# BREED AND SPECIES DIFFERENCES IN THE HAIR PROTEINS OF FOUR GENERA OF CAPRINI

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*Abstract*

A study has been made of the high-sulphur protein components of wools from goats and a variety of sheep using electrophoresis in starch gels at pH 2·4 and chromatography on DEAE–cellulose at pH 4·5 as methods for characterization. The high-sulphur proteins prepared from ovine wools, from domestic (10 breeds and strains), feral (Soay and Shetland), or wild (Bighorn, *O. canadensis*; Mouflon, *O. musimon*) sheep are very heterogeneous. Of the two methods gel electrophoresis gives the better resolution with a characteristic pattern in which upwards of 20 components can be seen and 12 are sufficiently well separated to be readily identifiable. This pattern is unaffected by the diet or by the age of the sheep (up to 5 yr) and would seem to be characteristic for ovine wools. One or other of two of these bands may be missing from the wool of some individuals, particularly of wild sheep.

Significant departures from this pattern were observed with high-sulphur proteins of non-ovine wools (Barbary sheep, *Amnotragus lervia*; Blue sheep, *Pseudois nayaur*) and the hair of the domestic goat (*Capra* sp.), although they were equally heterogeneous. This electrophoretic technique may be of use in clarifying the somewhat uncertain phylogenetic relationships within the sheep-like and goat-like animals. High-sulphur proteins from the wool of six sheep having the felting lustre mutation gave patterns which differed from the standard ovine pattern in characteristic individual ways.

## I. INTRODUCTION

All mammalian keratinous fibres appear to be constructed of aligned filaments which are composed largely of proteins of comparatively low sulphur content and high α-helix content (low-sulphur proteins). These filaments are surrounded by an unaligned matrix composed of non-helical proteins which are of high sulphur content (high-sulphur proteins) (Crewther *et al.* 1965). In spite of this common structure there are major differences in composition between keratin fibres which are mainly caused by differences in the amount and type of constituent high-sulphur proteins (Gillespie and Inglis 1965). Differences in the low-sulphur proteins (if these exist) must be small, for so far they have not been observed. Thus high-sulphur proteins may be a key in understanding variations in the physical properties of keratins (Gillespie 1967). However, the study of these proteins has been limited by lack of

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convenient methods for their identification and characterization. The improved electrophoretic and chromatographic fractionation procedures recently developed for these proteins (Darskus and Gillespie 1969; Darskus 1971), have now made it possible to obtain more reliable information on their variability in keratins, which may ultimately lead to a link with differences in physical properties.

As the high-sulphur proteins of wool appear to be species-specific, they may be useful genetic markers and used to supplement the data of Ryder (1964, 1971) on the lines of development of the domestic sheep from its wild ancestors. The classification and lines of demarcation of sheep-like and goat-like animals are confused, and in recent studies even domestic goats and sheep could only be distinguished with certainty by chromosome counting (Payne 1968; Higgs and Jarman 1969; Curtain 1971). Electrophoresis of the high-sulphur proteins of wools and hairs from these animals may be a useful supplementary tool to aid in their classification and identification.

Previous studies have shown that the high-sulphur proteins of Merino wool are extremely heterogeneous (Gillespie 1965; Joubert, de Jager, and Swart 1968; Darskus, Gillespie, and Lindley 1969). Therefore the opportunity was taken in these studies to estimate the part, if any, that cross-breeding has played in the evolution of this heterogeneity. In this paper we have examined by chromatography and gel electrophoresis the high-sulphur proteins of wools from various domestic, feral, and wild sheep. Proteins from wool grown by sheep with the lustre mutant fleece type and from domestic goats have also been examined.

## II. Experimental

### (*a*) *Preparation of Proteins*

The tips of domestic wool and all but 1–2 cm of the proximal end of fibres from feral and wild sheep were discarded and the remaining fibres then washed in the usual way (Gillespie and Reis 1966). High-sulphur proteins for the chromatographic studies and some of the electrophoretic experiments were prepared by the low-temperature preferential method of Gillespie (1962). If only small amounts of wool were available the urea–thioglycollate procedures of Harrap and Gillespie (1963) were used instead, either at 0 or 40°C. All three methods gave proteins which were indistinguishable by starch-gel electrophoresis.

