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The Composition and Reactivity of Medullated Keratins

By S. BLACKBURN, *Wool Industries Research Association, Torridon, Leeds*

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One of the features which distinguishes the coarser wools from the finer merino types is the presence of a medulla. Whereas the finer wools consist only of a cortex and outer scaly structure, the coarser wools contain also a medulla. The chemistry of wool and other keratins has been fairly intensively studied, but, apart from the considerable evidence which suggests a low sulphur content, relatively little is known about the composition of the medulla. Barritt & King (1931), from a comparison of the sulphur contents of medullated and non-medullated wools, calculated that the medulla substance contained little, if any, sulphur. Jordan Lloyd & Marriott (1933) found that the medullary cells of goat hair, after separation by treatment with 4*N*-sodium hydroxide at room temperature, contained only 0.23 % sulphur. Bekker & King (1931) showed that the medulla dissected from porcupine quill contained less sulphur than the cortex. The absence of cystine from the medulla was inferred from the appearance of cross-sections of the quill stained with sodium plumbite.

Bailey (1937) observed that scoured samples of kemp, which is a heavily medullated fibre, and to a much lesser extent wool, had a low cystine content. One such sample of kemp contained less than 2 % of cystine. He concluded that the cystine originally present had been destroyed, probably during the

scouring process. The cystine of kemp would therefore appear to be much more readily attacked by mild alkalis, i.e. the scouring liquor, than that of other keratins.

Stoves (1946*a, b*) has recently studied the medulla cells of a number of animal fibres, isolating them from bleached horse hair and other keratin fibres by chemical treatment. Observation of the change in appearance of the cells when treated with a 1 % tannic acid solution, 0.1 % sodium oleate or 1 % saponin suggested that the surface of the medulla cells contained a lipoprotein-cholesterol combination. Stoves further showed that when the medulla cells were refluxed with ethanolic sodium hydroxide the solution gave a positive reaction for phosphate, whilst a chloroform extract of the solution gave a positive reaction for sterols.

In the present paper the sulphur analyses of wool and kemp which had been scoured in the laboratory, and wool which had been scoured on a commercial scale, are compared with those of the same wool which had been degreased with solvents. The results of phosphorus determinations on a number of medullated and non-medullated keratins, and the examination of hydrolysates of medulla by two-dimensional partition chromatography are also given.

METHODS

Materials. All the keratins were first extracted with benzene, then with ethanol in a Soxhlet, repeatedly rinsed in water and dried in air. The medulla cells of wool fibres were isolated by the technique of Jordan Lloyd & Marriott (1933); those of feather rhachis and porcupine quill by dissection. Kemp fibres were separated from wool fibres by hand.

Analyses. Total sulphur estimations on keratins were carried out by the Benedict-Denis method (Barritt, 1934); disulphide S and thiol S estimations by Shinohara's (1935, 1936) methods; methionine by Baernstein's (1936) volatile iodide method; phosphorus estimations by Kuttner & Lichtenstein's (1930) method (see Gortner, 1945; Burmaster, 1946). Analytical results are given as a percentage of the dry weight of the keratin. Qualitative amino-acid examination of the keratins was carried out by two-dimensional partition chromatography on paper (Consden, Gordon & Martin, 1944). The keratins were hydrolyzed for 24 hr. with 5N-HCl, excess acid being finally removed by distillation *in vacuo*. The chromatograms were run in collidine for 2 days and then in phenol-ammonia for 1 day.

Scouring methods. Part of a Radnor fleece, containing a large proportion of kemp, and a sample of 64's Cape wool were scoured in the laboratory, the tips of the Cape-wool fibres being cut off as a preliminary step. The samples were treated with a solution containing 0.2% soap and 0.1% Na₂CO₃ for 3 min. at 47°. After passing through rollers, the wool was again treated with 0.1% soap solution for 3 min. at 47°, again passed through rollers and air-dried. Samples were also taken of wool at various stages during its passage through a commercial scouring set. A 70's quality Australian greasy wool was scoured in a five-bowl set. In the first bowl, the wool was given a water steep. The second bowl contained Na₂CO₃, the third soap and Na₂CO₃, the fourth soap, the final rinse being carried out in the fifth bowl. Hourly additions of soap were made to the third and fourth bowls, and the liquors were blown back at the end of a run in the usual way. The temperature of each bowl was taken during the run, and samples of the liquor removed for subsequent pH determination with the glass electrode. Samples of wool were removed before scouring, as it emerged from each bowl, and after passage through the dryer. The samples were removed shortly after the start of a run, so that the liquor in the bowls was probably in the most alkaline condition and the grease content low. Before analysis, they were extracted with light petroleum, then with ethanol, rinsed in water and dried.

RESULTS

The phosphorus content of keratins

Table 1 gives the phosphorus content of a number of medullated and non-medullated keratins. None of them contains more than a very small amount of phosphorus, which is possibly derived from small amounts of suint which have not been removed by the purification procedure employed, and shown by Freney (1934) to contain phosphates. Some support for this view is given by the fact that non-medullated wools may contain as much phosphorus as medullated wools and keratins.

