at 6 concentrations (10 mM, 20 mM, 50 mM, 75 mM, 100 mM, 200 mM), while two concentrations (100 mM, 150 mM) were tested on *E. coli* K12 A11 and K12 A46.

The toxic effect of the two products is shown in figure 1. For all *E. coli* strains utilized, chloroethylene oxide at 2.5 mM and chloroacetaldehyde at 100 mM showed a strong cytotoxic effect.

The mutagenic effect of the two compounds is shown in figure 2. In the case of chloroethylene oxide, all four strains tested are reverted to tryptophan prototrophy. *E. coli* K12 A46 showed the highest mutagenic response. In the case of chloroacetaldehyde, the three strains tested could also be reverted to tryptophan prototrophy and the highest reversion frequencies are observed at 100 mM.

These results show that chloroethylene oxide is more effective as a mutagen than chloroacetaldehyde in *E. coli* strains, although the durations of treatment are different. *E. coli* strains were treated for only 6 min with chloroethylene oxide because a rearrangement to chloroacetaldehyde occurs at room temperature[10]. Thus, assuming a linear extrapolation with respect to time, a treatment using chloroethylene oxide at 0.25 mM for 60 min could be as effective to obtain a similar mutagenic effect as a treatment using chloroacetaldehyde at 100 mM for the same time. Under such conditions, and with *E. coli*, chloroethylene oxide is approximately 400 times more effective as a mutagen than chloroacetaldehyde. Our results are consistent with those obtained in *Salmonella typhimurium*, especially for chloroethylene oxide[8]. However, in the *S. typhimurium* study, chloroacetaldehyde was more mutagenic than in our experiments.

In conclusion, we have been able to obtain a great number of *E. coli* revertants. Since reversion pathways to prototrophy of Yanofsky *E. coli* strains are known[14, 15], it is of interest to study the type of base pair substitutions or other DNA changes induced by CEO or CAA.

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Genetic differences between inbred strains of mice; a new source of variation in high-sulphur keratins[1]

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Summary. Proteins were extracted by improved techniques from the hair of inbred strains of Peru, C57BL/6 and CBA/Ca mice. The extracts were characterized by amino acid analysis and high resolution polyacrylamide gel electrophoresis, and previously unreported strain differences were observed. Genetic analysis confirmed them.
Key words. Keratins; mouse; electrophoresis; amino acid analysis.

Three major protein fractions can be extracted from mammalian keratins. These are termed high-sulphur (H-S), low-sulphur (L-S) and high tyrosine-glycine (H-T-G-) proteins[2, 3]. The L-S proteins, a high molecular weight fraction, are thought to include the α-helical proteins found in wool filaments extracted from wool fibers[3] and also the tonofilaments of mammalian epidermis and strateum corneum[4]. The matrix in which the filaments are embedded is made up of the lower mol. wt H-S and H-T-G proteins.

There exists substantial heterogeneity in all three types of protein extracted from hair, each class being made up of several components. The H-S proteins, in particular have been reported by many workers as being extremely heterogeneous[5]. The number of variants is not exactly known, but estimates vary from 20 up to 70[3].

The sulphur content of wool proteins has been shown to be related to the sulphur content of the sheep's diet; diets low in sulphur containing amino acids being associated with lower sulphur content of protein extracted from wool[6, 7]. This variation might arise by differential rates of production or complete repression of particular H-S proteins, or by some form of post translational control, but the actual mechanism is not known. No genetic variation in high sulphur keratins has been demonstrated previously[8] although the mouse mutants 'tabby' and 'naked' have been examined for differences in H-S keratins without success[9].

Mouse hair has been examined by Marshall and Gillespie[10, 11] from animals of unspecified genetic background. It contains a relatively large proportion of H-S proteins (30%), which Marshall and Gillespie reported to be extremely heterogeneous in size and charge. Both high and low sulphur keratins from mammalian hair are extremely insoluble, making them difficult to extract and very difficult to separate[3, 12]. The use of agents such as mercaptoethanol or dithioerythreitol during extraction of the proteins is necessary to reduce the disulphide linkages of cysteine. These are prevented from reoxidizing by alkalation of the thiol groups with iodoacetic acid to produce a carboxymethylated protein[12]. Conventionally, harsh conditions have

been employed to obtain the hair proteins. Keratin is reduced in the presence of 8 M urea at pH 11, 30 °C. It is possible that this procedure produces artefacts in the extraction mixture in the form of the heterogeneous H-S protein[13]. Previous techniques for separating hair proteins include moving boundary electrophoresis[14], DEAE cellulose chromatography[15] and polyacrylamide gel electrophoresis[9] but none of these have so far resolved between the different proteins sufficiently for any genetic differences to be apparent.

