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The Natural Fluorescence of Wool

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ABSTRACT

Wool has been examined by microspectrofluorimetry. Single merino fibres show a decrease in fluorescence intensity along the length of the fibre from tip to root, with similar fluorescing species present throughout the fibre. With fabric, oxidative bleaching caused an increase in fluorescence intensity, whereas laser-blue-light irradiation caused a decrease. A decrease was also found when fabric embedded in immersion oil or glycerol was irradiated with UV radiation. When bleached and unbleached wools were separated into their morphological components (cuticle, cortex, and cell-membrane complex), similar fluorescing species were found. From preliminary measurements, the fluorescent species appears to be most heavily concentrated in the cortex.

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1 INTRODUCTION

The exposure of wool to sunlight results in a number of chemical and physical changes.[1] The most sensitive change is a discoloration resulting from the formation of yellow photodegradation products. Other changes that become evident after prolonged exposure to sunlight include loss of abrasion-resistance and tensile strength as well as altered dyeing properties.[1]

The weathering of wool is most noticeable in tips of dense fleeces, where the weathered tips are yellow–brown and much weaker than the unweathered root sections.[2] This can result in 'tippy' dyeing, a phenomenon in which weathered fibres often accept more dye than unweathered fibres (although, depending on the dye used, the reverse can be true).[3–5]

Wool has an intrinsic fluorescence,[6–14] which is not fully understood.[10] Weathering of wool, both natural and artificial, leads to photoyellowing associated with the destruction of tryptophan and an increase in visible fluorescence.[9–11] Although a number of compounds have been proposed as causing this fluorescence, e.g. dityrosine,[15] *N*-formylkynurenine,[7] and carbolines,[12] the nature of the main chromophore responsible is still the subject of conjecture.[9]

Recently, a number of studies have sought to characterise more fully the natural visible fluorescence and quantify the effect of various treatments.[7–14] This paper examines the fluorescence of single wool fibres by observing the effect of various treatments on wool fabric and by characterising the fluorescence of the different morphological components of wool.

2 EXPERIMENTAL

2.1 Materials

Merino fleece of average diameter 21·5 μm was used for fibre studies after cleaning. This involved twice shaking with tetrachloroethene for five minutes at room temperature, followed by damping with paper towels before leaving the wool staples to dry at room temperature in the dark.

A woven 2/2-botany-twill serge (200 g/m^2) was used as supplied for the fabric studies.

Merino-wool tops of average diameter 21·0 μm were used after cleaning by rinsing in water before soxhlet extraction with dichloromethane for four hours, followed by rinsing and drying at ambient temperature.

2.2 Bleaching treatments

A number of chemical-bleaching treatments were employed:

In bleaching treatment 1, wool fabric was oxidatively bleached by using tetrasodium pyrophosphate decahydrate 2 g litre^{-1}, hydrogen peroxide (100 vol, 30%) 30 ml litre^{-1}, and Lissapol N (ICI) 1 g litre^{-1}. A liquor ratio of 25:1 was used and the pH adjusted to 8·5 with the addition of ammonium hydroxide. The temperature was raised to 40°C and held for four hours, this being followed by rinsing in cold water and drying at room temperature in the dark.

In bleaching treatment 2, wool tops were oxidatively bleached by using tetrasodium pyrophosphate decahydrate 1·5 g litre^{-1}, hydrogen peroxide (35%) 10 ml litre^{-1}, and Nekanil LN (BASF) 0·8 g litre^{-1}. Bleaching was carried out at a liquor ratio of 20:1 and pH of 9·1. The temperature was raised to 50°C in 15 minutes and held for five hours, this being followed by rinsing twice in warm water and several times in cold water before drying at room temperature.

