It soon became clear, however, that the condition was not infective to normal mice; and the fact that

hairless mice which have no protection of hairs at all do not seem to be particularly prone to the disease made this explanation seem very improbable. In order to find out if the snuffling had any obvious anatomical cause some dissections were made of the nasal cavities of crinkled mice. These revealed the astonishing fact that snuffling crinkled mice had the nasal cavities partly blocked by large masses of hair matted together by mucus. Ten crinkled mice were thus dissected, and hair mats were found in all except four which did not show any evidence of the disorder when they were killed. One of these was only 1 month old; the other three were 3 months, and of these one had a few hairs in each nostril, not yet matted nor blocking the nasal cavity. Three normal mice inhabiting cages with crinkled mice were similarly dissected and no hairs were found. The hairs in the nasal cavities were not growing in situ, but were ordinary body hairs. Most seemed to come from the mouse itself, but in at least one case hairs belonging to another crinkled mouse in the same cage were identified by their colour. Four crinkled mice sharing a cage with a normal mouse were carefully examined, but no hair belonging to the normal mouse was found in the nasal cavities. No explanation of this strange abnormality can be offered. It can only be said that the shape of the nostrils of crinkled mice appeared to be normal, and that protective hairs, which might have been absent from crinkled mice, were not found in the nostrils

of normal mice. It seems probable that the hairs enter the nostrils while the mouse is grooming itself.

(15) Growth, mortality, and fertility. Many of the mutant genes of mice are known to reduce viability, growth rate, and fertility, particularly when their effects are drastic. The effects were therefore not unexpected in crinkled mice, and no special attention was paid to them. Most of the deaths occurred soon after weaning at a time when the mortality of normal mice is at its highest. It is probable therefore that the mortality in crinkled mice, as well as the reduced growth rate and fertility, are due to an unspecific lowering of viability rather than to a specific pathological cause.

4. DISCUSSION.

Studies of the morphological effects of mutant genes have much to contribute to the general understanding of gene action. The most important problems that can be studied in this way are those connected with regional and temporal differences in the gene's action, which may be connected with the differentiation of tissues during morphogenesis. Regional differences are studied by the analysis of pleiotropy, and temporal differences by the determination of the times at which the gene acts during development. These two aspects of the present study must now be discussed.

Pleiotropy. The principle of the unity of gene action may be taken as a convenient working hypothesis to guide the study of the pleiotropy. The fundamental postulate that the primary product of the gene is a single chemical substance cannot be directly studied by the morphologist. It can only be inferred if a single primary effect of the gene is demonstrated. For the morphologist the most important postulate contained in the principle is that of tissue-specificity (Grüneberg, 1943b): the primary morphological effect of the gene must be shown to be either cell-specific or tissue-specific. Many genes are obviously cell- or tissue-specific in their main visible effects. A gene that influences pigmentation, for example, has visible effects only in tissues that contain pigment-forming cells. The question is whether genes that visibly affect several different tissues act directly

on each of them, or directly on only one and indirectly on the others. It is not our intention to review the previous work on this aspect of pleiotropy, but only to point out that few cases have been analysed and these have not all led to the same conclusion. Two genes analysed by Grüneberg (1938, 1943b), for example, were found to have primary effects that were tissue-specific; but the macrocytic anaemia of the mouse, studied by Russell (1949), appears to act directly on two unrelated tissues, though it is possible that the primary action of this gene has not yet been identified. Each case must be judged separately, and it is therefore important to add to the number of analyses of pleiotropic genes.

The pleiotropy of the crinkled gene involves a wide variety of organs and characters - the skeleton of the tail, the structure and colour of the coat, the eye, and the respiratory tract. The analysis of these abnormalities has shown with a greater or less degree of certainty that all are caused by a defect of the epidermis. The postulate of tissue-specificity has therefore been shown to hold in this case. The study of the pleiotropy may now be summarised in the pedigree of causes shown in Fig. 7. Though the primary effect of the crinkled gene has been shown to be confined to one tissue, the epidermis, a single primary effect has not yet been fully demonstrated, because two distinct effects on that tissue have been found and a causal

connection between them has not been proved. These are the failure to form certain hair follicles, and the slow growth of those follicles that do form. That a causal connection exists can hardly be doubted. The slow growth may itself be the primary effect andthe suppression of follicle formation merely a limit of zero growth. But it seems better, until more evidence is available, to postulate the existence of a primary effect not yet identified, of which the slow growth and the suppression of follicle formation are separate manifestations. This primary effect might, for example, be a reduced rate of division of the epidermal cells, or the lack of a single substance which might be the final product of the normal gene.

