Genet. Res., Camb. (1984), **44**, pp. 29–38
With 3 plates
Printed in Great Britain

Protein and amino acid composition of hair from mice carrying the naked (*N*) gene

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(*Received 4 January 1984 and in revised form 13 March 1984*)

SUMMARY

The protein and amino acid compositions of hair from mice carrying the naked (*N*) gene were compared with those of wild-type (+/+) mouse hair using two-dimensional polyacrylamide gel electrophoresis and amino acid analysis. In samples obtained from *N*/+ mice, the numbers of positions of the low-sulphur (LS), high-sulphur (HS) and high tyrosine (HT) protein spots were indistinguishable from those observed in +/+ samples but the amount of HT protein was reduced in *N*/+ hair. In samples obtained from *N*/*N* mice the protein spots were fewer than, and perhaps different from, those in samples from the other two genotypes; all protein classes were represented but the HS and HT protein content appeared to be less than that in +/+ and *N*/+ hair. Amino acid analyses of hair samples showed that the HS contents of +/+ and *N*/+ hair were approximately the same, whereas the HT protein content of *N*/+ hair was about half that in +/+ hair. *N*/*N* hair contained less half-cystine and tyrosine than +/+ and *N*/+ hairs, but because the contents of proline, threonine and glycine were unexpectedly high, it appears that protein components of unusual composition are present in *N*/*N* hair. It was concluded that the gene at the *N* locus is involved only indirectly in the synthesis of the structural proteins found in mouse hair.

1. INTRODUCTION

The naked (*N*) gene in the mouse is a semi-dominant gene which causes fragility of the hair. In heterozygous naked (*N*/+) mice the hairs grow to full length before breaking off close to the skin surface at the end of each growth cycle. In homozygous naked (*N*/*N*) animals the hairs remain trapped in the skin during the first two or three hair cycles and only a few hairs erupt during later hair cycles. These hairs break off at the skin surface after they reach 2–3 mm in length.

An electron microscope examination of hair and follicles from +/+, *N*/+ and *N*/*N* mice revealed abnormalities in cell differentiation and protein deposition in the mutants (Raphael *et al.* 1982). In *N*/+ hair follicles the deposition of the matrix in developing macrofibrils was irregular; in *N*/*N* hair follicles the cells adjacent to the inner root sheath sometimes contained both globular deposits, similar to those

seen in cuticle cells, together with fibrillar deposits, similar to macrofibrils seen in cortical cells.

Most of the protein present in hair is synthesized in the cortical cells. This cortical protein consists of microfibrils, composed essentially of low-sulphur (LS) proteins, embedded in a matrix of high-sulphur (HS) and high-tyrosine (HT) proteins (Fraser, Macrae & Rogers, 1972). Tenenhouse & Gold (1976) and Marshall, Frenkel & Gillespie (1977), who compared the HS and HT proteins extracted from $+/+$ and $N/+$ hair, were unable to find qualitative differences between the two genotypes but $+/+$ hair was found to contain twice as much HT proteins as $N/+$ hair (Tenenhouse & Gold, 1976). Hair from $+/+$ mice was also found to contain more tyrosine and glycine than $N/+$ hair (Tenenhouse *et al.* 1974); both amino acids are known to be present in comparatively large amounts in HT proteins.

The abnormalities in the keratinization of hair cells in mice carrying the *N* gene could be due to the direct involvement of the mutant in the synthesis of one or more of the structural proteins, or they could be due to an indirect effect of the mutant on the synthesis of all proteins in the cells of the developing hair. Biochemical analyses (Tenenhouse *et al.* 1974; Tenenhouse & Gold, 1976) of $N/+$ hair showed that it is unlikely that the *N* gene codes for a specific HT or HS protein but that it may act by affecting the production of the whole family of HT proteins. However in order to decide more conclusively between the possible influences of the *N* gene, it is necessary to examine hair from N/N mice. This paper describes the results of electrophoretic analysis of HS, HT and LS proteins from $+/+$, $N/+$ and N/N hair, and the results of amino acid analysis of the three hair types. Our results confirm the findings of Tenenhouse & Gold (1976), and from the analysis of N/N hair we conclude that the *N* gene exerts its effects on the synthesis of hair proteins via some indirect route.