The aims of this study were to devise methods of extracting keratins which did not produce additional heterogeneity, to use modern techniques of thin layer SDS polyacrylamide gel electrophoresis to separate the proteins and to use inbred lines of mice and genetically appropriate crosses between them to investigate the possible contribution of genetic variation to the known heterogeneity of the high sulphur proteins.

Materials and methods. The following inbred strains were used; C57BL/6, a mouse with black hair and normal hair morphology; CBA/Ca, a mouse with agouti hair and normal hair morphology and Peru/Ste, also with agouti hair and normal hair morphology. F1 crosses were constructed from the three strains (i.e. Peru × C57BL/6, C57BL/6 × Peru, Peru × CBA/Ca, CBA/Ca × Peru, CBA/Ca × C57BL/6, C57BL/6 × CBA/Ca). Backcrosses (i.e. (Peru × C57BL/6 hybrid) × C57BL/6 or Peru parent (C57BL/6 × CBA/Ca hybrid) × C57BL/6 or CBA/Ca parent and (Peru × CBA/Ca hybrid) × CBA/Ca or Peru parent) were also constructed, as were F2 crosses, Peru × C57BL/6 hybrids selfed, and Peru × CBA/Ca hybrids selfed.

Hair was shaved with fine scissors from a patch 1 cm by 2 cm on the backs of the mice, and cleaned by immersion in three changes of ether, two of ethanol and two of water. It was then dried in an oven at 60 °C. The hair samples were then solubilized using either a 'harsh' procedure (1, a modification of that given in Gillespie and Reis[7]) or one of two milder procedures, (2) or (3), as detailed below: 1) 0.2 M mercaptoethanol, in 8 M urea at pH 10.5, 30 °C for 4 h. 2) 20 mM DTE, 50 mM Tris in 8 M urea, pH 8, 18 h at 25 °C[16]. 3) 0.2 M mercaptoethanol, pH 10.5 at 4 °C for 18 h[17].

After the extraction was complete, the residual hair was filtered off and the filtrate alkylated with iodoacetate. The protein mixture was then dialyzed against distilled water before being freeze dried.

Amino acid analyses were performed on protein extracts from hair of three and a half week old mice; 5 individuals were used from the Peru strain and three individuals from each of the C57BL/6 and CBA/Ca strains. The protein was hydrolyzed with 6 N HCL at 110 °C for 24 h, and amino acids were then separated on a Rank Milger chromatography machine.

Electrophoretic characterization of the hair protein mixtures was performed on hair extracts from 20 mice of each inbred strain, 6 mice from each of the F1 crosses, 20 mice from each of backcrosses and 50 mice from each of the F2 crosses. Vertical slab SDS/urea polyacrylamide gels were used following the method of MacGillivray[118]. The system was modified in that a 3% stacking gel, and an exponential separating gel of 5–20% were used. The gels were 1.5 mm thick vertical slabs. To improve the migration of protein from the stacking to the separating gel, riboflavine/light was used to set the stacking gel and ammonium persulphate was used to set the separating gel. This was found to reduce the amount of protein precipitated at the interface between the gels. Additionally, the protein mixture was solubilized using 5% mercaptoethanol, 8 M urea and SDS, before being run on the gel at 4 °C. The gels were run at 300 V through the stacking gel and 200 V through the separating gel, until the bromophenol blue had run off the end of the gel. Staining was carried out using the method of MacGillivray[18]. Later, the techniques of O'Farrell[19] were used; the main differences were that no urea was used in the gel and that riboflavine gel setting methods were not used. Gels were also silver stained[20] to make sure that bands were not being missed.

Results and discussion. The amino acid compositions obtained from the mouse hair protein extracts (method 1), as shown in table 1 differed slightly from previous values where the whole keratin sample has been hydrolyzed and analyzed[8, 21]. In particular the serine and glycine, tyrosine and proline values were higher and the glutamic acid and lysine values lower than reported for whole mouse hair and sheep wool samples. The differences observed in the main are slight and may be due to the fact that a comparison is being made between analyses of the products of an extraction procedure which may not remove proteins in the proportion they are present in the keratin, and an analysis of whole hydrolyzed hair. Additionally, the values given for whole mouse hair were obtained from animals of unspecified genetic background[8].