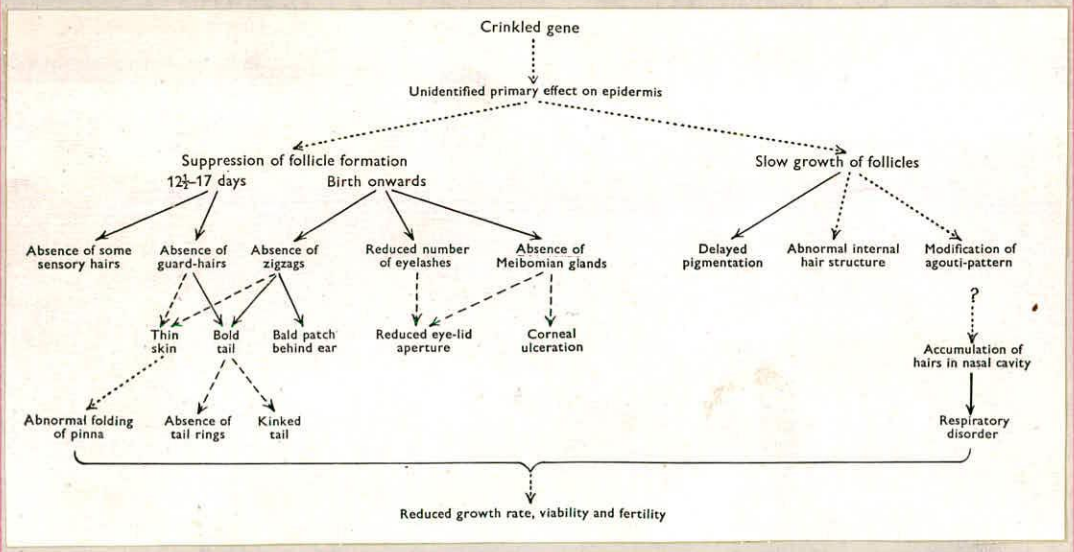

Fig. 7. Pedigree of causes showing the origin of the pleiotropy of the crinkled gene. Not all the causes have been demonstrated with equal certainty. Those that we regard as proved beyond reasonable doubt are shown with a solid line: those that we regard as only probable with a broken line, and those that are no more than conjectural with a dotted line.

Two more epidermal defects whose causal connection with the first can only be inferred are the abnormal internal structure of the hairs and the exaggerated dorso-ventral differentiation in the agouti-banding. The interrelations of these different epidermal effects should clearly form the starting point of further study.

The study of the regional differentiation of the effects of the crinkled gene has shown that the primary effects are confined to the epidermis. Regional differentiation within the epidermis must now be considered. Grüneberg's (1943b) third postulate is: The specificity of the gene for a particular cell or tissue being established, it must be examined whether all representatives of this tissue are uniformly

affected throughout the body. If it is found that certain regions of the tissue are either immune or more strongly affected than others, the reasons for these local differences of gene effect must be elucidated. The epidermis of all the regions examined were found to be affected by the crinkled gene. But, on the other hand, the different types of hair follicle in every region of the body were very differently affected. The reasons for these differences can only be understood by the study of the temporal differentiation of the gene's action, which will now be discussed.

Time of action. The action of many genes seems to be continuous from the time the tissue in which they produce their effect is differentiated. Some of the effects of the crinkled gene behave in this way. The slower growth of the hair-follicles is found in all the follicles no matter when they form, and the effects on the structure and colour of the hairs continue throughout the life of the animal. But in its effect of suppressing the formation of follicles the gene is remarkable for the changes that occur during development. At first no effect is apparent. The sinus follicles differentiate normally up to the foetal age of 12½ days. Then the effect appears and the differentiation of new follicles is suppressed, while one sinus follicle that has started its differentiation regresses. At 17 days, however, the effect disappears and new follicles start to differentiate. This continues till about the time of birth when the effect appears again and once more the differentiation of new follicles is suppressed. At about 3 or 4 days after

birth the differentiation of new follicles in the normal ceases, and this aspect of the action of the crinkled gene cannot be followed further. These changes are responsible for the different effects of the gene on the different types of hair-follicle because these start their differentiation at different times. Some sinus hairs and the guard-hairs are affected by the first period of the gene's effect, and the zigzag hairs are affected by its second period. The awls, however, escape suppression because they start their differentiation while the gene is not having any effect.

Much remains to be learned about the action of the crinkled gene, even on the morphological level, but the present study has at least mapped out the ground in some detail. It has shown that the action of the gene is tissue-specific, being confined to the epidermis, though the final effects are found in several different organs and tissues. It has shown also that there is no regional differentiation of action within the epidermis, but that temporal differentiation plays a very important part in deciding the final effects of the gene. The morphological analysis therefore shows clearly where attention should be directed if the analysis is to be carried forward by physiological or biochemical methods.

5. SUMMARY.

1. 'Crinkled' is a new recessive gene of the house mouse that segregates normally and does not reduce viability up to the age of classification.

2. It has many widespread morphological effects, the chief of which are: (i) Absence of guard-hairs and zigzag hairs. (ii) A bald patch behind each ear. (iii) Bald tail. (iv) Kinds at the tip of the tail. (v) Reduced aperture of the eyelids. (vi) Corneal ulceration. (vii) Respiratory disorder. (viii) Modification of the agouti colour pattern.

3. All these defects have been traced with more or less certainty to the gene's effects on te formation and growth of the hair follicles, the effects on tissues other than the epidermis being secondary. A pedigree of causes summarising the analysis of the pleiotropy is given in Fig. 7.