In bleaching treatment 3, wool tops were reductively bleached by using Blankit IN (sodium dithionite, BASF) 2·5 g litre^{-1} and Nekanil LN 1 g litre^{-1}. Bleaching was carried out at a liquor ratio of 20:1. The temperature was raised to 60°C in 15 minutes and held for one hour. The pH was adjusted to 3·7 with the addition of formic acid and held at 60°C for 20 minutes. The wool was rinsed once in warm water and several times in cold water (to the last rinse, 1·2 ml litre^{-1} hydrogen peroxide (35%) was added to remove sulphurous odours) before drying at 20–50°C.

In bleaching treatment 4, wool tops were fully bleached. Tops that had been oxidatively bleached (bleaching treatment 2) but not dried were reductively bleached by using Blankit IN 3 g litre^{-1} and Uniperol (BASF) 0·5 g litre^{-1}. Bleaching was carried out at a liquor ratio of 20:1. The temperature was raised to 60°C in 15 minutes and held for one hour. After 45 minutes at 60°C, the pH was adjusted to 3·5 by the addition of formic acid. The wool was rinsed once in warm water and several times in cold water (to the last rinse, 0·5 ml litre^{-1} hydrogen peroxide (35%) was added) before drying at room temperature.

2.3 Separation of morphological components

Wool tops were separated into their morphological components: cuticle cells, cortex, and cell-membrane complex (cmc). Tops that had been chemically bleached (oxidatively, reductively, and fully by using bleaching treatments 2, 3, and 4, respectively) were also separated.

Cuticle cells were liberated by mechanical treatment of fibres in a

sodium dodecyl sulphate solution after the method of Ley.[16,17] Cortex was prepared by shaking with carborundum (silicon carbide) in aqueous propanol (n- or *iso*-) after the method of Hüsken.[18] Cell-membrane complex was obtained by enzymatic degradation of wool with papain and dithioerythritol according to the method of Schwan.[19]

2.4 Irradiation methods

A number of irradiation methods were employed and are described below.

In irradiation method 1, fabric was irradiated with a helium-cadmium laser, model 4240 NB (Liconix, Sunnyvale, USA), wavelength 442 nm, output power 50 mW, for 390 minutes. A lens was placed between the laser and the fabric such that the diameter of the beam impinging on the fabric was 5 mm.

In irradiation method 2, fabric, embedded in immersion oil (Olympus, Japan), was irradiated for five minutes *in situ* on a Carl Zeiss LAB16 microscope equipped with a 5/0·15 objective by using a mercury lamp (HBO 50W) with a UV filter set for excitation (see Section 2.6.1).

In irradiation method 3, pieces of fabric (6 cm × 7·5 cm, held onto glass plates of similar dimensions by aluminium foil) were irradiated for three days by using an array of Blacklight lamps (Philips, 3 × 18W, 600 mm, emitting UV) as previously described.[11] During irradiation, the fabrics were covered by a solvent, either water or immersion oil. Before irradiation, the water sample was dipped in aqueous Lissapol N and rinsed, whereas the immersion-oil sample was squeezed in immersion oil. After irradiation, the water sample was rinsed in water, and the immersion oil sample was soxhlet-extracted by using toluene (AnalaR, BDH), this being followed by rinsing in pentane (hplc grade, Rathburn) and thorough rinsing in water. Both samples were air-dried in the dark. The irradiation was carried out in duplicate. In the second case, ordinary glass (5 mm) was used as a filter,[11] but this was placed so that it did not prevent air from moving between the top of the solvent container and the bottom of the filter.

2.5 Fluorescence spectrophotometer

Fluorescence measurements of fabrics that had been subject to irradiation method 3 were obtained by using a Perkin–Elmer Fluorescence Spectrophotometer (MPF-4) and are uncorrected. The attachment for solids was designed and made at City University and is similar to that described by McKellar and Allen.[20] The samples were placed at 50° to the incident beam to avoid scattered light. The samples were excited at

360 nm, and the fluorescence emission was measured between 370 and 610 nm. For each sample, six portions were measured.

2.6 Fluorescence microscopy

The samples were embedded on a glass slide before measurements were made. Embedding involved placing the sample on the glass slide, adding one or two drops of embedding medium (as described), and then pressing a cover slip (0·17 mm thick) on top. Two Carl Zeiss (Oberkochen, Germany) fluorescence microscopes were used: the LAB16 and the Scanning Photometer Microscope. These are described below.

