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Proteins of the Hard Keratins of Echidna, Hedgehog, Rabbit, Ox and Man

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Abstract

In the accompanying paper it has been shown that two major groups of proteins (low-sulphur and high-sulphur) of ovine wool, horn, and hoof contain similar components although the overall proportions of the groups of proteins and the relative proportions of components within the groups may show significant differences. In the present paper it has been shown for five other species (echidna, hedgehog, rabbit, ox and man) that the hard keratins produced by one animal contain the same groups of protein components but in different relative proportions. The wide apparent differences in the type and relative proportions of the low-sulphur components which comprise the major constituent proteins of the microfibrils suggest that microfibrils can tolerate a considerable variation in the constituent proteins and still produce functional structures.

The low-sulphur protein components are sufficiently well resolved by sodium dodecyl sulphate–polyacrylamide gel electrophoresis to make this procedure potentially useful for animal identification and classification.

Introduction

In the accompanying paper (Marshall and Gillespie 1977) it was shown that the three hard keratins (wool, horn and hoof) produced by the sheep are constructed of the same heterogeneous groups of low-sulphur and high-sulphur protein components. These conclusions were based on the identity of electrophoretic mobilities in polyacrylamide gels at pH 8·9 and in sodium dodecyl sulphate (SDS)–polyacrylamide gels at pH 7. Additional data were obtained for the high-sulphur proteins by electrophoresis in polyacrylamide gels at pH 2·6 and by moving boundary electrophoresis at pH 4·5. These electrophoretic data indicated that the three keratins contained the same components although these were often present in quite different proportions. In general, the proteins of horn and hoof (referred to as horny keratins) resembled each other more than those of wool.

In the present less detailed study, the proteins from two or three types of hard keratin from each of another five species of mammal have been examined in order to assess whether our findings with the sheep keratins may be applied to keratins in general. We have attempted to provide answers to two questions. Firstly, do the proteins from the keratins from one species contain the same array of components, and in what relative proportions? Secondly, do the low-sulphur proteins of these keratins exhibit species specificity so that their SDS–polyacrylamide gel electrophoretic patterns could be used in animal classification in conjunction with the procedures already worked out for the high-sulphur proteins (Marshall *et al.* 1977)?

Materials and Methods

The keratins from the following mammals were examined: echidna (*Tachyglossus aculeatus*), hair, quill and claw; hedgehog (*Erinaceus europaeus*), hair and quill; rabbit (*Oryctolagus cuniculus*), hair and claw; ox (*Bos taurus*), hair, horn and hoof; man, caucasian male child (*Homo sapiens*), hair and nail. The keratin samples for each species (called a set of keratins) were obtained from one individual.

The horny keratins were freed from non-keratinous constituents by careful dissection followed by extraction of the ground keratinous material with several changes of 0·15 M NaCl over 24 h. After washing with water and drying, the solid keratins were further treated by the procedure used for cleaning the hairs which involved successive washing with petroleum ether, ethanol, and water.

The procedures for the preparation and fractionation of the *S*-carboxymethylkerateines, their amino acid analysis and polyacrylamide gel electrophoresis, and for the densitometric scanning of the polyacrylamide gels have been described previously (Marshall and Gillespie 1977).

Low-sulphur proteins were not separated from the whole extract, as satisfactory procedures have been worked out only in the case of wool. However, we have found that the low-sulphur proteins are sufficiently well resolved from the other proteins by the SDS–polyacrylamide gel electrophoretic procedure that their electrophoretic properties can be obtained from patterns of whole extracts.

Results

Comparison of the Low-sulphur Proteins of Keratins from Five Species

The unfractionated *S*-carboxymethylkerateins isolated from the keratins of the five species were run electrophoretically in polyacrylamide gels in the presence of SDS. For the purpose of this comparison only the sections of the gels containing the dominant low-sulphur protein bands have been reproduced in Fig. 1. The complete patterns for the hairs, showing also the lower-molecular-weight high-sulphur and high-tyrosine protein bands, are reproduced in Fig. 4.