4. The two most important effects of the gene on the epidermis are: (i) The formation of new hair follicles is suppressed between 12½ and 17 days of gestation, and again from the time of birth onwards. (ii) The growth rate of those follicles that do form is slowed.

5. The first period of follicle suppression accounts for the absence of guard-hairs, and the second period for the absence of zigzags. The absence of these two types of hair accounts for the bald patch behind the ear and for the bald tail. The kinks in the tail and the reduced eyelid aperture are probably due to the absence of follicles which cause a tension in the skin.

6. The suppression of follicle formation after birth results in the absence of the Meibomian glands of the eyelids, and this probably leads to the ulceration of the cornea.

7. The respiratory disorder is caused by an accumulation of hairs from the body in the nasal passages. The reason for their presence there is unknown.

8. The modification of the agouti pattern is probably related to the slow growth of the hair follicles.

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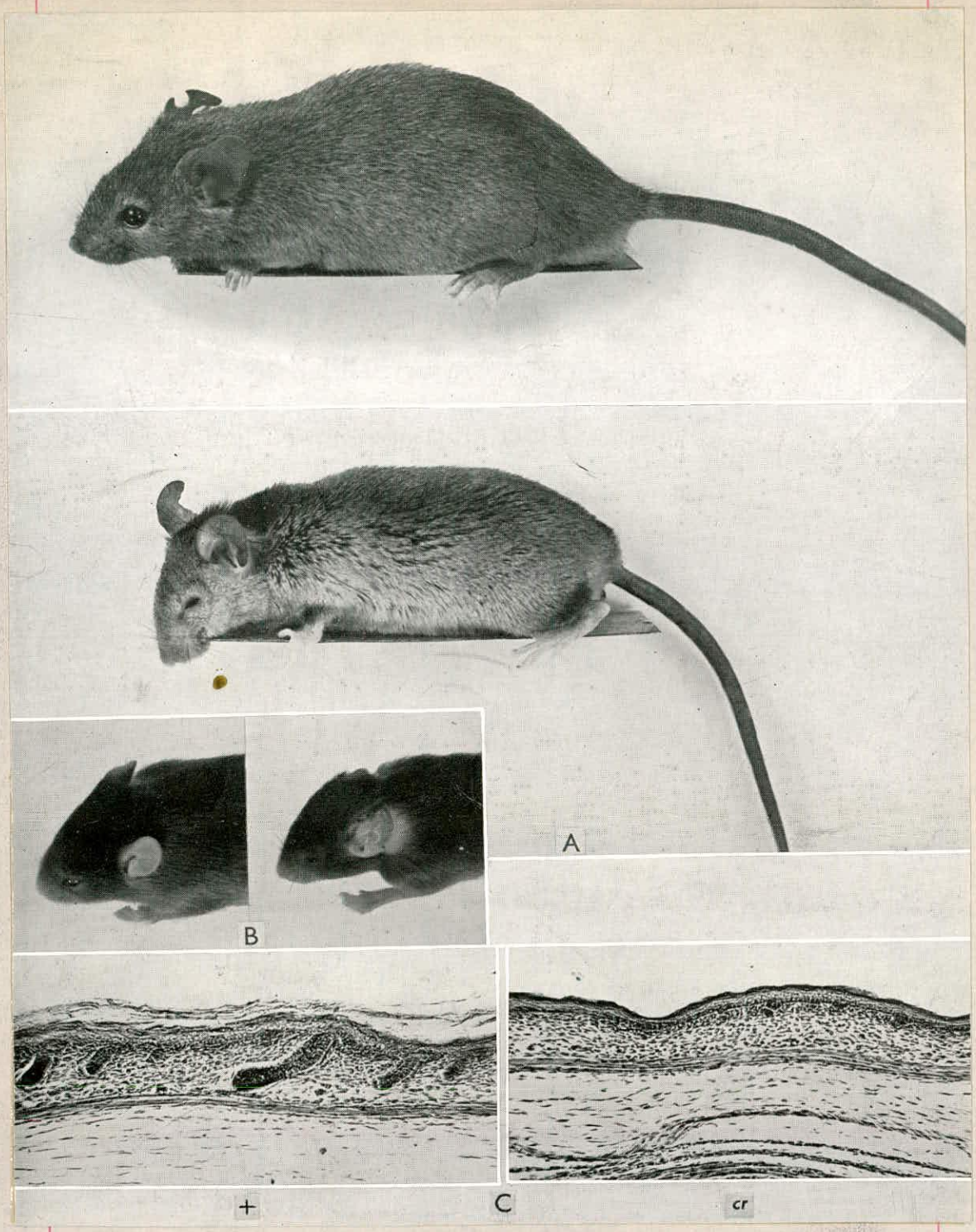

Plate 1. A. Normal and crinkled males, 4 months old. The normal (above) is from the CBA inbred line; the crinkled has 31/32 of the CBA genotype.