2. MATERIALS AND METHODS

(i) *The mice*

The $+/+$, $N/+$ and N/N mice were from a random bred stock formed by crossing an inbred naked stock from the Jackson Laboratory, Maine, U.S.A. to a local coloured, random bred strain. It was not possible to use mice from the inbred strain because mortality between birth and weaning among the N/N mice from this inbred line was 100%. The random bred strain was maintained by mating $N/+$ sibs of N/N mice which survived to adulthood.

(ii) *Hair samples*

Hair samples were plucked from the dorsum of adult mice of the three genotypes, namely 3 $+/+$ males, 4 $N/+$ males and one $N/+$ female and 3 N/N males and 3 N/N females. In addition, a sample was obtained from the rump of one of the N/N males which provided dorsum samples. In order to obtain sufficient hair from the N/N mice a sample was removed every few days over a period of about 10 days and these samples for each mouse were pooled.

(iii) *Cleaning of hair*

The hair was washed successively in petroleum ether, absolute ethanol, water and absolute ethanol, and then air dried.

(iv) *Reductive extraction of proteins*

The hair was extracted overnight under nitrogen at room temperature in a solution containing 8 M urea, 0·05 M Tris and 0·05 M dithiothreitol (pH 9·5) (Marshall & Gillespie, 1982). Hair sample weighing 3·5–4 mg from $+/+$ and $N/+$ mice were used. Because only a small amount of hair was available from N/N mice, a sample was first taken for amino acid analysis and the remaining sample was used for the extraction of keratins. For N/N samples weighing less than 0·2 mg, 20 μl of extracting solution was used, whereas for the remaining samples 100 μl of extracting solution was added for each mg of hair.

Because of the small amount of hair that was available for many samples, the extractabilities of the hairs were not determined. On the basis of experiments with other samples about 70–80 % of the hair would be expected to be solubilized.

(v) *Radioactive labelling of proteins*

The proteins in 50 μl aliquots were *S*-carboxymethylated by adding 2 μl of iodo [2-^{14}C]acetic acid aqueous solution (2·5 μCi) and 0·2 μl of 3 M Tris (Marshall & Gillespie, 1982). In the case of the small samples ($<$ 0·2 mg hair) 5 μl of iodo [2-^{14}C]acetic acid solution (6·25 μCi) and 0·5 μl of 3 M Tris were added to 20 μl of protein. After 10 min 25 μl of a 1 M iodoacetic acid, 2·3 M Tris solution (10 μl for small samples) was added to complete the alkylation of SH groups. After a further 10 min the excess iodoacetic acid was reacted with 5 μl of 2-mercaptoethanol (2 μl for small samples). The samples were then frozen until ready for use.

(vi) *Electrophoresis*

The proteins were separated using two-dimensional polyacrylamide gel electrophoresis when separation in the first dimension was carried out at pH 3 in 8 M urea and separation in the second dimension was by SDS electrophoresis (Marshall & Gillespie, 1982). After electrophoresis the proteins were detected by fluorography (Bonner & Laskey, 1974).

Identification of protein spots as HS, LS or HT proteins was based on their position in the gel. HS, LS and HT proteins extracted from wool take up characteristic positions in SDS gels (Marshall, 1981) and it was assumed that the proteins from mouse hair would behave like those from wool.

The presence or absence of specific proteins in hair samples from $N/+$ and N/N mice was determined by comparing the spots obtained for these two genotypes with those obtained from $+/+$ hair. For this purpose the HS, LS and HT spots in a $+/+$ sample were allotted numbers (Figs 1 and 2) and the spots in all other samples were numbered according to this nomenclature. In order to make satisfactory

comparisons between patterns, several exposure times were used so that resolution of specific protein spots was optimal.

Although it was not possible to make direct quantitative comparisons between the same protein in hair from different mice because of differences in amounts of protein loaded on the gel, it was possible to make relative comparisons. For this purpose the LS group of spots was selected as a standard. When the intensity of these spots in the sample of interest was approximately the same as that in the sample from the reference animal it was possible to say whether the amounts of other proteins were greater or less than those observed in the standard sample.

(vii) *Amino acid analysis*

Cleaned hair samples were hydrolysed in batches for 24 h *in vacuo* at 108 °C with constant boiling HCl and 2 mM phenol, and then freeze-dried. In order to oxidize any cystine produced by reaction between phenol and cysteine the hydrolystates were dissolved in 0·1 M borate and shaken in contact with air before being freeze-dried. The hydrolysate was analysed with a modified Beckman amino acid analyser. Only single analyses were carried out, it was assumed that the usual accuracy of $\pm 2\%$ would be applicable to most values. For those samples of N/N hair where only a very small amount was available, the accuracy of the cystine values was about $\pm 5\%$.