2.6.1 LAB16

The LAB16 was equipped with a 40/0·75 Neofluar objective unless stated otherwise. A high-pressure mercury lamp (HBO 50W) was used for 365 nm UV excitation, together with an excitation-band pass filter G365, a selective mirror FT395, and an emission long-pass filter LP420. For visible (laser) light excitation (no excitation filter), a selective mirror FT510 and an emission long-pass filter LP520 were used. Both continuous-wave lasers were gated by an acoustooptical shutter device, rise time 50 ns, (A.A., France), controlled by a microcomputer C64 (Commodore, Braunschweig, Germany). The diameter of the laser beam impinging on the sample was 4 μm. The fluorescence-emission spectra were recorded with a monochromator, grating 1200 grooves/mm (Bausch & Lomb, USA). The fluorescence intensity was determined with a photomultiplier tube R446 (Hamamatsu, Japan), connected to a Tektronix digitiser TD20T (Beaverton, USA) and/or via an 8-bit A/D interface to the microcomputer. The microscope and associated equipment are illustrated in Fig. 1. Details of the measurements are given below.

2.6.1.1 Variation in fluorescence intensity along a fibre. A single wool fibre (approximately 6 cm long) was embedded on a glass slide by using immersion oil. (The coverslip was not large enough to cover the whole length of the fibre.) An argon-ion laser (model LH 1232 KS (Toshiba, Japan), wavelength 488 nm, output power 10 mW) was used for excitation and the fluorescence emission monitored at 550 nm. Measurements were made at approximately 0·2 mm intervals except for the first 1·4 mm (tip end), where they were made at approximately 0·1 mm intervals.

2.6.1.2 Fluorescence spectra of tips and roots of fibres. Portions of tips and roots were embedded in a glycerol/carbonate buffer solution. Fluorescence emission spectra were obtained by using UV excitation.

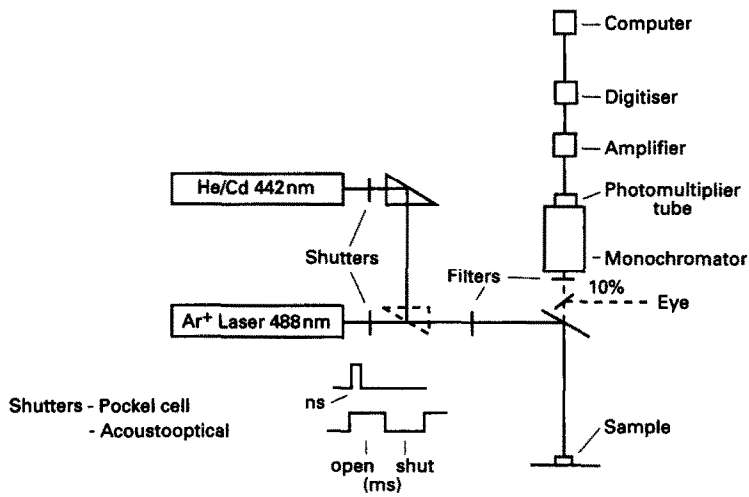

Fig. 1. Schematic diagram of LAB16 microscope and associated equipment.

2.6.1.3 Fading of the fluorescence exhibited by wool tips/roots. Portions of tips and roots were embedded in glycerol for studying the 'fading' and recovery of the fluorescence intensity. The 'fading' of the tips and roots is defined as the decrease in the fluorescence intensity during laser irradiation, and the 'recovery' is the increase in fluorescence intensity after switching off the continuous irradiation, measured by repetitive laser pulses (pulse length 1·1 μs, pulse-dark interval 2·3 ms). A helium–cadmium laser (wavelength 442 nm) was used, with the fluorescence being monitored at 550 nm.