B. Heads of normal and crinkled mice 12 days old, to show the ears. Normal on left.

C. Sections through the skin of the back of normal (left) and crinkled (right) embryos of 18 days foetal age.

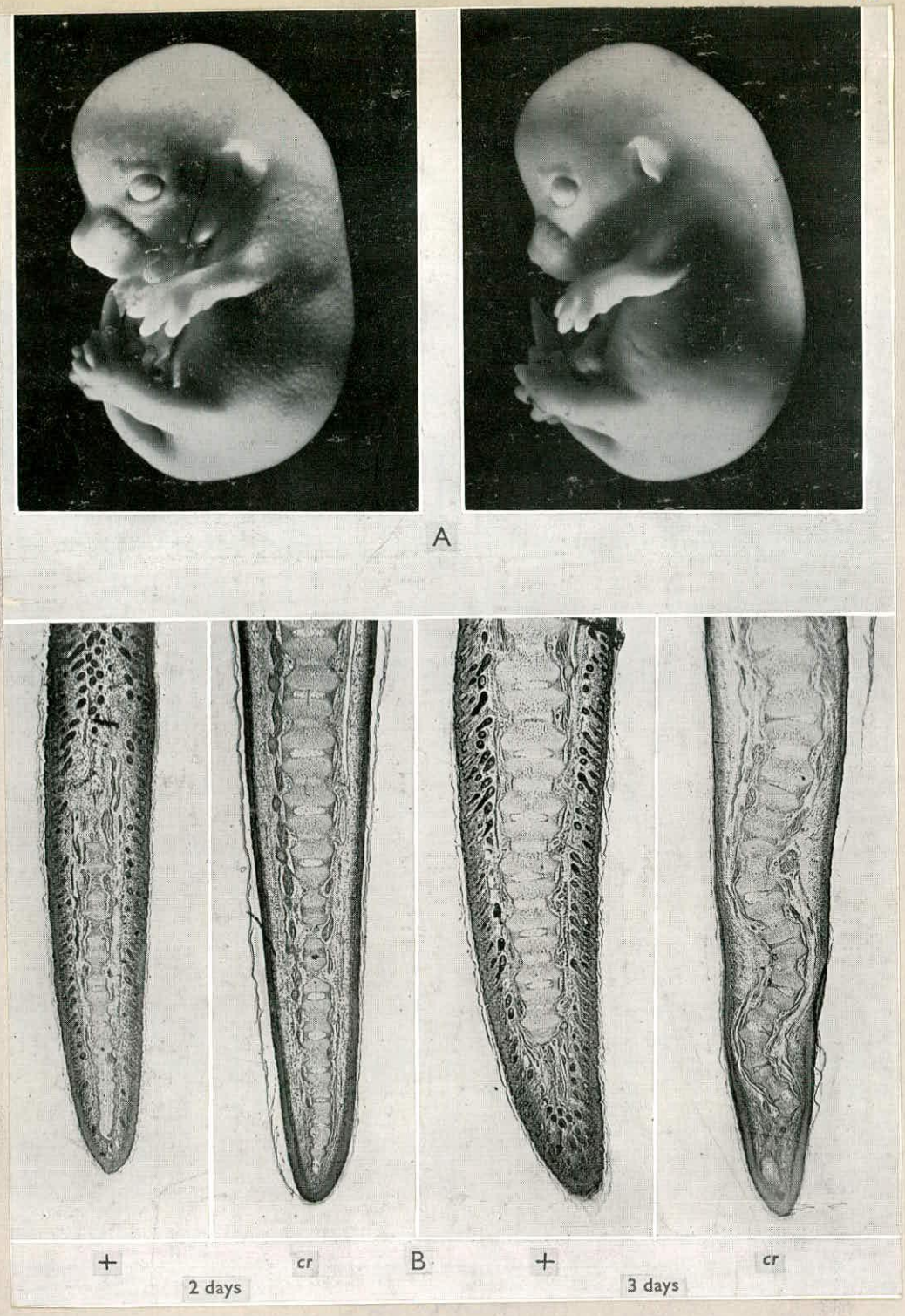

Plate 2. A. Embryos from a litter of 15 days gestation. Normal on the left, crinkled on the right.

B. Longitudinal sections through the tails of normal and crinkled mice, 2 and 3 days after birth.

GROWTH OF THE REX AND ANGORA COATS

by

A. S. Fraser.

In rabbits, as in most mammals, the skin follicles form a succession of complete hairs, alternating each growth period with a rest period during which the follicle decreases in size. After forming a hair a follicle may immediately shed it, or retain it passively for some time, thus retaining continual cover. Apart from the growth of the first pelage it is not usual for more than a small region of skin to be in the growth phase at any one time. Dry (1926) in the mouse, and Haddow et al. (194) in the rat have shown that the occurrence of the growth phase passes like a wave in an anter-posterior direction over the main trunk. Examinations of new born mice and rabbits show that the commencement of growth of the coat passes in a wave from head to tail. The cessation of growth of the first pelage presumably also passes in a wave from head to foot (Fraser 1951).

Variation of the length of the coat can therefore occur from variation of two causes: the fibre growth rate and the duration of the growth phase. Two genes occur in the rabbit which affect the length of the coat. In Rex rabbits the coat is shorter, and in angora rabbits the coat is markedly longer. Both the rex (r) and the angora (l) genes are recessive, and they show independent segregations indicative of a lack of, or a loose linkage.