3. RESULTS

(i) *Two-dimensional polyacrylamide gel electrophoresis*

Wild-type hair. A two-dimensional gel obtained from one + / + hair sample is given in Fig. 1 while Fig. 2 is a schematic diagram of this pattern showing the positions of the three protein classes and the number allotted to each spot in each of the three classes. The proteins with the highest apparent molecular weights were the LS proteins, the proteins with the lowest weights the HT proteins. The remaining spots were HS proteins.

Among the LS proteins, a small fraction sometimes failed to enter the first dimension gel, but separated in the second dimension. The LS proteins which entered the first dimension gel separated into seven spots but one of these, LS 3, was larger than the others and probably contained two or more components. The same numbers of LS spots were found in all the + / + hair samples examined and the positions of these spots appeared to be identical.

PLATE 1

Fig. 1. Polyacrylamide gel electrophoretic pattern of the proteins extracted from a hair sample from a + / + mouse.

Fig. 2. Tracing of the protein spots illustrated in Figure 1 showing the position of the three classes of keratins and the numbers allotted to each spot in each of the three classes. Numbers were not allotted to the components of the LS spots which failed to enter the first dimension of the gel but which migrated in the second dimension, or to the ill-defined spots near the origin of the second dimension. These latter spots were probably aggregates of LS protein. LS, low-sulphur proteins; HS, high-sulphur proteins; HT, high-tyrosine proteins; 0, origin; SDS, sodium dodecyl sulphate.

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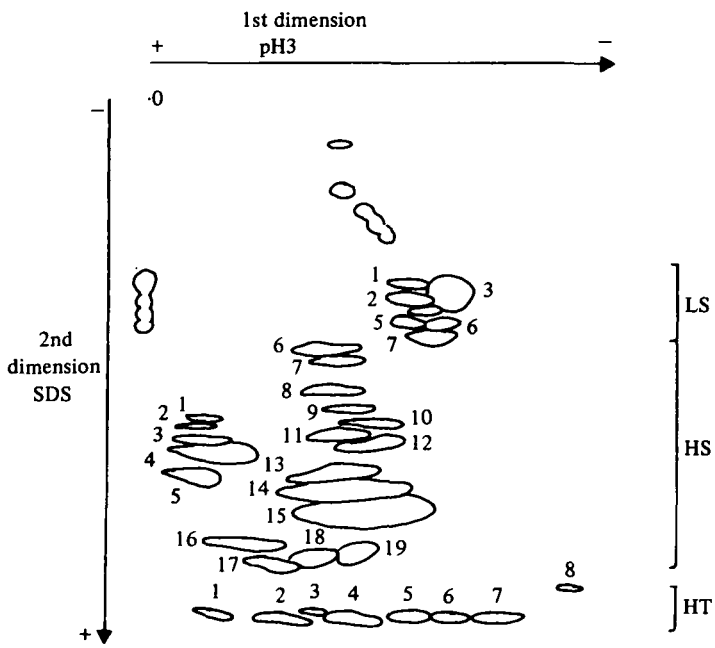

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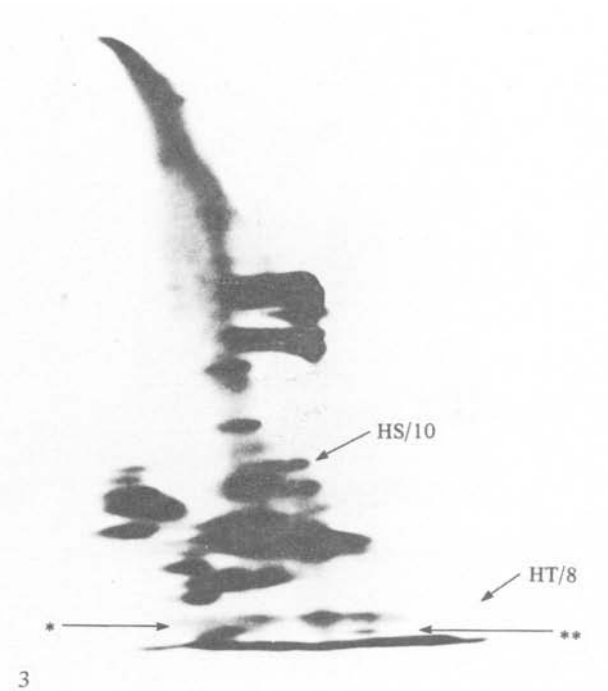