2.6.1.4. Fluorescence spectra of fabric subjected to various treatments. Untreated fabric, fabric that had been oxidatively bleached (bleaching treatment 1), fabric that had been irradiated with blue laser light (irradiation method 1), and fabric that had been irradiated with UV radiation *in situ* (irradiation method 2) were used. Small pieces of the fabrics were embedded on a glass slide by using immersion oil. The fabrics were examined by using a 5/0·15 objective. UV excitation was used and the fluorescence intensity at various wavelengths recorded. The results are the average of three readings, except for the *in situ* irradiation with UV excitation, which was carried out only once, the fluorescence intensity being measured straight after the irradiation. The results are corrected for the decrease in the fluorescence intensity that occurred during the period of measurement and are expressed as the mean ± standard error of the mean (standard deviation/$\sqrt{n}$).

2.6.1.5. Fluorescence intensities of the morphological components. Portions of the morphological components were embedded in a glycerol/water (3 : 1) solution. An argon-ion laser (wavelength 488 nm) was used for excitation, the fluorescence being monitored at 550 nm. The results are the average of 15 measurements.

2.6.2 Scanning photometer microscope

The Scanning Photometer Microscope 03 with MPC 64 control unit was equipped with an Ultrafluar 10/0·20 objective. A high-pressure mercury lamp (HBO 100 W) was used for 365 nm UV excitation, together with an excitation band-pass filter G365, selective mirror FT395, and emission long-pass filter LP420.

Fluorescence spectra of the morphological components, embedded in immersion oil, were obtained by using UV excitation.

2.7 Other measurements

Fourier-transform infra-red (FTIR) spectra were recorded by using a Bio-Rad FTS 60 equipped with a diffuse-reflectance attachment. Amino-acid analyses were carried out by Dr J. Föhles, of the Deutsches Wollforschungsinstitut, Aachen, Germany.

Yellowness Index (YI) values were calculated by using the formula given in eqn (1).[11]

$$\mathrm{YI} = \frac{100\,(1{\cdot}316X - 1{\cdot}164Z)}{Y} \tag{1}$$

where X, Y, and Z are the CIE tristimulus values (D65/10° illuminant/observer) obtained from an ICS–Texicon Macbeth Micro-match Reflectance MM9000 system equipped with a Macbeth 2020 Plus measuring head. The lower the YI value, the whiter is the wool.

3 RESULTS AND DISCUSSION

3.1 Single fibres

In a previous paper,[9] we showed qualitatively that the natural visible fluorescence of merino fibres ranges from highly fluorescent tips to barely fluorescent roots. To quantify the variation in the fluorescence from tip to root more accurately, the fluorescence intensity along single wool fibres was measured. The result from one fibre is shown in Fig. 2. The

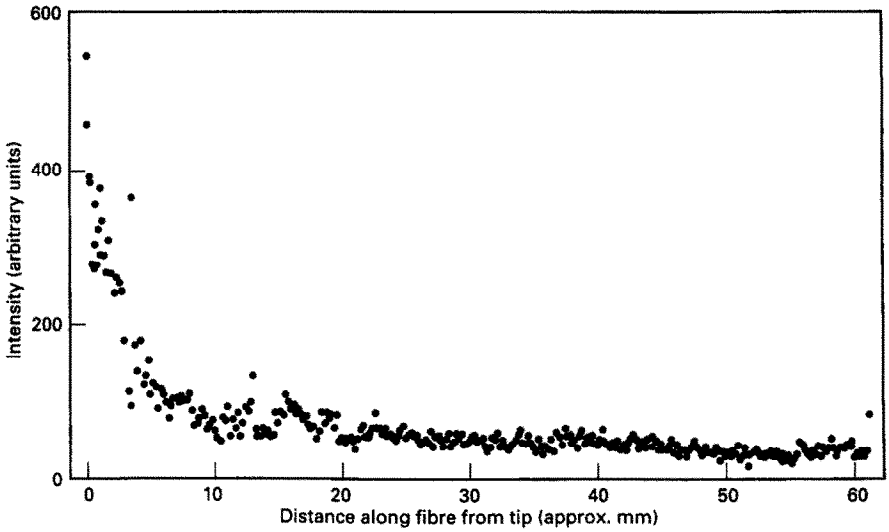

Fig. 2. Variation in fluorescence intensity along the length of a single merino wool fibre (488-nm excitation, fluorescence monitored at 550 nm).