It is clear (Fig. 1) that within the set of keratins from each species corresponding low-sulphur protein components have identical mobilities and hence molecular weights. Marked differences in the relative proportions of components from each keratin of a set can be readily seen. On the basis of number and relative proportion of components, the low-sulphur proteins of ox horn and hair appear similar as do the low-sulphur proteins of echidna quill and hair. In each case there are marked differences from the third member of the keratin set.

Comparison of the High-sulphur Proteins within Each set of Keratins

High-sulphur proteins, prepared by fractionation of the extracted proteins studied in the previous section, were compared by electrophoresis in polyacrylamide gels at pH 2·6 (Fig. 2). Previous work has shown that this procedure gives maximum resolution of high-sulphur protein components (Marshall and Gillespie 1976). It can be seen (Fig. 2) that there is a striking resemblance between the patterns of a keratin set from one species, both in the number of bands and in the apparently identical mobilities of corresponding bands. It is possible that certain components may actually be missing from some keratins but a close examination of the gels generally suggests that traces at least of most components are common to all members of a set. A feature of certain keratins, most commonly the hairs, is the presence of material which tends to give a smear superimposed upon a pattern of resolved bands. Previous work has shown that this material consists of proteins which are extremely rich in sulphur (Marshall and Gillespie 1976).

There are significant differences in the relative proportions of components in each keratin of a set which can best be seen in the densitometer tracings reproduced in Fig. 3. Echidna quill and claw have a similar relative distribution of components in contrast to the hair which is enriched in the slower-moving components. For