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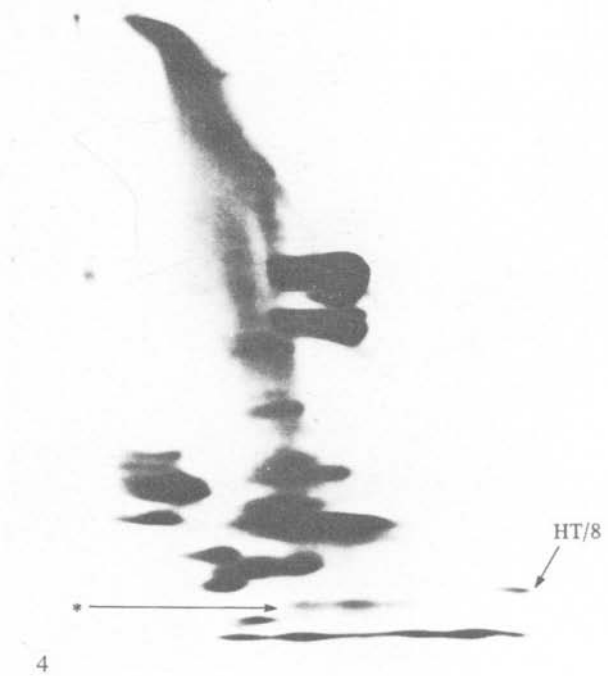

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The HS proteins separated into 19 separate components. Some of the large spots, for example HS 14 and HS 15, probably contained several different components for Marshall & Gillespie (1976) found more than 20 HS proteins in mouse hair using a combination of ion exchange chromatography, gel filtration chromatography and polyacrylamide gel electrophoresis. Eighteen of the 19 HS proteins were present in all +/+ samples examined although HS 2, HS 7, and HS 9 tended to be faint in some. HS 10 was present in only two out of the three +/+ samples examined.

The HT proteins, which had very similar apparent molecular weights, separated into eight spots on the basis of charge. Some of the spots may have contained more than one component, for Gillespie (1972) found about 20 HT proteins in mouse hair using starch gel electrophoresis and Tenenhouse & Gold (1976) found 12 HT proteins using polyacrylamide gel electrophoresis. In our gels HT 2, HT 4, HT 6 and HT 7 were the most intense spots, HT 1, HT 3 and HT 5 were less intense and HT 8 was very faint. The same numbers of HT spots were observed in the hair samples from the three +/+ mice, and the relative positions of these spots appeared to be identical.

N/+ hair. The pattern of protein spots in the *N/+* hair samples was very similar to that seen in +/+ hair samples (Figs 3 and 4). The LS spots were identical in number and position and similar in intensity to the LS spots in +/+ samples. The HS spots, too, appeared to be similar in the two genotypes, and, like +/+ samples, the *N/+* samples showed heterogeneity for HS 10: two of the four had a spot in this position and two did not. The HT spots, although identical in number and position in samples from the two genotypes, were reduced in intensity in the *N/+* hair samples; this reduction being more readily apparent in patterns where the major protein spots were not over-exposed (shorter exposure times). The effect of the *N* gene on the hair proteins of *N/+* mice, therefore, appeared to be restricted to causing a reduction in the amount of HT proteins.

N/N hair. In all hair samples from all *N/N* mice the protein spots were fewer than in samples from +/+ and *N/+* mice but all protein classes were represented. There was a comparatively large amount of unresolved low molecular weight protein in the HS–HT region (Figs 5 and 6), and the nature of the protein being unknown. The *N/N* patterns were of two types, those with six or seven LS spots (Group A) (Fig. 5) and those with four or fewer LS spots (Group B) (Fig. 6). Group A mice tended to have more hair than Group B animals.

PLATE 2

Fig. 3. Polyacrylamide gel electrophoretic pattern of the proteins extracted from a hair sample from a +/+ mouse. The positions of HS 10 and HT 8 are indicated. The spots lying between the HS and the HT proteins (* →) are artifacts. The spot immediately above the HT spots (← **) could be an extra HT protein in this mouse; it was absent from the +/+ 2 sample (Fig. 1) and the *N/+* 1 sample (Fig. 4).