decrease in fluorescence on going along the fibre from tip towards the middle region is very rapid, with the first 5 mm the most intense. There is then a relatively small decrease on going along the fibre from the middle region to the root. Compared with the root, the tip is about ten times as intense. (Other fibres inspected showed different ratios.) Although the degree of scatter is larger at the tip, there is still a very large difference between the two regions. Interestingly, the very end of the root region (approximately 0·2 mm) shows an increase in fluorescence intensity. Whereas this may, at least partly, be due to natural scatter, it could also be due to the photo-oxidation of the exposed fibre end that had occurred since shearing.

Schäfer[10] has recently reported on the fluorescence intensities of different parts of the fibre of a Texel sheep. Exciting at 365 nm and monitoring at 440 nm, she observed an increase in intensity of approximately fourfold upon going from root to tip. The extent of weathering of wool along a fibre depends on the density of the fleece.[21,22] Hence, in a densely packed fleece, such as the merino one used for Fig. 2 (and previously[9]), weathering may be observed only in the extreme tips, whereas in an open fleece, the effect of weathering is more widespread. Examination of the coarser open fleeces from Scottish blackface sheep has shown no differences in visible fluorescence from tip to root, the whole fibre exhibiting significant intense fluorescence.[9]

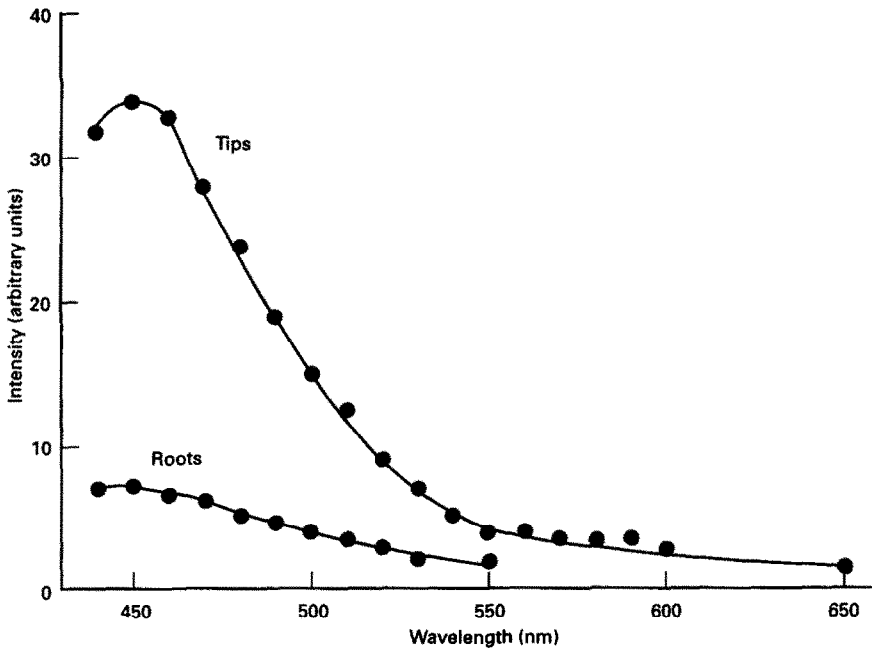

Fig. 3. Fluorescence spectra of tips and roots of wool fibres (UV excitation).

The increase in fluorescence from root to tips could be due to an increase in the (same) emitting species and/or the production of a new type of fluorescent species. Figure 3 shows the fluorescent spectra of roots and tips of wool fibres. The spectra are similar, each having a maximum at approximately 450 nm (UV excitation) and demonstrate that the nature of the fluorescing materials in the tips and roots is similar. This was confirmed by looking at the 'fading and recovery' of fluorescence at the tips and roots of the wool fibre.