### (*b*) *Gel Electrophoresis and Column Chromatography*

Darskus (1971) gives in detail the procedures used for starch-gel electrophoresis in urea–acetic acid at pH 2·4 and for chromatography on DEAE-cellulose between pH 4·5 and 3·85.

## III. Results

### (*a*) *Variations within a Breed*

#### (i) *Individual Differences*

A comparison was made of the high-sulphur proteins from the wool of five related English Leicester–Merino crossbred wethers which were housed indoors and kept on a maintenance diet designed to give wool of a low sulphur content (Reis 1965).

The first comparison was made by chromatography at pH 4·5 (Fig. 1). For ease in discussion the chromatograms have been divided into four regions, A–D, and region

B has been further subdivided into peaks I–VIII. Many components can be seen in regions A and B; there is worse resolution in regions C and D, even though these also contain many components. Where peaks are resolved in regions A and B they have corresponding elution positions within experimental error in the five protein preparations. There are, however, differences in the relative proportions of many of these components, as can clearly be seen for peaks I and III. Larger differences can be seen in region D, both in the degree of resolution and in the proportion of components.

These proteins were also compared by starch-gel electrophoresis at pH 2·4 (see Fig. 4). In these particular experiments at least 17 components can be resolved, although only the 12 best-resolved ones have been numbered. Within experimental error, the mobilities of corresponding components are the same in each preparation, although band 12 is lacking in (*a*) and band 11 is split in (*c*), (*d*), and (*e*). There are conspicuous differences in intensity of staining of corresponding bands (e.g. band 1).

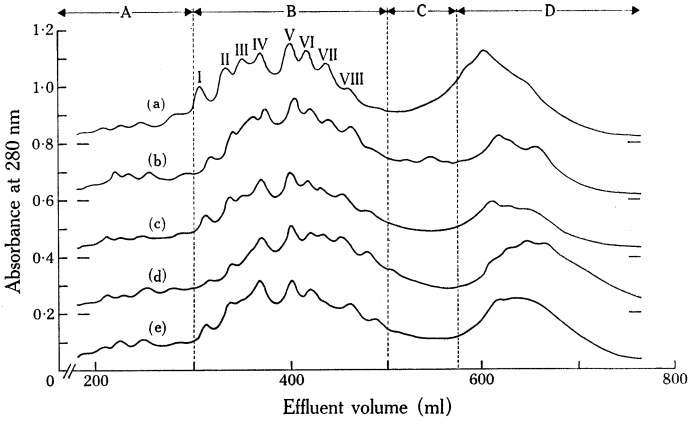


Fig. 1.—Elution pattern of high-sulphur proteins from the wool of five English Leicester-Merino crossbred wethers pen-fed an identical diet. 250 mg protein was loaded on to the column and was eluted under the conditions described in Darskus (1971). Curves (*a*), (*b*), (*c*), and (*d*) displaced 0·8, 0·6, 0·4, and 0·2 absorbance unit respectively.

(ii) *Strain Variations*

Similar comparisons were made between the high-sulphur proteins of wool from several strains of Australian Merino sheep (Fig. 2). Sample No. MW148 was a mixed sample from a commercial flock whilst the others were from individual sheep in experimental flocks in the field. The "primitive" Merino represents an original strain of Merino imported to Australia in 1797 and propagated at Camden Park, near Sydney.

The chromatographic comparisons (primitive Merino, medium non-Peppin, and MW148) give qualitatively similar patterns to those of the English Leicester–Merino crosses and all the peaks resolved in regions A and B have corresponding elution positions. There are, however, more substantial differences in the proportion

of components in region B, the primitive Merino and the medium non-Peppin being enriched in components I and II and relatively poorer in components IV–VIII.

The samples mentioned above, as well as additional samples from a further 10 Merino sheep representing four well-defined strains, were also studied by starch-gel electrophoresis. The patterns were almost indistinguishable, each showing the same number of components with the same mobilities, except for the deletion of band 12 in some samples. Some intensity differences were also found.