Fig. 4. Polyacrylamide gel electrophoretic pattern of the proteins extracted from a hair sample from a *N/+* mouse. The spot HS 10 was missing in this sample (compare with Fig. 3) and most of the HT proteins appeared to be reduced in amount relative to those observed in +/+ mice. Only HT 8 appeared to be present in greater quantities in the *N/+* sample than in the +/+ sample. The spots lying between the HS and HT proteins (* →) are artifacts.

Of the samples from the three Group A mice two had seven LS spots and one had six (LS 5 was missing). HS spots were present in all samples; in the sample from the mouse with the most profuse hair growth a pattern of well defined HS spots similar to that seen in $+/+$ and $N/+$ hair was produced; in a mouse with rather less hair growth although HS spots were present they were very blurred compared to the spots from $+/+$ and $N/+$ hair (Fig. 5). HT spots were present but, with the exception of HT 8, the HT spot of slightly higher molecular weight and charge, these spots were very faint. Another spot, HT 9, of even higher charge but similar apparent molecular weight was sometimes observed. This latter spot was never seen in $+/+$ or $N/+$ samples.

In samples from the three Group B mice three of the seven LS spots were missing (LS 1, LS 2 and LS 5). The amount of HS protein was considerably reduced; some of the lower molecular weight HS protein spots appeared to be present, for example HS 17 and HS 18, but even these spots were very blurred (Fig. 6). The amount of HT protein was also reduced, but, like samples from Group A mice, these samples from Group B mice had a prominant HT 8 spot and, in two mice, an HT 9 spot was also present.

The effects of the N gene on the hair proteins of N/N mice, therefore, were much more profound than its effects in $N/+$ mice. In N/N mice the synthesis of proteins in all classes was affected but the effects of the gene on the HS and HT proteins were more marked than its effects on the LS proteins. In some mice a new protein, HS 9, was produced but this spot was not observed in all N/N hair samples.

(ii) *Amino acid analyses*

In $N/+$ hair the amounts of the two amino acids known to be present in large amounts in HT proteins, namely tyrosine and glycine, were smaller than those found in $+/+$ hair (Table 1). On the basis that the tyrosine contents of hair samples from a number of different species are approximately linearly related to the amounts of HT proteins in the samples (Gillespie & Frenkel, 1974), the HT protein content was calculated to be reduced by about 40% for one dose of the N gene (Table 1). Because the average half-cystine contents of $+/+$ and $N/+$ hair samples were approximately the same, it appeared that the HS contents of the two hairs were similar, although unexpectedly the proline contents of the two samples were somewhat different. Because hair samples from $N/+$ mice contained

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PLATE 3

Fig. 5. Polyacrylamide gel electrophoretic pattern of the proteins extracted from a hair sample from a N/N mouse (group A). In this sample all the LS proteins appeared to be present, the HS proteins were reduced in number and amount and the HT proteins, with the exception of HT 8, were markedly reduced in amount. The position of the HT protein peculiar to N/N mice, HT 9, is indicated.

Fig. 6. Polyacrylamide gel electrophoretic pattern of the proteins extracted from a hair sample from a N/N mouse (group B). In this sample several of the LS proteins appeared to be missing, many of the HS protein were also absent and most of those that were present were reduced in amount. All the HT proteins, with the exception of HT 8, were reduced in amount, HT 9 was present; its position is indicated.