Irradiation of wool (tips) led to 'fading' (a decrease in fluorescence intensity), i.e. the fluorescent chromophores are being destroyed by irradiation (Fig. 4), the high rate of degradation being due to the high intensity of the light beam. If the fibre is irradiated for a short period of time, e.g. 1 ms, a reduction in fluorescence intensity occurs. If this is due to chemical reduction of the chromophore, then, when the light is turned off, it is possible for the reduced chromophore to be oxidised by air and consequently, on reillumination, the fluorescence is of a greater intensity than when the irradiation was originally terminated. The rapidity of the recovery experienced would indicate that this is indeed the process occurring in the fibres; such behaviour is compatible with the presence of quinone chromophores.

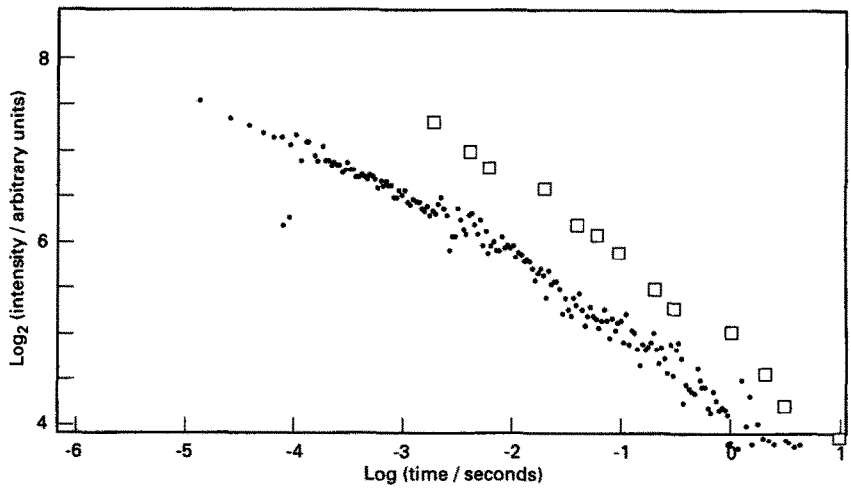

Fig. 4. Fading of the fluorescence exhibited by wool tips (442 nm excitation, fluorescence monitored at 550 nm), showing fading (●) and recovery (□) of the signal.

The rate of destruction of the natural fluorescence of the tips (Fig. 4) and the roots (Fig. 5) is very similar. Moreover in both cases, the fluorescence recovers during a dark period to similar extents. These facts suggest that the natural fluorescing species in the tips and roots are similar if not identical.

As mentioned above, Schäfer[10] has recently reported on the fluorescence intensities of different parts of a fibre of a Texel sheep. Exciting with UV excitation, she observed that the fluorescent-emission spectra were similar at different positions along the fibre, including tip and root. Consequently, the increase in fluorescence from root to tip is due to an increase in the (same) emitting species, rather than the production of a new type of fluorescent species.

3.2 Wool Fabric

A comparison of the fluorescence spectra and intensities of wool fabric subjected to various treatments was undertaken. The fabrics used were untreated fabric, oxidatively bleached fabric, laser-blue-light-irradiated fabric (50 mW, 442 nm helium–cadmium laser, 390 min), and UV-radiation irradiated fabric (irradiated *in situ* on the microscope) (Fig. 6).

The spectra of all the treatments are very similar, which agrees with the report[23] that the fluorescence spectra of wool are almost independent of the treatment they receive. The fluorescence intensity of the wool after