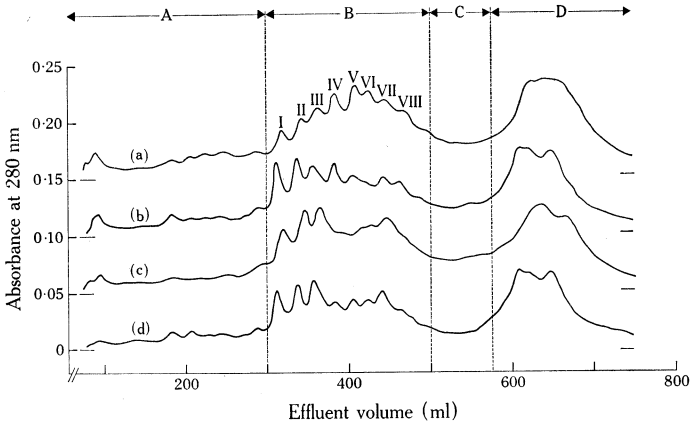


Fig. 2.—Elution pattern of high-sulphur proteins from different Merino wools: (*a*) a mixed sample (No. MW148); (*b*) primitive Merino from Camden Park flock (wether); (*c*) felting lustre mutant; (*d*) medium non-Peppin strain (ram). 75 mg protein was loaded on to the column. Curves (*a*), (*b*), and (*c*) displaced 0·15, 0·10, and 0·05 absorbance unit respectively.

### (iii) *Effect of Diet*

An English Leicester–Merino cross [this sheep also provided high-sulphur proteins for the experiments shown in Figures 1(*a*) and 4(*a*)] was kept indoors on a constant low level of diet to produce low-sulphur wool. The diet was then enriched by the abomasal infusion of methionine (0·5 g/day) by the procedure of Reis and Schinckel (1963). The control wool contained 3·0% of sulphur and the sulphur-enriched wool 3·98%. A comparison by both electrophoresis and chromatography showed no differences in the number of components or in their mobilities. This confirms the findings of Gillespie, Reis, and Schinckel (1964), Gillespie and Reis (1966), and Broad, Gillespie, and Reis (1970) that the ultra-high-sulphur proteins produced during the sulphur-enrichment process, which run in the D region of the chromatograms, cannot be resolved at pH values below about 10. However, the present work reveals in addition that the sulphur-enrichment process causes marked changes in the proportions of many components, for changes can be seen in all regions of the chromatogram but especially in peaks VI and VII in region B and in the peak of highest charge in region D.

### (*b*) *Variations between Breeds*

(i) *Domestic Breeds*

Wool samples representative of the main Australian domestic breeds of sheep were solubilized and their high-sulphur proteins compared by electrophoresis and, in some cases, chromatography. Representative results only are described below. Chromatograms of the high-sulphur proteins of Lincoln, Border Leicester, Southdown, and Corriedale sheep are shown in Figure 3. The patterns show the same components with the same elution positions as already seen for Merino and the English Leicester–Merino crossbred wool. There are major differences in the proportion of components in the B region and also marked but ill-defined differences in region D. For the few individuals examined, Lincoln, Border Leicester, and Corriedale [Figs. 3(*a*)–3(*c*) respectively] resemble the primitive Merino and medium non-Peppin wools [Figs. 2(*b*) and 2(*d*)] in having a greater relative amount of components BI–III than BIV–VIII, whilst the Southdown pattern in the B region [Fig. 3(*d*)] resembles that of the mixed Merino and the English Leicester–Merino crosses [Figs. 2(*a*) and 1(*a*) respectively].