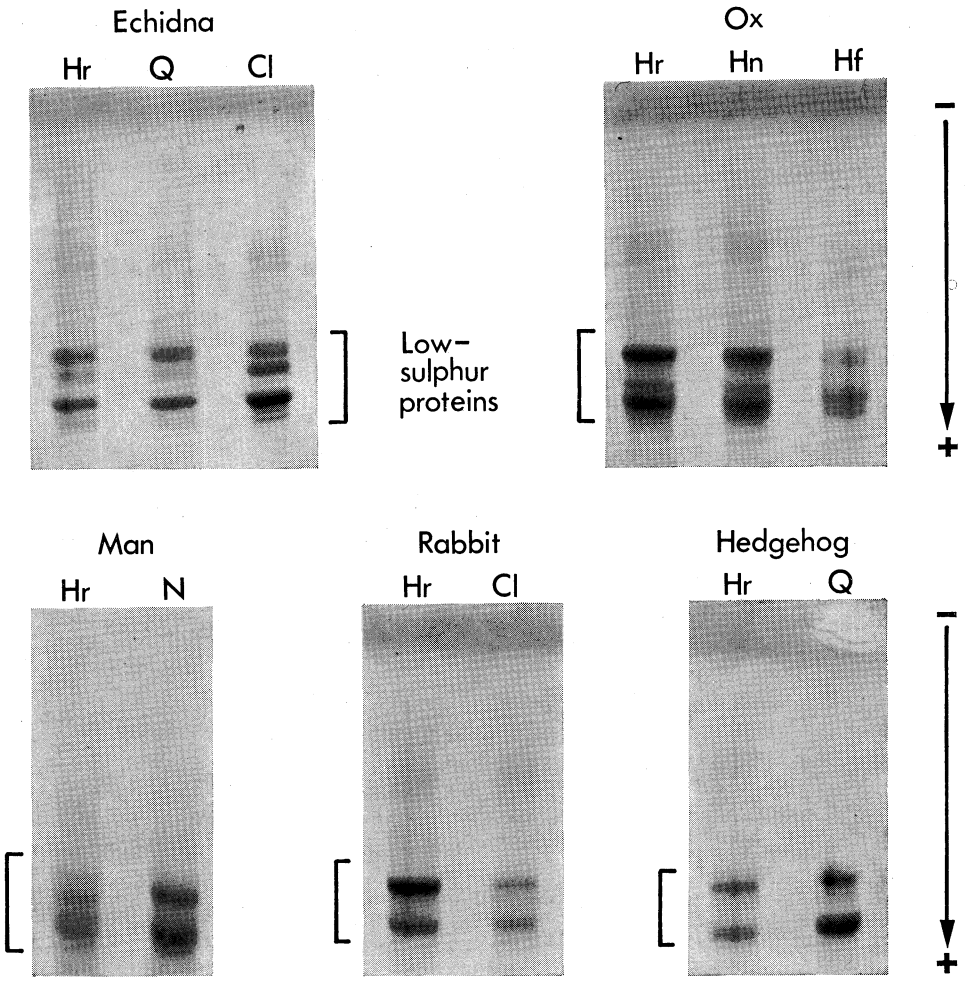

Fig. 1. Polyacrylamide gel electrophoretic patterns in the presence of SDS (4–27% continuous gradient gel, 0·1% SDS, 0·05 M phosphate pH 7) showing the low-sulphur bands of sets of keratins from echidna (hair, quill, claw), ox (hair, horn, hoof), man (hair, nail), rabbit (hair, claw), and hedgehog (hair, quill). Hair (Hr), quill (Q), claw (Cl), horn (Hn), hoof (Hf), nail (N). About 10 μg protein loaded.

the ox proteins, hair is similarly enriched in the slower-moving components compared to hoof, but the proportion of components in horn is more nearly like those in hair than like those in hoof. For the pairs of keratins from rabbit, hedgehog and man the high-sulphur proteins contain the same set of components but in each case the hairs contain the highest proportion of the lower-mobility components.