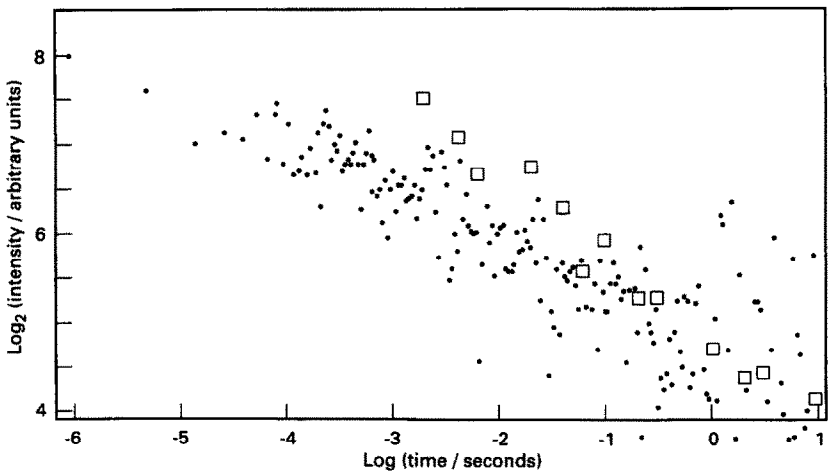

Fig. 5. Fading of the fluorescence exhibited by wool roots (442 nm excitation, fluorescence monitored at 550 nm), showing fading (●) and recovery (□) of the signal.

the various treatments is a different matter. Oxidative bleaching caused an increase in intensity, which agrees with our earlier finding,[9] and this increase can be attributed to conversion of quenching cystine to non-quenching cysteic acid.[9] Laser-blue-light irradiation caused a decrease in fluorescence intensity. This was accompanied by the wools becoming whiter (to the eye). It was thought that the blue light would cause the oxidation of cystyl residues via the intermediacy of singlet-oxygen or peroxy radicals and consequently lead to an increase in the fluorescence. Clearly, this is not the case. This has been further investigated[11] and the function of the blue light identified as destroying some of the unidentified coloured species present. Irradiation of fabric (embedded in immersion oil (a hydrocarbon oil[24])) on the microscope stage with a UV excitation source led to a decrease in fluorescence intensity. This was surprising, since previous results[9,10] had shown an increase in fluorescence intensity upon UV irradiation. Consequently, a study of the role of the embedding medium was undertaken.

Wool fabric immersed in immersion oil or water was irradiated (*non in situ*) by using Blacklight lamps producing UV light (Table 1). Compared with both the water sample and a blank, the immersion oil caused a significant difference in fluorescence (t-test, $P = 0{\cdot}05$ and $P = 0{\cdot}01$). The irradiation in water caused the Yellowness Index (YI) of the fabric to increase from 20·20 to 30·85 and the fluorescence-emission maximum to shift to 460 nm. However, the YI of the fabric irradiated in immersion oil increased by

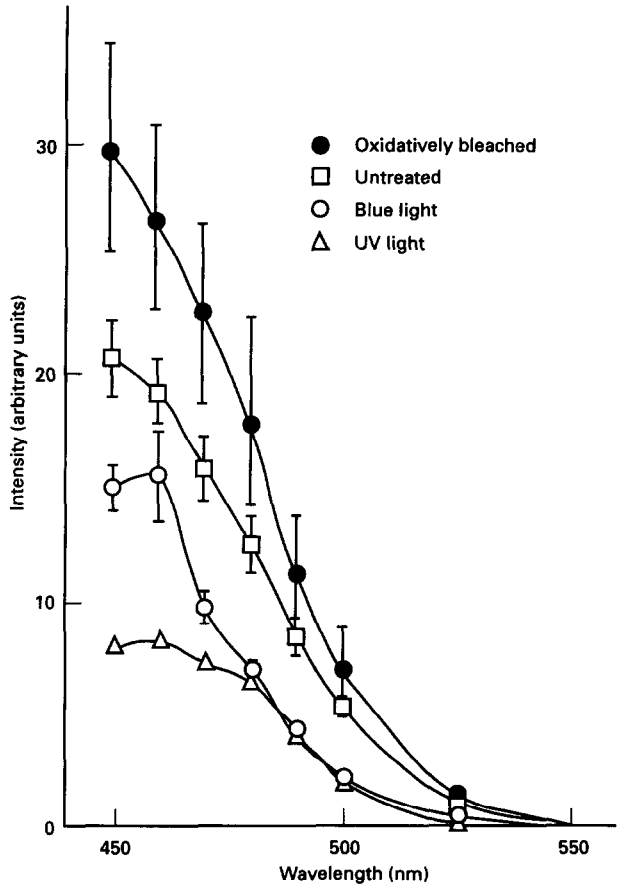

Fig. 6. Fluorescence spectra of wool fabric that had been subjected to various treatments (UV excitation, mean ± standard error of the mean).