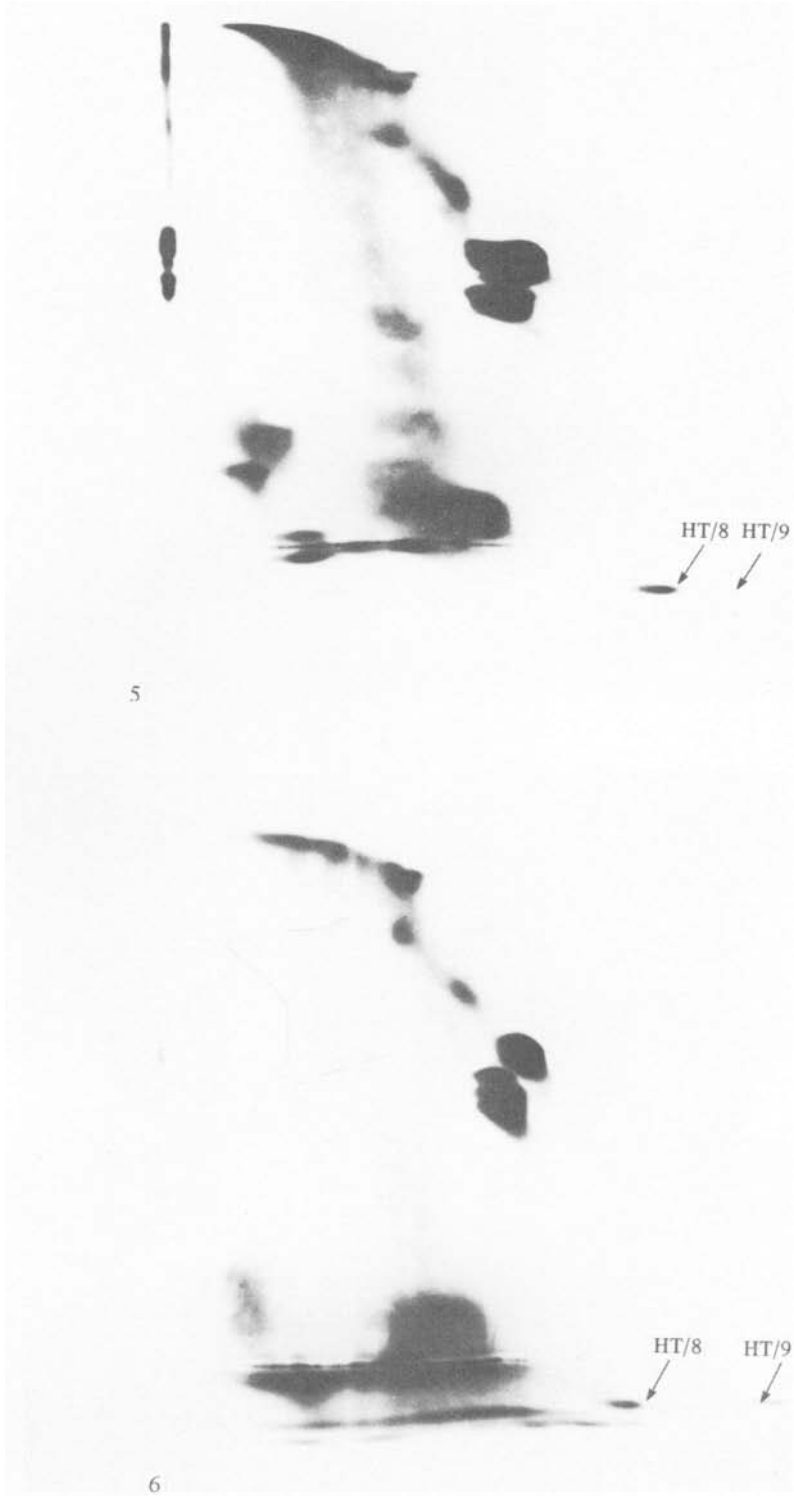

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Table 1. Average amino acid content and high-sulphur and high-tyrosine protein contents of hair samples from the dorsum of two +/+, two N/+, two N/N (group A) and three N/N (group B) mice

(Amino acid protein content is expressed as residues per 100 residues. Protein content, as grams per 100 g of hair, is calculated from regression lines of Gillespie & Frenkel (1974) on the assumption that the proteins are of normal composition. High-sulphur protein content = 2·8 C−7 and high-tyrosine protein content = 5 (T−2) where C = half-cystine (residues %) and T = tyrosine (residues %).)