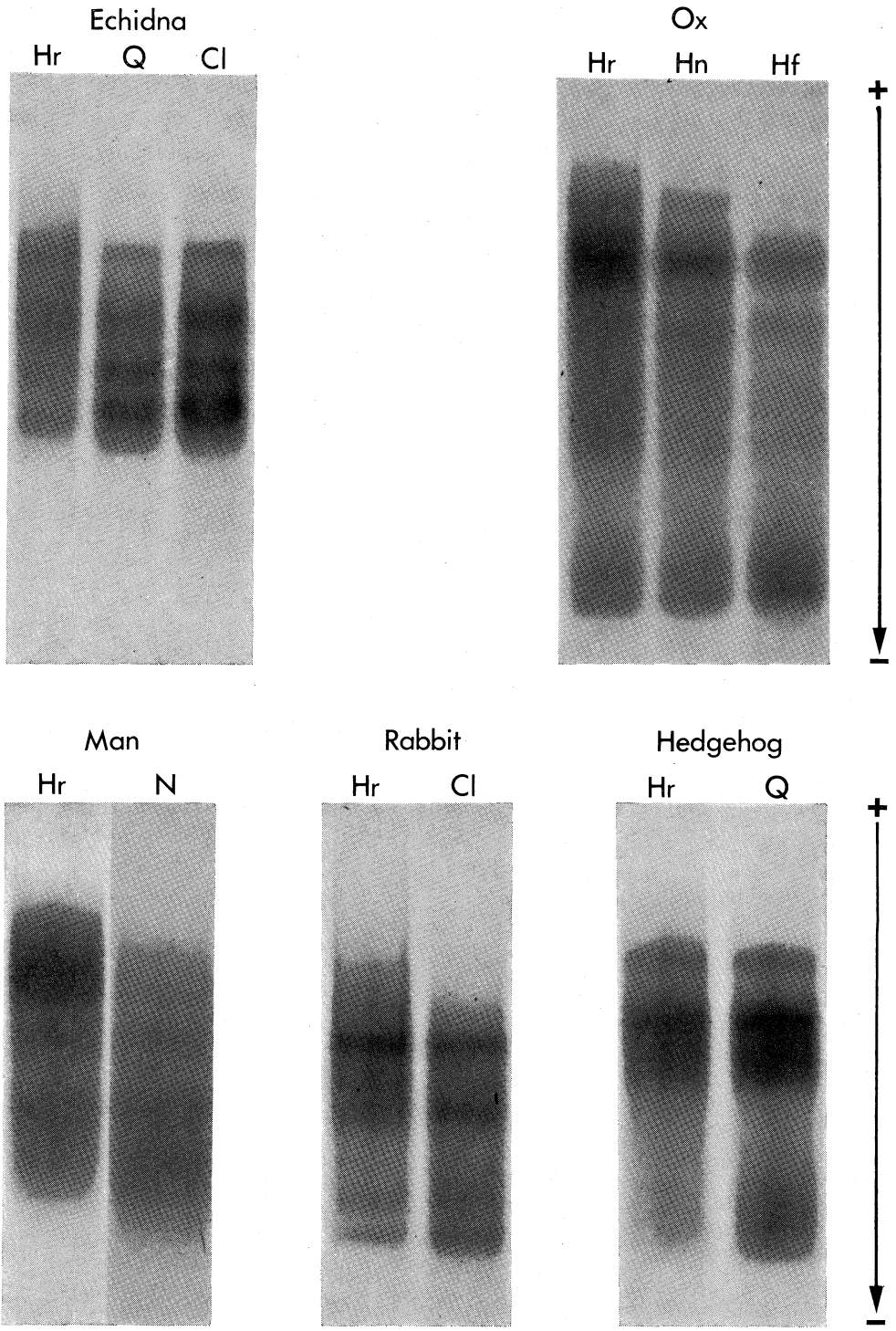

Fig. 2. Polyacrylamide gel electrophoretic patterns at pH 2·6 (10% polyacrylamide, acrylamide–bisacrylamide 27 : 1, 4·8 M acetic acid, 2·75 M urea) of the high-sulphur proteins isolated from sets of keratins from echidna (hair, quill, claw), ox (hair, horn, hoof), man (hair, nail), rabbit (hair, claw), and hedgehog (hair, quill). Hair (Hr), quill (Q), claw (Cl), horn (Hn), hoof (Hf), nail (N). About 70 μg protein loaded.

Comparison of the Amino Acid Compositions of High-sulphur Proteins from Three Sets of Keratins

The amino acid compositions of the high-sulphur protein fractions isolated from the keratins of echidna, ox and rabbit are given in Table 1. The compositions are typical of the high-sulphur protein group showing richness in *S*-carboxymethylcysteine [cysteine(Cm)] proline and serine (Gillespie and Inglis 1965). Within each set of keratins there are pronounced differences in amino acid composition which undoubtedly reflect the differences in electrophoretic composition referred to in the preceding section. The hair proteins contain significantly more cysteine(Cm), and significantly less aspartic acid, glycine, leucine, tyrosine, and phenylalanine than the comparable horny keratin proteins. This relation is also true for the high-sulphur proteins of sheep keratins (Marshall and Gillespie 1977)—wool is enriched, compared with hoof and horn, in those high-sulphur proteins which are richer than average in cysteine(Cm).