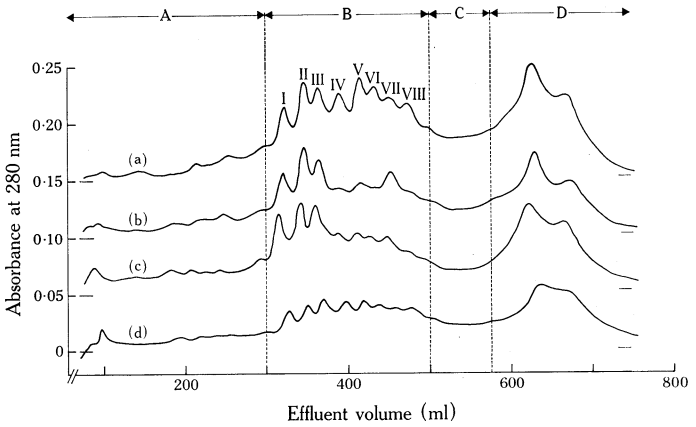


Fig. 3.—Elution pattern of high-sulphur proteins from various breeds of sheep: (*a*) Lincoln; (*b*) Border Leicester; (*c*) Corriedale; (*d*) Southdown. 75 mg protein was loaded on to the column. Curves (*a*), (*b*), and (*c*) displaced 0·15, 0·10, and 0·05 absorbance unit respectively.

The electrophoretic patterns of these proteins [Figs. 5(*a*)–5(*d*)] are identical with those obtained with Merino wool [Fig. 5(*e*)] in the number of components resolved and in their relative mobilities. There are differences in staining intensities, notably in band 11, which is much weaker in Southdown and Lincoln wools. Wool obtained in five successive yearly clippings of the Corriedale yielded high-sulphur proteins with identical starch-gel patterns.

(ii) *Feral Sheep*

The high-sulphur proteins of wool from two breeds of feral sheep (Shetland and Soay) were examined by gel electrophoresis [Figs. 6(*a*) and 6(*b*)]. Both proteins give the characteristic wool pattern, the only peculiarities being that in Shetland

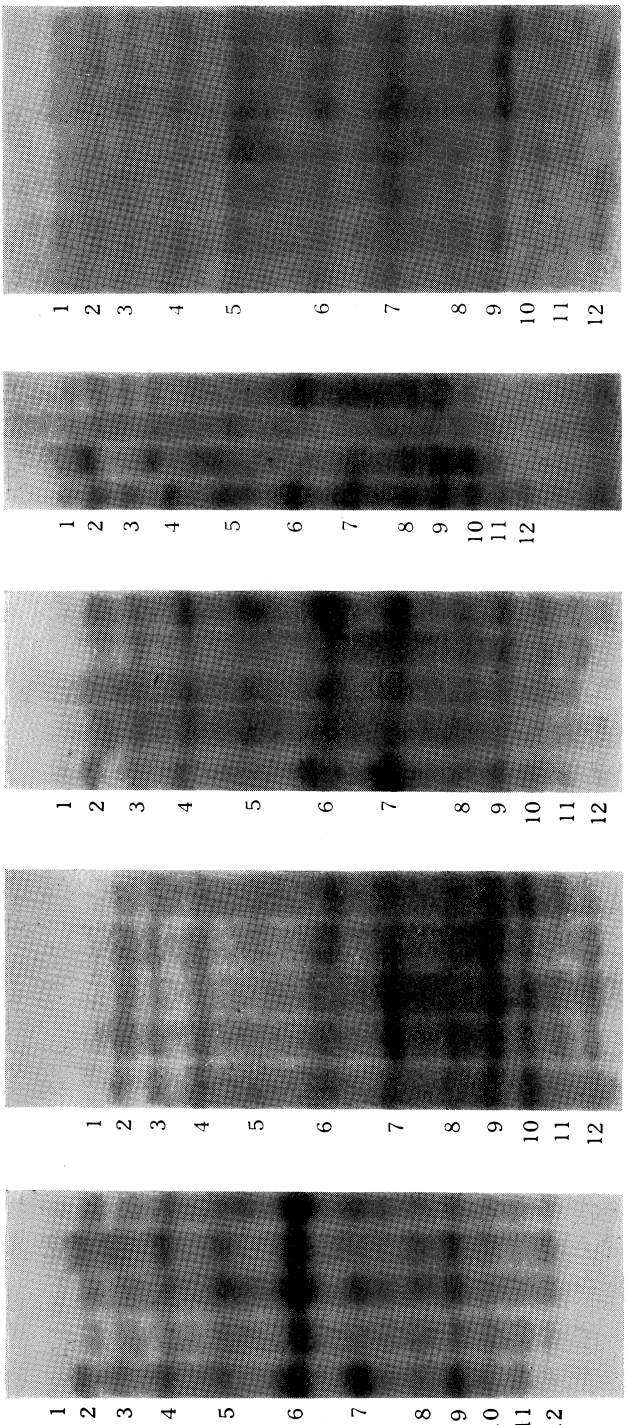


Fig. 4 Fig. 5 Fig. 6 Fig. 7 Fig. 8

Figs. 4–8.—Starch-gel electrophoresis patterns at pH 2·4 of high-sulphur proteins of goat hair and different types of wool.