Preliminary observations were made of the effects of these genes from crosses segregating for both Rex and Angora. The rex gene in addition to the shortening of the coat, also

causes a curling of the whiskers from which litters can be separated into Rex and non-Rex soon after birth, before growth of the coat has commenced. Once the coat has commenced growing it becomes increasingly obvious that the Rex coat grows more slowly than the non-Rex coat. No differences could be seen within the non-Rex group, either of age of commencement or rate of growth until 3-4 weeks after birth when it becomes increasingly apparent that the growth of the angora coat continues for a longer time than the normal coat. This is first noticeable around the head, and later on the rest of the body. The growth of the Angora coat continues until about 8 weeks after birth at least. Observations of the growth of the angora coat were not made after 2 months.

It can therefore be adopted as a working hypothesis that the rex gene causes a decrease of the rate of growth of the coat without affecting the duration of the growth phase, and the angora gene causes an increase of the duration of the growth phase without affecting the rate of growth. The latter point was deduced by Iljin (1945) from results of thallium treatment. If an animal is treated with a thallium compound the coat ceases to grow and after a few days it is shed, leaving the skin naked. This occurs only for those regions which are actively in the growth phase, no effect being discernible in regions in which the follicles in the resting phase. Iljin found that thallium treatment of Angoras always results in complete shedding all over the body, whereas the same treatment of normals results in only small regions shedding their hairs i.e. the growth period may be interrupted in various regions by thallium treatment of normals depending on age at treatment, but in angoras the growth phase occurs continually all over the

animal.

These observations indicate two methods by which useful data could be collected on the modes of action of the rex and angora genes. (1) Thallium treatment of rex, angora and normal sibs made at a sequence of ages, which should allow the duration of the growth phase to be estimated since if animals of a certain age shed their coat after treatment they can be concluded to have been in the growth phase at that time, whereas if they show no effect of treatment they can be concluded to be in the resting phase; (2) Comparison of the lengths of the coat at a sequence of ages. This will allow measurement of rate of growth, and estimation of the duration of the growth phase, and has been used in the mouse by Fraser (1951).

These two methods have been used in studies of a limited number of Rex, Angora, Rex-Angora and Normal rabbits. To simplify, only the growth of the first pelage is considered.

Treatment with Thallium.

Throughout the experiments involving Thallium treatment, thallous acetate was used, at a concentration of 1 mgm. per cc. of distilled water, to give a dosage of 10 mgm. per kilogram of body weight. The intra-peritoneal route was used.

The first experiment was to inject a litter of 2 Angoras and 2 normals, 28 days after birth. After a lag of about 6 days the Angoras completely shed their coats. No effect was discernible in the normals, showing that growth of the first coat has ceased in normals at some age before 28 days, but not in the Angoras.

The second experiment was designed to test whether the growth phase ceased at the same age in the Rex and normal types.

A series of sib pairs of Rex and normal rabbits were treated at ages ranging from 8.30 days. In all the pairs injected before 16 days the coat was completely shed in both Rex and normal. There was a lag between treatment and shedding of about 7-9 days in the normals and 8-10 days in the Rexes, as reported by Iljin (1945), and shedding commenced on the face. The effect of treatment of two pairs injected at 17 days is interesting since in both Rex and normal only the coat of the posterior trunk was shed, that on the head and shoulders showing no effect of treatment. This can be interpreted as showing that the coat had ceased to grow on the head and on the shoulder at 17 days, but was still growing on the back and rump. The pairs of animals injected at later ages (23, 25 and 30 days) showed no effect of treatment. Therefore the coat ceases to grow on the back between 18-22 days.

These results allow an estimate of the age of cessation of growth of the first pelage, but it is not certain whether the day of treatment or of effect is the pertinent age since on the former growth ceases on the back soon after 17 days, and on the latter soon after 24 days. Considering all the results, it can be said that growth of the coat on the back ceases either at 18-22, or 24-28 days. The growth data given below clarify this. However, regardless of the actual date of cessation it is certain that in both Rex and normal the duration of the growth phase is similar or identical.

Structure of the Coat.

Cursory inspection of the coats of normal rabbits shows that two types of hairs occur. Guard-hairs which project above the rest of the coat, and down-hairs which form the bulk of the

coat. If samples of the coat are separated into their individual hairs, this heterogeneity is very obvious, and it can be seen that guard-hairs in addition to their greater length, have a coarse tip, and are less markedly crimped, or not crimped at all. The down-hairs back this coarse tip, and are markedly crimped throughout their length.

As stated above, no differences can be seen between Angoras and normals till after 30 days from birth, when it becomes increasingly apparent that the angora coat continues to grow after this age whereas the normal coat ceases to grow. Before 30 days it is not possible to distinguish Angoras from normals, either macroscopically or microscopically.

The Rex coat grows at a slower rate than the normal coat, and further there is an absence of the guard-hairs which are so noticeable a feature of the normal coat. However, if samples of Rex coats are separated into their individual hairs, two types can be seen which correspond to the guard-hairs and down-hairs of the normal coat, apart from the differences in length. In Rex coats the guard-hairs are only slightly longer than the longest down-hairs, but they can be easily distinguished from the down-hairs by the occurrence of a coarse tip. This tip is not as coarse or as long as in normal guard-hairs, and the crimping of the basal part of the Rex guard-hairs is more marked than in normal guard-hairs.