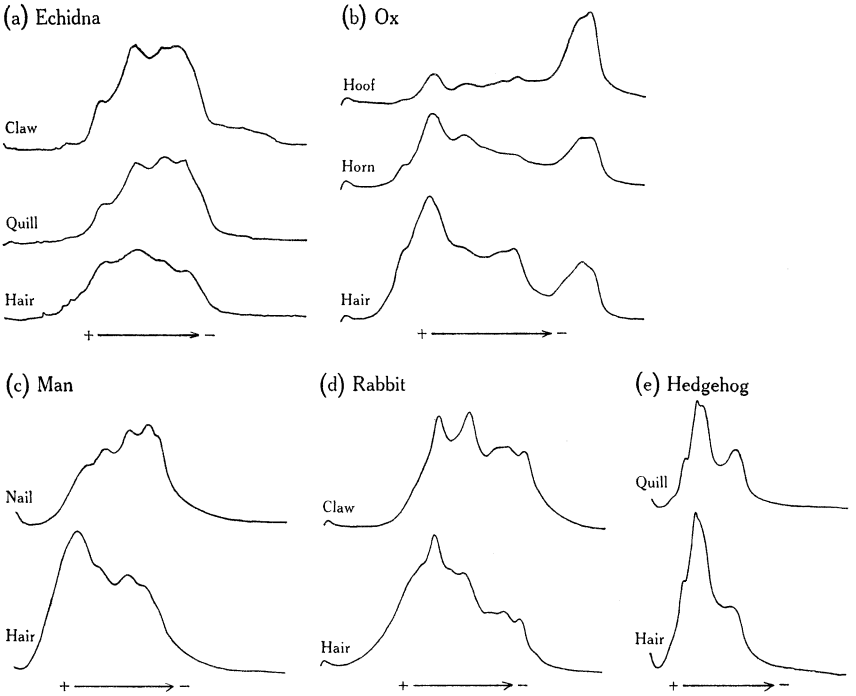

Fig. 3. Densitometric tracings of the polyacrylamide gel electrophoretic patterns (Fig. 2) obtained at pH 2·6 of the high-sulphur proteins isolated from sets of keratins from echidna (hair, quill, claw), ox (hair, horn, hoof), man (hair, nail), rabbit (hair, claw), and hedgehog (hair, quill). Sample wavelength 590 nm, reference wavelength 400 nm.

Comparison of the SDS Electrophoretic Patterns of the Proteins of Five Hairs and Wool

In the preceding sections it was shown that the various keratins from one animal contain the same proteins, but the variations between species were not examined. In Fig. 4 the SDS–polyacrylamide gel electrophoretic patterns for the hair proteins of echidna, ox, man, rabbit and hedgehog are compared with wool. The major banding regions of the low-sulphur proteins of wool are labelled according to the nomenclature

of O'Donnell and Thompson (1964) and Crewther *et al.* (1976). It can be seen (Fig. 4) that the low-sulphur protein components from each keratin span about the same range of molecular weights but there are considerable differences in the number of components, their relative proportions and actual molecular weights. Hedgehog and rabbit low-sulphur proteins give the simplest patterns, apparently containing only two major components in about equal proportions with apparent molecular weights similar to component 7 and the largest of the component 8 complex of wool. The very heterogeneous high-sulphur and high-tyrosine proteins are of higher mobility than the low-sulphur protein components. The high-tyrosine proteins probably run near the front and it is of interest to note that human hair, thought to be devoid of these proteins, also lacks these bands (Fraser *et al.* 1973).

Table 1. Amino acid compositions (as residues per 100 residues) of the high-sulphur protein fractions isolated from sets of keratins produced by three animlas

Amino acid	Echidna			Ox			Rabbit	
	Hair	Quill	Claw	Hair	Horn	Hoof	Hair	Claw
Lys	0·45	0·35	0·35	0·75	0·60	1·66	0·41	0·53
His	0·45	0·58	0·61	0·83	0·84	0·88	0·88	1·10
Arg	5·89	5·38	5·78	6·38	6·56	6·30	7·03	6·17
Cys(Cm)	27·80	24·50	24·70	25·70	21·90	16·30	28·50	24·20
Asp	2·33	2·61	2·66	2·03	3·17	3·82	1·78	2·93
Thr[A]	7·64	6·59	7·02	10·20	9·80	8·92	8·96	8·17
Ser[A]	9·82	10·20	10·90	12·30	11·40	11·70	11·90	10·80
Glu	6·13	5·86	5·73	7·22	6·56	6·07	7·87	7·24
Pro	15·80	14·10	13·90	13·50	13·50	11·60	14·90	14·40
Gly	6·90	10·10	9·19	5·25	6·40	10·60	4·76	8·41
Ala	3·41	3·48	3·31	2·56	3·15	3·72	2·21	2·56
Val	5·54	4·86	4·97	4·68	5·01	5·32	3·91	3·66
Met	0·17	0·21	0·22	Trace	0·05	0·07	0·08	0·05
Ile	0·96	1·11	1·16	2·80	3·37	3·13	2·47	2·71
Leu	3·39	4·26	4·11	2·74	3·60	4·33	2·17	3·51
Tyr	1·24	3·05	2·74	1·81	2·05	2·68	1·10	2·56
Phe	2·10	2·77	2·61	1·08	1·87	2·60	0·84	0·93