Table 1. *The phosphorus content of keratins*

Keratin	Phosphorus (% of dry wt.)
Blackface wool	0.020
Welsh mountain wool	0.015
Deccani wool (Poona)	0.021
Horsehair (Argentine black mane hair)	0.039
Kemp from Radnor fleece	0.022
Cape 64's merino wool*	0.021
New Zealand 56's crossbred wool*	0.023
Blackface wool medulla	<0.02
Porcupine quill cortex	0.011
Porcupine quill medulla	0.002
Hen feather	0.018

* A non-medullated keratin.

The present results enable an upper limit to be assigned to the phosphorus of keratins present as phosphatide. The medulla constitutes only a small proportion of the total weight of wool: Barritt & King (1931) found 5.5% by microscopical observations; Jordan Lloyd & Marriott (1933) 13.5% in goat hair by isolation. If wool possesses the lipoprotein-cholesterol complex only on the surface of the medullary cells, its phosphorus content should be lower than that of the medulla. Such cells, isolated from blackface wool in amounts of 5–10 mg., after digestion with sulphuric acid, contained no phosphorus detectable by the Kuttner & Lichtenstein (1930) procedure. By this method, very small amounts (<0.02% on the weight of the medulla) should have been detected.

These results are in contrast to those of Stoves (1946 *a, b*), who obtained a 'positive tartaric acid-ammonium molybdate-benzidine reaction' for phosphate on an extract prepared by refluxing medulla cells with 10% ethanolic sodium hydroxide for 5 hr. It is difficult to explain the difference between these two results, unless the lipoprotein-cholesterol complex is only found in a very few types of medullated fibres. Similar amounts of medulla cells of the rhachis of hen feather also contained no detectable phosphorus, and the medulla of porcupine quill only a small amount. In contrast, the cortex of the quill had a definite phosphorus content. The reverse would be expected if the medulla contained appreciable amounts of lipoprotein. It is interesting that medullary cells isolated from the rhachis of hen feather, or from porcupine quill by dissection, do not show the swelling and other reactions on treatment with tannic acid and sodium oleate observed by Stoves on medulla cells from Kolinsky fibres.

The cystine content of scoured keratins

Table 2 compares the analyses of the laboratory-scoured wool and kemp with those of the solvent-degreased specimens. The similarity of the analyses indicates that the laboratory method of scouring

causes little destruction of the combined cystine of wool and kemp. A similar conclusion can be drawn from Table 3, which gives the analyses of commercially scoured wool; the cystine sulphur and total sulphur contents of the wool after treatment in the various bowls of the scouring set are similar to those of the original wool. The cystine of wool thus seems to be stable to a commercial scouring process.

Table 2. *The sulphur analyses of laboratory-scoured and solvent-degreased wool and kemp*

(Results as percentage of protein weight)

	Total S	Cystine S	Methionine S
64's Cape wool:			
(a) Scoured	3.45	3.14	0.12
(b) Solvent degreased	3.48	3.09	0.12
Radnor kemp:			
(a) Scoured	3.10	2.45	0.22
(b) Solvent degreased	3.24	2.45	0.22

Table 3. *The sulphur analysis of wool, before, during and after a commercial scour*

	Temp. of bowl (°)	pH of bowl	Total S (%)	Cystine S (%)
Original wool	—	—	3.48	2.97
Wool emerging from: 1st bowl	52	9.85	3.53	3.02
2nd bowl	48	10.35	3.52	2.88
3rd bowl	48	10.70	3.46	2.82
4th bowl	40	9.40	3.48	2.95
5th bowl	38	8.95	3.50	2.92
Wool emerging from dryer	—	—	3.48	2.91

These results contrast with those of Bailey (1937). He found that while wool and kemp, obtained from the raw fleece which had been degreased with solvents, gave analyses in which 98–99% of the total sulphur was accounted for as cystine and methionine, only 90% of the total sulphur of scoured wool could be accounted for in this way, and only 36% in the case of scoured kemp. The present scoured kemp sample in particular does not show the strikingly large percentage of sulphur which is not present as cystine or methionine shown by Bailey's sample. Unfortunately, Bailey's analyses were carried out on different specimens of wool and kemp, the scoured and solvent-degreased samples being derived from different sources and possibly being of different types. Unless this author's kemp had received exceptionally severe damage during processing, its unusual analysis can probably be attributed to some other cause than alkali damage during scouring.

Virgin wools and other keratins which have been degreased with solvents, and show no obvious signs of damage, often contain varying amounts of sulphur which is not present as cystine or methionine. Bailey similarly found that 6% of the sulphur of edestin and 7% of the sulphur of gliadin could not be accounted for in this way. This sulphur does not seem to be derived from lanthionine, which might be formed by the action of alkali on the combined cystine, since two-dimensional partition chromatographic analysis (Consden *et al.* 1944) of such wools

often shows no detectable amount of lanthionine. Schoberl & Rambacher (1940) have suggested that the extent of damage by scouring might be determined by comparing the total sulphur with the cystine content. This suggestion should be treated with caution, since their criterion cannot be upheld in the present instance.