	+/+		N/+		N/N (group A)		N/N (group B)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Amino acids								
Lysine	2·9	2·8–3·0	3·3	3·2–3·3	4·2	4·1–4·3	4·3	4·0–4·6
Histidine	1·0	—	1·0	—	1·5	1·4–1·6	1·6	1·3–1·8
Arginine	6·3	—	5·8	5·7–5·8	6·5	6·3–6·7	6·6	6·0–7·1
Cysteic acid	0·3	0·2–0·3	0·3	0·1–0·5	0·1	0·0–0·1	—	—
Aspartic acid*	5·4	5·3–5·4	5·7	5·5–5·8	6·5	6·3–6·7	6·4	6·2–6·6
Threonine	4·8	—	5·1	4·9–5·3	5·2	5·1–5·2	5·1	4·9–5·3
Serine	9·6	9·4–9·7	9·9	—	10·1	9·8–10·3	10·2	9·5–11·1
Glutamic acid†	12·6	12·4–12·7	12·9	12·7–13·1	13·0	12·4–13·5	12·6	12·3–12·9
Proline	7·2	7·1–7·3	7·8	7·6–7·9	7·5	7·1–7·8	7·3	6·5–7·9
Glycine	10·9	10·7–11·0	9·0	8·6–9·4	8·3	8·0–8·6	9·7	8·0–11·2
Alanine	4·2	—	4·4	4·3–4·5	5·6	5·5–5·7	5·9	5·4–6·1
Half-cystine	13·8	13·7–13·9	13·7	13·2–14·2	9·8	9·2–10·4	8·0	5·9–11·6
Valine	4·1	4·0–4·1	4·3	4·2–4·3	4·9	4·6–5·2	4·7	4·5–4·9
Methionine	0·6	—	0·7	—	1·0	0·9–1·1	1·1	0·8–1·5
Isoleucine	2·4	2·3–2·4	2·5	—	3·0	2·8–3·1	3·1	2·8–3·3
Leucine	6·2	—	6·2	6·1–6·3	6·9	6·8–7·0	7·0	6·7–7·1
Tyrosine	4·7	—	3·6	3·3–3·9	2·9	2·8–3·0	3·0	2·9–3·1
Phenylalanine	2·5	2·4–2·5	2·6	2·4–2·7	2·4	2·2–2·6	2·7	2·5–2·9
Ornithine‡	0·4	—	0·6	0·5–0·6	0·6	0·5–0·6	0·3	0·0–0·5
Citrulline§	0·6	—	0·9	0·8–0·9	0·5	0·3–0·7	0·5	0·3–0·7
Proteins								
High-sulphur	31·6	—	31·4	—	—	—	—	—
High-tyrosine	13·5	—	8·0	—	—	—	—	—

* Includes asparagine. † Includes glutamine. ‡ From the breakdown of citrulline. § From medullary cells.

smaller amounts of the matrix proteins (HS + HT) than +/+ hair, the proportion of microfibrillar protein (LS protein) in $N/+$ hair should be greater, and this was in fact suggested by the amino acid analyses of the hair samples. $N/+$ hair contained larger amounts of the amino acids known to be richest in LS proteins: namely lysine, aspartic acid, glutamic acid, alanine, methionine, isoleucine and leucine, although the alanine, aspartic acid, glutamic acid and leucine contents were elevated to smaller degrees than expected.

The half-cystine contents of the N/N hair samples was very variable, the range being from 5·9 to 11·6 residues %. This variability may have been related to the amount of erupted hair, for half-cystine content was higher in samples from mice with relatively luxuriant hair growth than in samples from mice with sparse hair growth; for example hair samples from group A mice had higher half-cystine contents than those from group B mice (9·8 v. 8·0 residues %) and a hair sample from the rump of one N/N mouse, where hair growth was denser than in other body regions, had a higher half-cystine content than a sample from the dorsum of the same animal (11·8 v. 10·4 residues %).

Apart from differences in half-cystine and glycine contents, the average amino acid compositions of N/N hair samples from group A and group B mice were similar. In N/N hairs, the tyrosine and half-cystine contents were smaller than the corresponding values in +/+ and $N/+$ hairs. However on the basis of the known compositions of normal HS and HT mouse proteins (Gillespie, 1983), the contents of proline, threonine and glycine were unexpectedly high. Moreover some N/N samples contained more lysine and methionine, and less half-cystine, than purified LS proteins. The unusual composition of N/N hairs therefore suggests the presence of protein components of composition different from those normally found. Thus it was not meaningful to estimate the amounts of HS and HT protein contents from published regression lines.

4. DISCUSSION

The results of our analyses of $N/+$ hair confirm the findings of Tenenhouse and co-workers (Tenenhouse *et al.* 1974; Tenenhouse & Gold, 1967), namely, that $N/+$ hair contains all the HT proteins found in +/+ hair but that total HT protein is reduced in amount in hair from the heterozygotes. Moreover the degree to which the HT protein content was reduced was similar in the two studies, for Tenenhouse & Gold (1976) estimated that the HT protein contents +/+ and $N/+$ hair were 16 and 8 % respectively, while our estimates of the average amount of HT protein contents in the two hair types were 13 and 8 % respectively.