Fig. 4.—English Leicester–Merino crossbred sheep maintained on a standard minimal diet. See Figure 1 for chromatographic runs on these samples, curves (*a*)–(*e*) there respectively corresponding to (*a*)–(*e*) in this figure.

Fig. 5.—Various breeds of sheep: (*a*) Corriedale; (*b*) Southdown; (*c*) Border Leicester; (*d*) Lincoln; (*e*) Merino.

Fig. 6.—Feral and wild sheep: (*a*) Shetland; (*b*) Soay; (*c*) Mouflon; (*d*) Bighorn; (*e*) Merino.

Fig. 7.—Wild sheep and goat: (*a*) Merino; (*b*) goat; (*c*) Barbary sheep; (*d*) Blue sheep.

Fig. 8.—Felting lustre mutants: (*a*) Merino; (*b*)–(*g*) mutants.

the faster component in band 2 is missing and there is a split in band 6. Shetland wool gave a chromatographic pattern similar to that of the Corriedale shown in Figure 3(*c*), with the same elution positions and similar proportion of components.

(iii) *Wild Sheep and Goats*

High-sulphur proteins extracted from wool grown by four types of wild sheep and from goat hair were compared by electrophoresis. The results for Mouflon (*Ovis musimon*) and Bighorn (*O. canadensis*) are shown in Figures 6(*c*) and 6(*d*). It can be seen that the patterns are similar to that of standard Merino wool, exceptions being the absence of band 12 from both wild sheep and the apparent insertion of an extra component between bands 2 and 3 in Mouflon, apart from differences in relative staining intensities. In Figures 7(*b*)–7(*d*) respectively can be seen the patterns of high-sulphur proteins of the domestic goat (*Capra* sp.), the Barbary sheep (*Amnotragus lervia*), and the Blue sheep (*Pseudois nayaur*). Although in a general way these patterns are similar to those of the domestic sheep, there are some important differences between them. For example some or all of the non-ovine patterns show major differences in the mobilities of bands 2, 3, and 4, with smaller differences in bands 6, 7, 10, and 11. Considerable differences are observed in the intensities of bands 2, 6, and 7. Band 12 is not visible in the patterns of goat and the wild sheep; in addition, band 11 is absent or very faint in the Blue sheep. These differences combine to make the non-ovine patterns readily distinguishable from those of true sheep and to a lesser extent from each other.

(*c*) *Sheep showing the Felting Lustre Mutation*

The high-sulphur proteins of lustre mutant wools (Short 1958) have been compared with normal Merino proteins. Only one preparation was studied by chromatography [Fig. 2(*c*)]; within experimental error this mutant wool contains components with identical elution characteristics to those of normal Merino wool. The distribution of components in region B was similar to that observed with the primitive Merino [Fig. 2(*b*)].

The high-sulphur proteins from wools of six other mutant sheep of this type, from an experimental flock in the field, were examined by starch-gel electrophoresis with a standard Merino protein used for comparison. It can be seen (Fig. 8) that, with some exceptions mentioned below, the numbers and mobilities of the components in each preparation appear to be the same as those of the Merino wool. There are, however, some important differences. For example, band 12 is absent from samples 8(*d*) and 8(*g*), there is an extra band between components 2 and 3 in samples 8(*c*) and 8(*e*), and band 11 has a somewhat slower mobility in samples 8(*b*), 8(*c*), and 8(*f*) or is replaced in these samples by one or two new components. The variability in proportion of components as manifested by differences in intensity of staining is similar in these mutant proteins to that observed with the set of English Leicester–Merino crossbred sheep [Section III(*a*)(i)].