Two Rex-Angora rabbits have occurred in one of our crosses. These initially were scored as Rexes since they were identical with Rex rabbits, having the curled whiskers, short coat, and apparent lack of guard-hairs which are diagnostic of the rex gene. However, after 30 days from birth their coats continued to increase in length until it became obvious that

they had coats of the Rex-Angora type. The length of their coats is intermediate between normal and Angora, and there is a lack of the coarse guard-hairs which are a feature of the Angora coat; instead the shorter, less coarse guard-hairs occur, as in the Rex coat. In figure X are shown two samples of the coats, taken at 20 and 53 days, of normal, Rex, Angora, and Rex-Angora rabbits.

The structure of the different types of coats corroborates the suggestions that the angora gene affects only the duration of the growth phase, and the rex-gene affects only the rate of growth of the coat. The identity of normal and Angora, and of Rex and Rex-Angora coats before 30 days, the continuing of growth of the Angora and Rex-Angora coats after this age, and the difference of length of normal and Rex, and Angora and Rex-Angora coats all support this suggestion.

Rate of growth of the coat.

Measurements were made of the lengths of a number of hairs from samples taken at various ages, from 7-60 days. All the samples were taken at or near the standard back sampling region which is located at the point of attachment of the last rib. The measurements were made by the same method as that used for the analysis of the growth of the mouse coat (Fraser 1951).

Several rabbits were sampled sequentially, around the same region, at ages from 7-60 days, and in figure M are given the average lengths of guard-hairs and down-hairs plotted against age. These show that growth ceases at or before 21 days since no further increase of length occurs after that age.

The average lengths of the samples taken before 25 days lie on reasonably straight lines, showing that for each type of hair, within a rabbit, the rate of increase of length is constant. This has also been found for mouse hairs (Fraser 1951). As expected the guard-hairs grow faster than the down-hairs, both in Rex and normal, with the difference less in Rex.

The results of thallium treatment gave an estimate of the cessation of growth of the coat as at 17+ or 24+ days, depending on whether the day of treatment or of effect is pertinent. The measurement of the rate of growth of the coat shown in figure M indicates that the coat ceases growing before 21 days, and therefore considering the results from both methods it appears that the growth of the first pelage ceases on the back at about 17-21 days.

These comparisons of Rex and normal can be extended by including all the various rabbits which were sampled in the analysis. In figure N are shown the growth rates separately for guard-hairs and down-hairs for all four types of coat.

These data show that the normal and Angora coats grow at the same rate, and that the growth of the normal coat ceases at about 21 days, whereas that of Angora continues at the same rate until 60 days, after which no further measurements were made. Similarly the Rex and Rex-Angora coats grow at the same rate, and the growth of the Rex coat ceases at about 21 days, whereas that of Rex-Angora continues until 60 days, after which no further measurements were taken. This fully corroborates the hypothesis of the different bases of effect of the rex and angora genes.

Discussion and Summary.

The data given above have shown fairly conclusively that the normal coat of rabbits is made of two types of hairs: long guard-hairs, and short down-hairs. The growth of the coat ceases at about 18-21 days. The rex gene causes the hairs to grow at a slower rate, but does not affect the duration of the growth phase. The angora gene causes no change in the rate of growth of the hairs but causes an increase of the duration of the growth phase, the hairs continuing to grow till about eight weeks after birth. This is illustrated in Figure 4.

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Rex

Normal.

Plate I. Rex and normal sibs injected at 17 days with thallium acetate, showing the partial molt referred to in the text.