The amino-acid composition of medulla from keratins

In view of the evidence that the medulla of wool and porcupine quill has a sulphur content different from that of the cortex, two-dimensional chromatograms were run on acid hydrolysates of medulla to obtain some idea of their amino-acid composition. The chromatogram derived from the medulla of blackface wool differed from that of normal wool. For the medulla, the spots corresponding to glutamic and aspartic acids were strong, as were those corresponding to the basic amino-acids. The leucine spot

was very strong, but those of the remaining amino-acids were weak. Similar chromatographic analysis was made on the medulla of porcupine quill. The spots corresponding to glutamic acid, leucine, valine, alanine and glycine were very strong, those of the remaining amino-acids being very weak, many not being present in sufficient amount to give a readily observed spot. The chromatogram given by the cortex of the same quill (the quill tip) differed greatly from that of the medulla and closely resembled that given by normal wool. Analysis showed the medulla of a porcupine quill to contain 0.66% of disulphide + thiol S, as compared with a value of 1.47% for the cortex, which had a total sulphur content of 1.97%. (Bekker & King (1931) found that the medulla of porcupine quills had a total sulphur content of 1.24% as compared with a value of 1.50% for the cortex.)

DISCUSSION

Previous work had indicated that the medulla of keratins has a lower sulphur content than the cortex. The present results support this conclusion and provide evidence that the medulla differs in composition from the cortex with respect to other amino-acids. The cystine of a heavily medullated kemp fibre, however, considered as a whole, does not

seem to differ from that of a normal keratin in its stability to the alkalis used in scouring. On the other hand, the treatment with sodium hydroxide used in isolating the medulla from wool probably involves some chemical degradation (Jordan Lloyd & Marriott, 1933; Stoves, 1946*a*). The very low sulphur content of the medulla of goat hair, found by Jordan Lloyd & Marriott (1933), may in part be due to the destruction by alkali of any cystine present. The medulla of porcupine quill can be isolated without such treatment.

The X-ray diagram of medullary cells differs from that given by the cortex (Rudall, cited by Stoves, 1946*a*). Keratins in general give two types of X-ray diagram, wool and hair an α -keratin diagram, corresponding to folded polypeptide chains, while feather keratin gives a β -keratin diagram, corresponding to almost fully extended polypeptide chains (Astbury & Marwick, 1932). The X-ray diffraction pattern of medulla cells from porcupine quill shows the presence of β -keratin and some unidentified material; the cortex gives a well-defined α -keratin diagram (MacArthur, 1943).

The main polypeptide chains of the medulla of porcupine quill are thus in the extended condition, in contrast to the folded chains of the cortex, and it is noticeable that the amino-acids which are predominant in the former are those with non-polar, often short, side chains, i.e. glycine, alanine, valine and leucine. This might suggest that one factor favouring the α - or β -keratin structure in nature may be the proportion of amino-acids with relatively short side chains present. Astbury has pointed out that the structure of feather keratin may be thought of as intermediate between the structure of hair keratin (folded polypeptide chains) and that of silk fibroin (fully extended polypeptide chains). The latter protein contains a large proportion of short (glycine and alanine) side chains, and hence the polypeptide chains can pack closely together and assume an extended form. In α -keratin, which has a large number of polar side chains, the polypeptide chain assumes a folded configuration. As a generalization, it is possible that β -keratins contain a higher proportion of amino-acids with short non-polar side chains than α -keratins, and that the larger pro-

portion of longer side chains favours the α -configuration. The chromatographic analysis of porcupine quill tip (α -keratin) and tortoise shell and snake skin, both of which give a β -keratin X-ray diagram, lent some support to this idea. Tortoise shell seems to be richer in glycine, alanine, valine, leucine, serine and tyrosine than porcupine quill tip, whereas snake skin was richer in glycine, alanine, serine and threonine.

The difference between the reaction of the medulla and cortex of wool fibres towards 4*N*-sodium hydroxide is probably largely dependent on structural differences. The cortex of porcupine quill is similarly attacked more rapidly than the medulla. When pieces of quill are immersed in 4*N*-sodium hydroxide at room temperature, a considerable part of the cortex has dissolved after 24 hr., leaving the medulla largely intact. In 3 days, however, a considerable part of the medulla has also dissolved. Similar differences were observed between other keratins. Thus a 56's New Zealand wool was almost completely dissolved in a few hours, while duck feathers were largely undissolved. In general, the keratin structure with the α -configuration is attacked most rapidly. It may be that the fully extended polypeptide chains of the medulla can pack closer together than those of the folded chains of the cortex, and are less accessible and less affected by reagents such as sodium hydroxide.

SUMMARY

1. The phosphorus content of a number of medullated and non-medullated keratins, and of the isolated medulla of keratins, has been measured. None contains more than traces of phosphorus.

2. The combined cystine of a medullated keratin (kemp) or a normal wool is stable to the alkali employed in scouring. Two-dimensional partition chromatography indicates that the medulla of wool and porcupine quill differs greatly from the cortex in amino-acid composition.

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