Using arcsin transformation[22] on the % residue values it is possible to test whether the protein extracts from the three strains differ significantly. One way analysis of variance shows that at the 5% level the three strain extracts only differ significantly in their serine and tyrosine content (table 2). There is a difference at the 10% level in arginine, glycine, valine and leucine content. However there are technical difficulties involved in

Table 1. Replicate extractions on strain*. The amino acid composition of protein extracted by method 1 (harsh) from the hair of CBA/Ca, C57BL/6 and Peru mice at three and a half weeks of age, by solubilizing in 0.2 M mercaptoethanol, 8 M urea at pH 10 for 4 h at 30 °C, expressed as residues per 100

Amino acid	CBA/Ca			C57BL/6			Peru				
	1	2	3	1	2	3	1	2	3	4	5
Threonine	5.1	5.1	5.0	5.2	5.0	5.2	5.4	5.5	5.4	5.8	5.6
Serine	9.2	9.4	9.4	9.6	9.6	9.5	9.8	9.8	10.3	10.7	9.7
Glutamate	9.6	10.3	10.3	10.2	10.0	10.3	10.5	11.1	10.1	10.4	10.1
Glycine	14.1	13.4	14.4	13.6	13.7	13.5	13.0	12.8	14.0	13.3	13.4
Alanine	4.9	4.8	4.9	4.6	4.8	4.3	4.6	4.8	4.0	4.3	4.7
Valine	4.4	4.2	4.0	4.1	3.9	3.9	4.2	4.2	4.1	4.4	4.6
Methionine	Destroyed by oxidation										
Isoleucine	2.5	2.4	2.5	2.4	2.3	2.3	2.5	2.6	2.4	2.4	2.9
Leucine	5.9	5.9	6.2	5.7	5.6	5.6	6.1	6.0	6.2	5.6	6.0
Tyrosine	6.7	6.5	7.2	6.7	6.6	6.4	6.1	6.1	6.3	5.6	6.2
Phenylalanine	2.8	2.7	3.1	2.5	2.6	2.6	2.8	2.9	2.6	2.7	3.2
Lysine	2.0	1.8	1.8	1.7	1.4	1.6	2.0	2.0	2.2	1.5	1.6
Arginine	6.1	6.3	5.7	6.0	7.1	5.9	4.6	4.7	5.1	4.9	5.1
Proline	7.0	7.1	6.4	7.4	6.7	8.3	7.9	6.8	6.5	6.3	4.9
CMcysteine**	5.9	7.3	8.0	8.0	6.4	8.7	7.7	7.2	8.1	8.7	8.2
Aspartate	5.3	5.3	5.4	5.0	5.1	5.0	5.3	5.5	5.9	5.0	5.6
Histidine	8.0	7.0	5.5	7.0	8.8	6.6	7.3	7.9	6.9	8.4	7.6

* Each extraction represents a hair sample from one mouse. [a] As the protein was carboxymethylated with iodoacetic acid, cysteine appears here as CMcysteine.

Table 2. Summary of 1 way analysis of variance of amino acid composition of hair from different mouse strains

Variable	Between strain mean square (df = 2)	Within strain mean square (df = 8)	F, variance ratio
Serine	0.49391	0.08900	5.55*
Tyrosine	0.90634	0.12663	7.16*

* $p < 0.05$.