[A] Uncorrected for decomposition.

It is evident from the SDS–polyacrylamide gel electrophoretograms shown in Fig. 4 that each keratin provides a unique arrangement of bands. This applies both for the major low-sulphur bands and for the many minor high-sulphur and high-tyrosine protein bands. This method might well provide an additional electrophoretic procedure for use in the identification and classification of mammals to supplement that reported by Marshall *et al.* (1977).

Discussion

It is clear from the results presented in this paper and the accompanying one (Marshall and Gillespie 1977) that for the six mammalian species which have now been examined the various keratins from one species are constructed of the same complex of high-sulphur and low-sulphur proteins, although individual components within either of these protein groups may be present in different relative proportions and certain components may be missing. The horny keratins appear to be enriched

in those high-sulphur proteins which are at the low-sulphur end of the spectrum of components. There is evidence from other studies (Gillespie and Inglis 1965; Marshall and Gillespie 1977) that superimposed upon these differences in individual component proportions is a change in the overall ratio of amounts of high-sulphur to low-sulphur protein groups in the keratins from one animal. It is probable that these conclusions apply to sets of keratins in general, and such sets of keratins would provide useful material for studying the relation between protein composition and function.

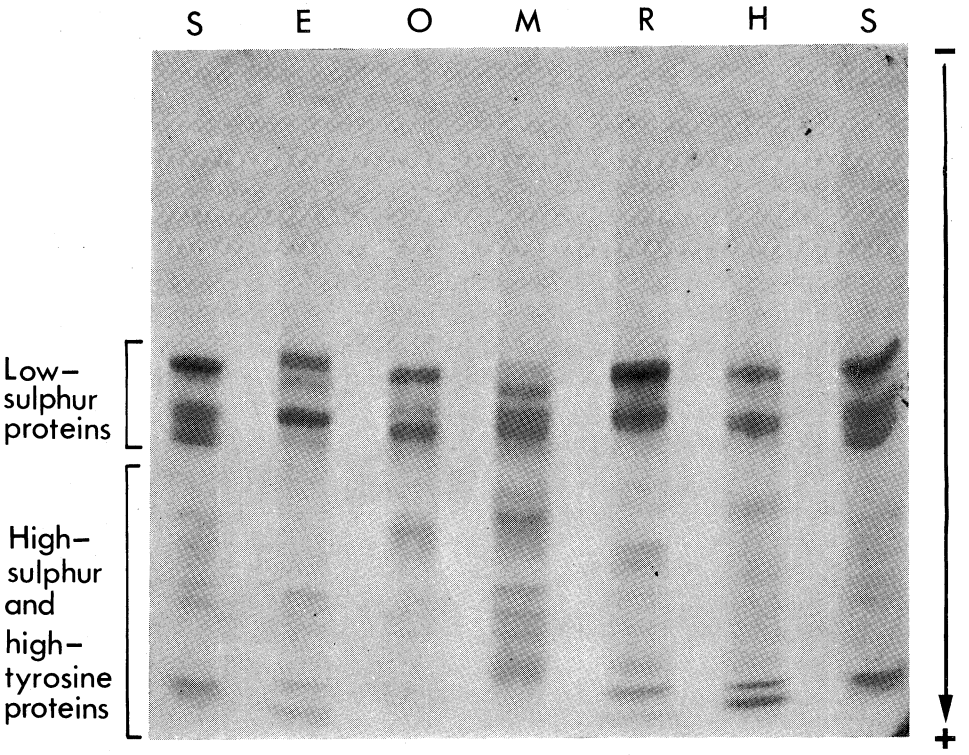

Fig. 4. Polyacrylamide gel electrophoretic patterns in the presence of SDS (4–27% continuous gradient gel, 0·1% SDS, 0·05 M phosphate, pH 7) of whole protein extracts from the hairs of sheep (S), echidna (E), ox (O), man (M), rabbit (R), and hedgehog (H). About 10 μg protein loaded.

In the extraction procedure used the keratins were solubilized to variable extents, usually about 70%, and the question may be asked whether the differences in the observed proportion of low-sulphur protein components are due to preferential extraction. This is considered unlikely for no such variations have been observed in the component proportions when the low-sulphur protein fraction was prepared at different levels of extraction (Gillespie 1964; Gillespie *et al.* 1964). Furthermore it seems unlikely that a microfibril could be preferentially solubilized without complete disruption taking place.

The low-sulphur proteins from ox hair and horn, or echidna hair and quill, are more similar to each other than they are to hoof or claw respectively. This is different from the situation for the sheep keratins (Marshall and Gillespie 1977) where the low-sulphur proteins from the horny keratins are alike and are different from those of

wool. High-sulphur proteins from sheep or echidna horny keratins are more similar to each other than they are to those from the respective hair; this is in contrast to the situation for the ox proteins where the proportion of components in horn is more similar to that in hair than it is to that in hoof.