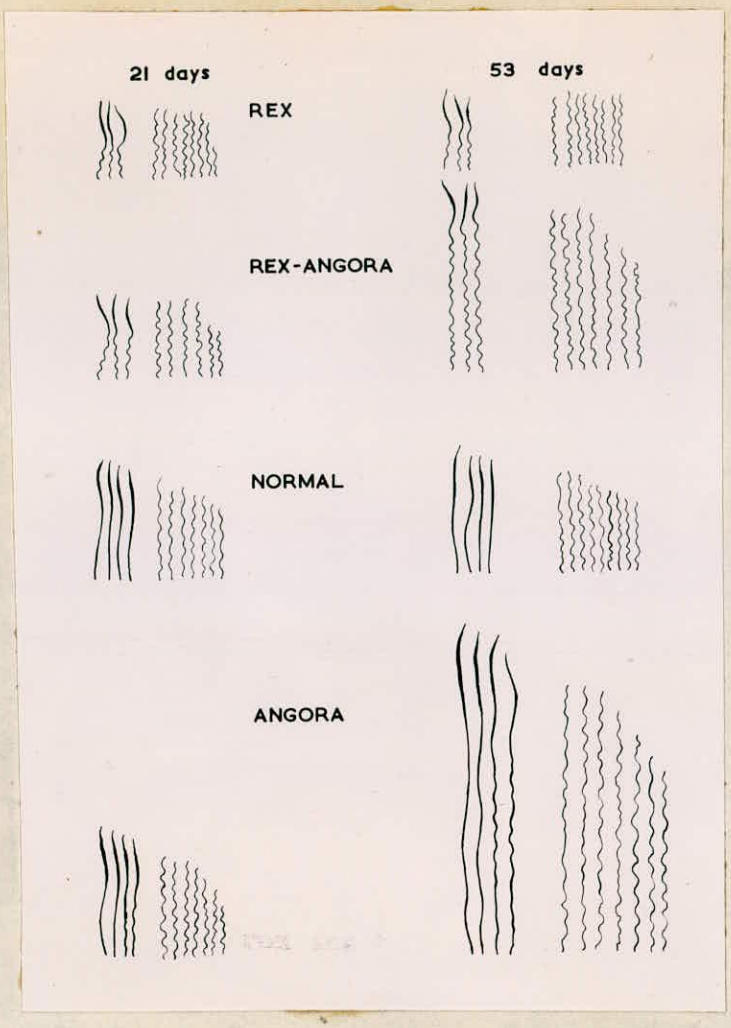

Figure 1. Line drawings of samples from the backs of the various types of rabbits studied, taken just after the cessation of growth (21 days) and well after the the cessation of growth (53 days). The samples have been separated to show the differences between guard and down hairs.

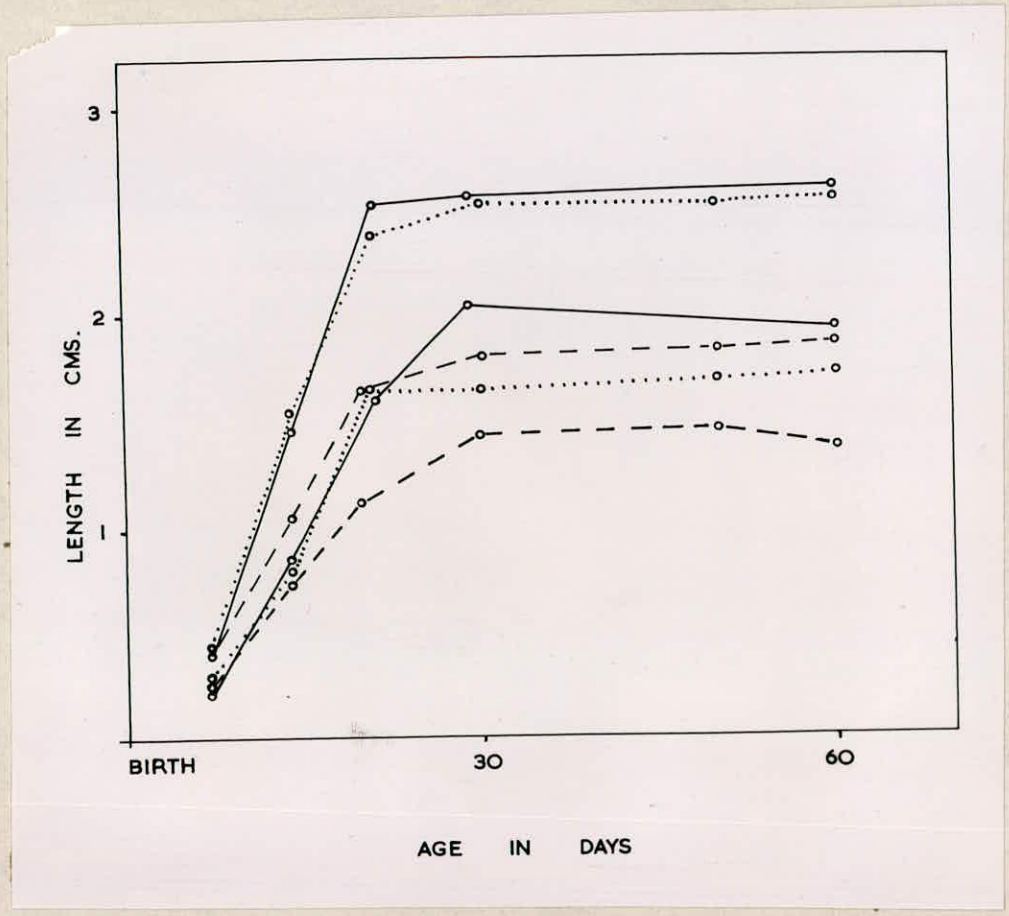

Figure 2. Groth rates of guard and down hairs, from samples taken in sequence from the same rabbits (two normal and one Rex) to show growth rates and cessation of growth in individual animals.