## IV. Discussion

The great heterogeneity of the high-sulphur proteins of Merino wool has attracted interest ever since it was first observed (Gillespie 1963), because it is unusual for a particular function in an organism to be performed by a family of related proteins

having such a wide distribution of compositions and molecular sizes. It is now evident that this complexity is not confined to Merino wool, but is a property of the high-sulphur proteins of wool in general and, as will be shown in a later publication, of most animal hairs. The fact that wild sheep such as the Bighorn and Blue sheep, which presumably represent pure strains, also contain many high-sulphur protein components indicates that cross-breeding has not contributed materially to this heterogeneity. The contribution which bilateral differentiation makes to heterogeneity cannot be eliminated at present, for even the relatively crimpless Lincoln and lustre mutant wools contain differentially staining cells (Fraser and Macrae 1956). As it is now generally conceded that artefacts of preparation can be excluded from consideration (Lindley, Gillespie, and Rowlands 1970), it must be concluded that heterogeneity is an inherent characteristic of wool high-sulphur proteins.

All wool high-sulphur proteins give a gel electrophoretic pattern with approximately 17–20 bands in a definite arrangement which can be regarded as typical of ovine wool. This pattern, with minor variations, has now been obtained with the high-sulphur proteins of wool from 50 sheep irrespective of sex, age, breed, or diet. The electrophoretic pattern is not affected by the nutritionally induced sulphur-enrichment process, presumably because the ultra-high-sulphur proteins produced (Gillespie and Reis 1966) are a heterogeneous minor fraction running in the region of poorest resolution (bands 5–9). It should be pointed out that electrophoretic patterns of the high-sulphur proteins from the hair of other animals, even of other Bovidae, bear no resemblance to those patterns shown in this study (Gillespie 1970; Gillespie and Darskus, unpublished data).

Parts of the gel pattern are variable, in that certain bands may be missing or slightly displaced in some individuals. These aberrant sheep have not been observed over a period of time to see if this is a constant characteristic, but as it seems unrelated to any environmental effect it may be assumed to be genetic in origin. It may provide a useful marker in the characterization of sheep in genetic experiments, particularly if it proves to be linked with some valuable characteristic. Deletions of component 12 have been observed in different breeds; it may, however, be of some significance that this deletion occurs in all the wild sheep and goats examined. The most obvious explanation for the overall pattern and its variability is the presence of multiple genes, some of which exhibit polymorphism.

Comparison of the gel patterns shows marked resemblances between the high-sulphur proteins of the sheep-like and goat-like animals, although a number of components differ in mobility. These differences between the four genera are sufficiently great to make electrophoresis of their high-sulphur proteins of potential use in distinguishing between them, and for clarifying their rather uncertain phylogenetic relationships. With the resolution obtainable it is unfortunately still not possible to distinguish one breed of sheep from another and, therefore, it is unlikely at present that this work can supplement Ryder's (1964, 1971) views on the evolution of domestic sheep. Such studies will have to await further improvements in resolution, although the small interbreed differences which can be seen in freshly stained gels suggest that this is almost within reach.

The effects of the presumed mutation responsible for the felting lustre characteristic are obvious from the appearance and the mechanical properties of the fibre (Short 1958), but virtually no information has been available on the effects at a

molecular level. Previously it had been shown that in one mutant fleece, not one of those examined here, the protein fraction SCMKB2 contained one mutated peptide (Gillespie, Haylett, and Lindley 1968). In the present work the changes in high-sulphur protein components suggest that some of the wools contain mutated proteins. There are, however, no constant differences from the proteins of normal wool but if each lustre mutant sheep represents a unique mutational event, then these would not be expected. These changes do not appear to be of sufficient magnitude to account for the changed properties of the wool. In other experiments not reported here, no changes were found in the low-sulphur proteins of these wools, although changes could be found in the proportions of the minor group of proteins which are rich in glycine and tyrosine.