Because Tenenhouse & Gold (1976) were only able to analyse $N/+$ hair they were unable to determine whether the gene at the N locus is directly involved in the production of hair proteins or whether the mutation causes a much more generalized tissue malformation. Our analyses of N/N hair suggest that the latter is the case for all three protein groups were affected in N/N hair and the extent of the abnormalities varied between animals. For example, the number of LS spots was either 4, 6 or 7, and the pattern of HS spots and the quantity of HS proteins were variable. Only the HT proteins were consistently reduced in $N/+$, and

perhaps N/N, hair compared with $+/+$ hair. However, the HT proteins are known to be sensitive to environmental factors such as diet and depilatory agents (Frenkel, Gillespie & Reis, 1974, 1975; Gillespie, Frenkel & Reis, 1980), so a reduction in HT protein content cannot necessarily be attributed to a direct involvement of the N gene in HT protein synthesis. Although an additional protein, HT 9, was present in some N/N samples it was not represented in all samples. There is, therefore, no evidence that N codes for one of the structural proteins in hair or that it regulates the synthesis of one particular protein class.

In hair from $+/+$ and $N/+$ mice most of the protein is synthesized in the cortical cells of the hair shafts with small contributions from the cuticle and medullary cells (Raphael *et al.* 1982). In transmission electron microscope pictures, the deposition of matrix in the macrofibrils in the cortical cells of $N/+$ hair is sometimes irregular (Raphael *et al.* 1982). HT proteins are thought to contribute to the matrix proteins (Fraser, Gillespie & McRae, 1973; Bendit & Gillespie, 1978); thus, the irregularity in the deposition of the matrix in $N/+$ hair may reflect the reduction in HT protein content of $N/+$ hair.

In hair samples from N/N mice, like those from $+/+$ and $N/+$ mice, most of the protein was probably from cortical cells but some may have come from the abnormal cells seen in transmission electron microscopy of N/N hair follicles. These cells, which abut onto the inner root sheath of the follicle, contain amorphous aggregates which are found alone in the cell or in association with macrofibrils (Raphael *et al.* 1982). These protein aggregates may represent abnormal accumulations of cuticle keratin, for the cells in which they are found, are in a position normally occupied by cuticle cells. The only protein to increase in amount in N/N hair relative to $+/+$ and $N/+$ hair was HT 8 and the only protein unique to N/N hair was HT 9, and therefore both these proteins could be derived from the amorphous aggregates.

Examination of the electrophoretic patterns and the half-cystine contents of the hairs from the 3 genotypes certainly suggest that the N/N hairs are deficient in HS proteins and enriched in LS proteins. However, on the basis of known compositions of normal mouse proteins (Gillespie, 1983), the amino acid analytical results were not consistent with this idea because the anticipated decreases in proline and threonine contents and the anticipated increases in glutamic acid, leucine and aspartic acid, were not observed. If it is assumed that the interpretations of the electrophoretic and amino acid analyses are correct, then it appears that a simple change in the proportion of protein types has not occurred. Perhaps either a 'new' set of HS proteins of abnormal composition is produced or a protein(s) rich in proline and threonine and poor in glutamic acid, leucine and aspartic acid, and possibly deriving from the amorphous aggregates seen in electron microscopy (Raphael *et al.* 1982), is present in relatively large amounts. An alternative hypothesis is that a set of apparently normal HS proteins is synthesised but these proteins are partly degraded by enzymes in the follicle with the loss of some peptide fragments and the production of low molecular weight protein components as seen in the lower part of the electrophoretic patterns (Figs 5 and 6). Unfortunately, because of the minute amounts of hair from the N/N mice, it was impractical to fractionate the proteins to distinguish between these possibilities.

Taken together with the results of the electron microscopic study of $+/+$, $N/+$ and N/N hair, the results of these protein and amino acid analyses suggest that the fragility of the hair in mice carrying the N mutation is due to generalized tissue malformation rather than to the absence of (or abnormality of) one particular structural protein or one group of proteins. This generalized tissue malformation is presumably responsible for the reduction in the number of follicle cells being channelled into hair and for the abnormal differentiation of some of these cells which was observed by electron microscopy. The changes in the amounts of the various structural proteins in hair from $N/+$ and N/N mice may be a reflection of this disturbance of the differentiation of the follicle cells.

We are indebted to Dr J. M. Gillespie for helpful discussions, to Miss Monica Davis who assisted with the electrophoretic analysis of the hair proteins, to Dr C. M. Roxborough who carried out the amino acid analyses and to Ms Barbara Threlfall and Mr Vince Tongue who maintained the mouse stocks.

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