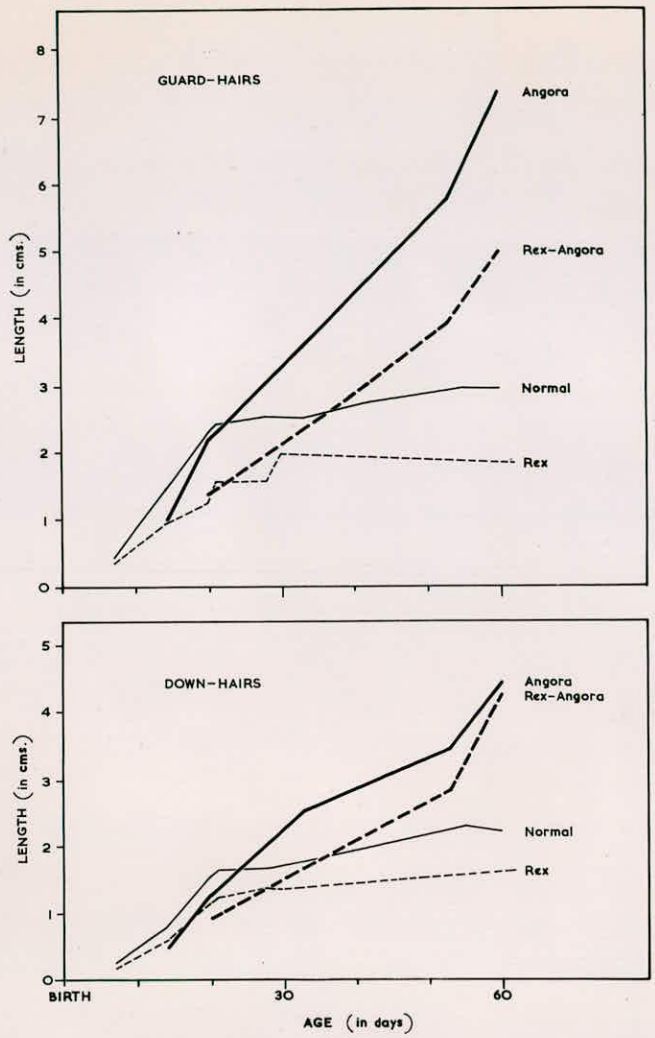

Figure 3. Growth rates of guard and down hairs averaged overall the available data to show the differences of rate of growth between r and + and of age of cessation of growth between l and +.

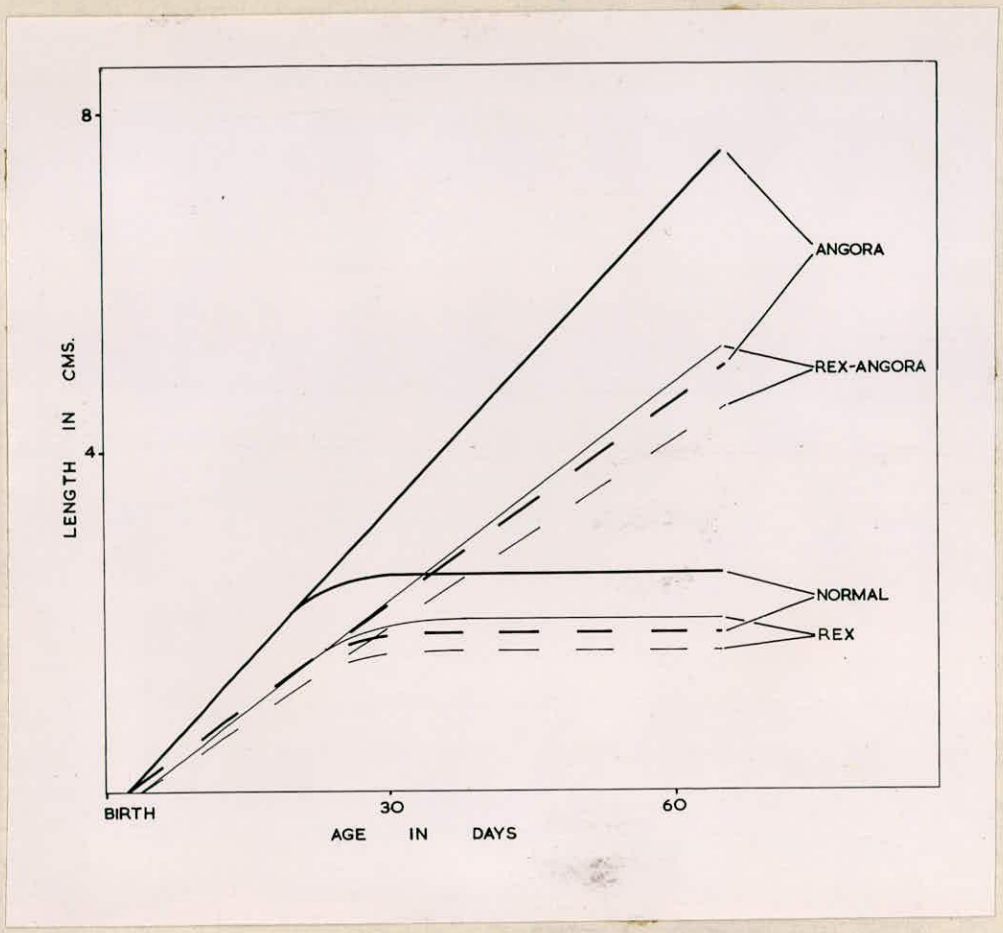

Figure 4. As in figure 3, but simplified to remove the scatter around the lines of the actual estimates. Guard hairs are shown with thick, down hairs with thin lines.

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