The chromatographic patterns and, to a lesser extent, the gel patterns show that, although the same components are present, with minor exceptions, in the high-sulphur proteins of all ovine wools, they may be present in rather different amounts. It is of considerable interest to uncover the significance of this variability, for it may help ultimately to distinguish between breeds and it may be relevant to the problem of the molecular basis of physical properties. The effects of dietary, genetic, and environmental influences on this variability are not easy to separate but the following conclusions can be made. There is a variation in proportion of components from animal to animal of the same breed even when these sheep are on the same diet (cf. English Leicester–Merino crossbred sheep). This may well be a genetic difference. Genetic effects may also be responsible for the quantitative differences observed between other individual sheep, both within a breed and between breeds. However, in these cases dietary and environmental effects may also be involved. The large effects of diet are illustrated by the changed chromatograms of the high-sulphur proteins from sulphur-enriched wool [Section III(*a*)(iv)]. To sort out the variables in this situation completely would require a large number of sheep to be housed indoors and maintained on a uniform diet; such facilities are unfortunately not available to us. Whatever the cause, this variation in the proportion of components has not previously been recognized as a source of variability in composition between wools, and consequently has not been taken into account in considerations on the molecular basis of the mechanico-chemical properties of wool.

## V. Acknowledgments

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## IV. References


Broad, A., Gillespie, J. M., and Reis, P. J. (1970).—*Aust. J. biol. Sci.* **23**, 149.

Crewther, W. G., Fraser, R. D. B., Lennox, F. G., and Lindley, H. (1965).—*Adv. Protein Chem.* **20**, 191.

Curtain, C. C. (1971).—The origin of domesticated sheep. *Antiquity* (In press).

Darskus, R. L. (1971).—Electrophoretic and chromatographic characterization of sulphur-rich proteins from wool. *J. Text. Inst.* (In press.)

Darskus, R. L., and Gillespie, J. M. (1969).—*Proc. Aust. biochem. Soc.* **2**, 77.

Darskus, R. L., Gillespie, J. M., and Lindley, H. (1969).—*Aust. J. biol. Sci.* **22**, 1197.

Fraser, R. D. B., and Macrae, T. P. (1956).—*Text. Res. J.* **26**, 618.

Gillespie, J. M. (1962).—*Aust. J. biol. Sci.* **15**, 262.

Gillespie, J. M. (1963).—*Aust. J. biol. Sci* **16**, 259.

Gillespie, J. M. (1965).—In "Biology of the Skin and Hair Growth". (Eds. A. G. Lyne and B. F. Short.) pp. 377–98. (Angus and Robertson Ltd.: Sydney.)

Gillespie, J. M. (1967).—*J. Polymer Sci.* C **20**, 201.

Gillespie, J. M. (1970).—*Science, N.Y.* **170**, 1100.

Gillespie, J. M., Haylett, T., and Lindley, H. (1968).—*Biochem. J.* **110**, 193.

Gillespie, J. M., and Inglis, A. S. (1965).—*Comp. Biochem. Physiol.* **15**, 175.

Gillespie, J. M., and Reis, P. J. (1966).—*Biochem. J.* **98**, 669.

Gillespie, J. M., Reis, P. J., and Schinckel, P. G. (1964).—*Aust. J. biol. Sci.* **17**, 548.

Harrap, B. S., and Gillespie, J. M. (1963).—*Aust. J. biol. Sci.* **16**, 542.

Higgs, E. S., and Jarman, M. R. (1969).—*Antiquity* **43**, 31.

Joubert, F. J., Jager, P. J. de, and Swart, L. S. (1968).—In "Symposium on Fibrous Proteins." Australia, 1967. (Ed. W. G. Crewther.) pp. 343–52. (Butterworths: Australia.)

Lindley, H., Gillespie, J. M., and Rowlands, R. J. (1970).—*J. Text. Inst.* **61**, 157.

Payne, S. (1968).—*Proc. Prehist. Soc.* **34**, 368.

Reis, P. J., (1965).—*Aust. J. biol. Sci.* **18**, 671.

Reis, P. J., and Schinckel, P. G. (1963).—*Aust. J. biol. Sci.* **16**, 218.

Ryder, M. L. (1964).—*Agric. History Rev.* **12** (1), 65.

Ryder, M. L. (1971).—On the status of non-domesticated sheep. *Antiquity* (In press.)

Short, B. F. (1958).—*Nature, Lond.* **181**, 1414.