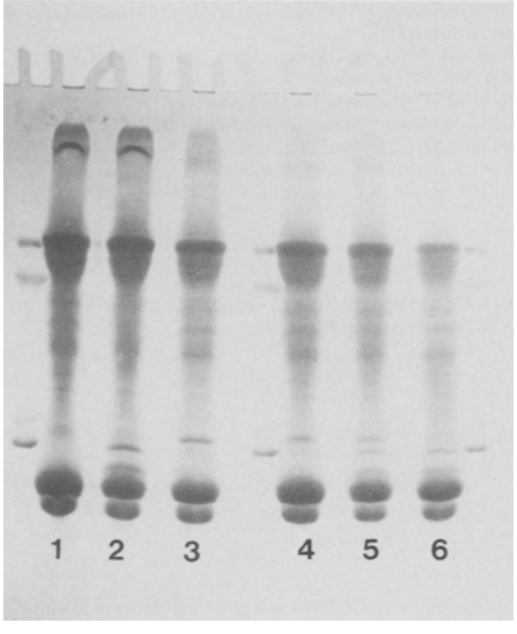

Figure 1. Sodium dodecyl sulphate polyacrylamide gel electrophoresis showing proteins extracted from mouse hair by method 1 (harsh) on the inbred lines and F1 crosses.
1 = CBA/Ca, 2 = Peru, 3 = C57BL/6, 4 = F1 cross between C57BL/6 and CBA/Ca, 5 = F1 cross between C57BL/6 and Peru, 6 = F1 cross between CBA/Ca and Peru. The gel was prepared, electrophoresed, and stained as described in the text. The mol. wts of the proteins were estimated using myosin (205,000), β-galactosidase (116,000), phosphorylase (97,400), bovine albumin (66,000), egg albumin (45,000), glyceraldehyde 3-phosphate dehydrogenase (36,000 subunit), carbonic anhydrase (29,000), trypsinogen (24,000), typsin inhibitor (20,100) and α-lactalbumin.

the accurate measurements of these amino acids (e.g. arginine is more difficult to measure accurately because of the relative peak positions or arginine and ammonia on the elution profile), so that the differences observed at the 10% level can only be regarded as suggestive.

Figure 1 illustrates the banding pattern observed for protein extracts from the hair of the inbred lines of mice using method 1 (harsh). All three extracts contain two high molecular components and have the same banding pattern for very low molecular weight protein (less than 10,000).

Differences in the banding pattern between strains were observed however around the molecular weight range 14,100 to 16,500. The CBA/Ca mice have only a 14,100 band whereas Peru has the 14,100 band and a 15,400 band and C57BL/6 has the 15,400 band and a 16,500 band. It is not likely that these are H-G-T proteins since the mol. wts of the two classes do not overlap in this region[2]. The bands involved are probably not L-S proteins as the lowest molecular component of this group is 40,000. Some breakdown products of the L-S proteins have been reported in 8 M urea, but these are in the range of 3000[23].

Figure 2 illustrates the banding patterns observed for protein

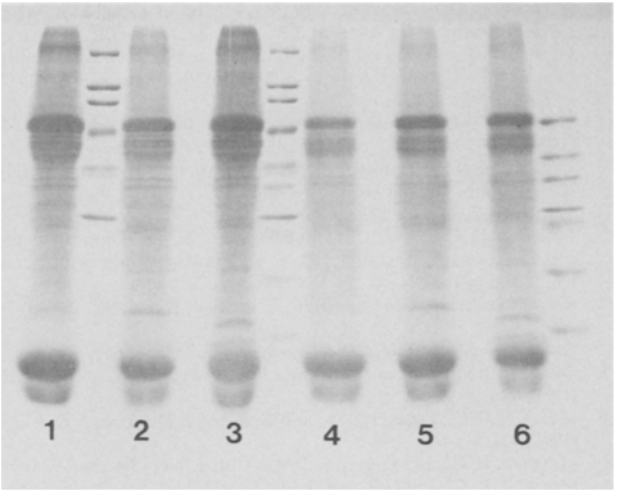

Figure 2. Sodium dodecyl sulphate polyacrylamide gel electrophoresis showing proteins extracted from mouse hair by method 2, DTE extraction (tracks 1–3) and method 3, plasmolysis (tracks 4–6).
1 = CBA/Ca, 2 = C57BL/6, 3 = Peru, 4 = CBA/Ca, 5 = C57BL/6, 6 = Peru, standards as in figure 1.

extracts using methods 2 and 3. Since these are the same in the 14,100–16,500 region as those obtained using method 1, this precludes the possibility that the heterogeneity seen in the proteins between strains is merely an artefact of the harsh extraction procedure. Previous work has not achieved this level of resolution[9–11,16,24,25]. In particular, exponential concentrations of acrylamide in the gels and thin layer gels have not been used before and this combination of techniques is clearly of value.

The amino acid analysis and gel electrophoresis results show that strain differences exist in the hair proteins of mice. From mol. wt determinations of the banding patterns obtained it is not possible to identify conclusively the proteins involved, but it is likely that they are H-S keratins. It is known that two types of melanin are present in agouti hair, pheomelanin having a relatively large sulphur content (9–12%),[26] and eumelanin which contains less sulphur. The melanins have lower mol. wts than the variant proteins we have found and the different patterns seen for the two agouti mice (CBA/Ca and Peru) exclude them from being involved in the strain differences. Peru mice have been reported as being very different from other strains (e.g. Wallace[27]) but in this case they have proteins in common with two other well known strains; it is the C57BL/6 line that has a unique keratin variant.