Microfibrils, the filamentous structural entity of mammalian keratins, consist largely of low-sulphur proteins (Fraser *et al.* 1972; Jones 1975). Our work suggests that the microfibrils, although being of similar dimensions in different keratins (Fraser *et al.* 1972), are by no means constant in the number and molecular sizes of the low-sulphur protein components they contain. We have already suggested (Marshall and Gillespie 1977) that in sheep keratin microfibils, components 5 and 7 are interchangeable. The results presented here further support the idea that for a set of keratins from a species there can be a substitution amongst components within the low-sulphur protein complex which does not affect the integrity of the microfibril.

L. G. Sparrow (unpublished data), by isolation and amino acid analysis, has shown that corresponding components from different animal species may differ in molecular sizes although they are of similar composition. This variability in molecular size is also evident in our studies. Since the low-sulphur proteins contain helical and non-helical sections, the corresponding component proteins from different species may contain homologous 'structure-forming' sections which would be expected to be helical. The dissimilarity in size would be correlated with changes in the lengths of the non-helical units.

It is of interest that Steinert and coworkers (Steinert 1975; Steinert *et al.* 1976) found that tonofilaments of bovine epidermal α-keratin could be reconstituted *in vitro* from different selections of subunit proteins. Reconstitution experiments similar to those of Steinert need to be conducted on purified low-sulphur protein components isolated from wool and other keratins. Since rabbit and hedgehog low-sulphur proteins appear to contain fewer components than wool, as judged by SDS–polyacrylamide gel electrophoresis, it would probably be easier to isolate pure components from these non-ovine keratins. Also with fewer components a study of their stoichiometry and re-assembly into microfibrils might be more readily undertaken than a similar study with wool proteins.

Without a very large amount of work it is not possible to say with absolute certainty that the electrophoretic patterns reported here for individual animals are characteristic for the particular species. However, this is likely to be true in view of the body of accumulated evidence which shows, for the high-sulphur proteins of wool, mouse hair, kangaroo hair, and fingernail and for the low-sulphur proteins of wool and fingernail, that there are characteristic banding patterns for each particular species of animal (Gillespie 1964; Gillespie *et al.* 1964; O'Donnell and Thompson 1964; Marshall *et al.* 1977; Marshall and Gillespie, unpublished data; I. J. O'Donnell personal communication).

Acknowledgments

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