Further, as the mice were kept on a constant diet the differences between strains seen in this study are not likely to be due to environmental plasticity, as in the case of the sheep diet dependance previously discussed, but to genetic differences between strains.

This is confirmed by the genetic analysis described in the methods. Gel electrophoresis of protein extracts from F1 hybrid hair reveals that the proteins behave as non allelic and codominant e.g. a cross between Peru and C57BL/6 shows all three bands in all animals (fig. 1). There is no difference between reciprocal crosses, indicating an absence of sex linkage. Analysis of the F2 progeny has revealed no recombinant phenotypes indicating that the variant genes may be closely linked.

In order to provide confirmation that these are indeed H-S proteins, amino acid compositions of the contents of each band are being obtained by electroeluting them and analyzing them on the Rank Milger machine. N-terminal amino acid sequences of the same eluted material will be obtained by the solid phase technique of Ward et al.[28] in cooperation with Dr J. Findlay, Dept of Biochemistry, University of Leeds.

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Supernumerary chromosomes in *Drosophila nasuta albomicana*

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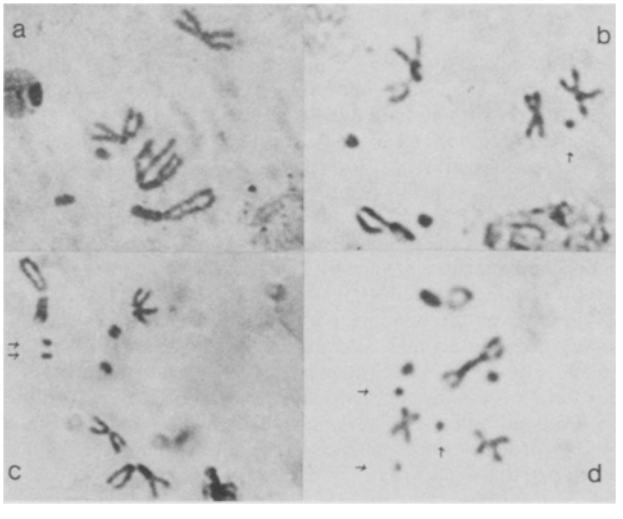
6 April 1984

Summary. Supernumerary chromosomes have been detected in the karyotype of *D.n.albomicana.* Their number varies from one to three. They are the smallest elements in the karyotype. Karyotypes of *D.n.albomicana* with and without supernumerary chromosomes have been presented.
Key words. Drosophila nasuta albomicana; chromosomes, supernumerary; karyotype.

Karyotypic variation is frequent in *Drosophila.* It may be due to inversions[1,2], heterochromatin variation[3,4], and the nature of microchromosomes and sex chromosomes[5]. Karyotypic plasticity due to the presence of extra chromosomes or supernumerary chromosomes is rare in *Drosophila*[2]. The presence of such chromosomes in *Drosophila nasuta albomicana* was first detected by Kitagawa (personal communication). We present here the preliminary cytology of supernumerary chromosomes in the Chiangmai (Thailand) strains of *D.n.albomicana* provided by Prof. O. Kitagawa.

Materials and methods. D.n.albomicana is a chromosomal race in the *nasuta* subgroup of the *immigrans* species group of *Drosophila*[6,7]. The Chiangmai strains were maintained at 21 °C on wheat cream agar medium seeded with yeast. Neural ganglia from third instar larvae were pretreated in 1% sodium citrate hypotonic solution for 15 min and fixed in alcohol/acetic acid (3:1).Air dried preparations were made following the procedure adapted by Lakhotia and Kumar[8] with slight modifications.

Results and discussions. The normal chromosome complement of *D.n.albomicana* is 2n = 6 as reported earlier[9]. It has two pairs of metacentrics – one of them represents chromosome 2 and in the other sex chromosomes and chromosome 3 are united – and a pair of long dots (fig., a). The present analysis showed the presence of extra chromosomes. Their number ranges from 1 to 3 (fig., b–d). They are the smallest elements in the karyotype. They resemble the basic dot chromosomes of other *Drosophila* species. Within an individual, the number of supernumerary chromosomes remains constant in all the cells. The relative frequencies of karyotypes in the strain under investigation with the normal complement and with one, two and three supernumeraries are 33%, 36%, 26% and 5% re-



A Normal karyotype of *D.n.albomicana; B, C* and *D* karyotypes of *D.n.albomicana* with one, two and three supernumerary chromosomes (arrows).