only a small amount to 22·50 and the emission maximum shifted only to 445 nm. Similar fluorescence results were obtained when glycerol was used instead of immersion oil (not shown). The fabric irradiated in glycerol then increased in YI from 18·07 to 19·39 and gave a sulphurous odour, while the YI of the water-irradiated fabric increased to 34·07. This lack of build-up of long-wavelength-absorbing and fluorescing species may be due to the glycerol and immersion oil reducing these degradation products. Such an effect is seen when wool is irradiated in the presence of reductants such as zinc formaldehyde sulphoxylate.[25]

Bailey and Launer,[26] using a Westinghouse Blacklight fluorescent lamp, found that, if the strongly yellowing spectral impurity at 313 nm was filtered out, the main radiation near 365 nm caused photobleaching. Since 365 nm light is generally regarded as causing yellowing[11,27,28] and

TABLE 1
Yellowness Index Values, Fluorescence Intensities, and Peak Emissions of Wool Fabrics Irradiated with UV Radiation in Different Embedding Media with or without a Glass Filter

Irradiation condition	*Yellowness Index*	*Approximate peak emission*[a] *(nm)*	*Fluorescence Intensity*	
			(Mean[b]*)*	*(s.d.*[c]*)*
Blank	20·20	440	100·0	13·6
Immersion oil	22·50	445	67·3	14·6
Immersion oil +filter	22·54	445	75·7	12·9
Water	30·85	460	115·4	12·9
Water + filter	35·44	460	113·1	12·8

[a] Excitation 360 nm.
[b] Arbitrary units.
[c] Standard deviation.

was used as such here, this was a cause for concern. At the same time as the immersion oil was investigated *non in situ*, the effect of the 313- and 365 nm wavelength radiations was investigated. The experiment was done in duplicate—once with glass to filter out the 313 nm radiation, and once without any filter. Table 1 shows the results obtained.

With a filter used, the irradiation in water caused the YI of the fabric to increase from 20·20 to 35·44 and the fluorescence-emission maximum to shift to 460 nm, whereas the YI of the fabric irradiated in immersion oil increased by only a small amount to 22·54 and the emission maximum shifted only to 445 nm, i.e. the same results as those obtained without using a filter. For the filtered fabrics, the immersion oil caused a significant difference in fluorescence (t-test, $P = 0{\cdot}05$ and $P = 0{\cdot}01$) when compared with both the water sample and the blank, again the same as for the unfiltered fabrics. Additionally, there was found to be no significant difference (t-test, $P = 0{\cdot}05$) between the two immersion-oil samples or between the two water samples. Hence 365 nm radiation, and consequently Blacklight/UV lamps as well as microscope mercury lamps, do cause photoyellowing of wool as expected, and the claims of Bailey and Launer are disproved.

It is appropriate here to comment on the measurement techniques used. During the comparison measurements on the microscope (Fig. 6), it was found that fabrics are not good materials for microspectrofluorimetry. Since fabrics are usually made of yarns, a relatively high depth of field was required to view them, which means a low-power objective (5×) had to be used. Hence the advantages of microscope analysis are largely lost,

and thus the use of a fluorescence spectrophotometer is more suitable. Consequently, (whole-) fabric-fluorescence analysis (of the *non-in-situ* irradiated fabrics) was restricted to the fluorescence spectrophotometer. This reiterates the need for care to be taken in sample preparation for microspectrofluorimetry.

3.3 Morphological components

It has been reported[9] that cross-sections of wool fibres reveal the presence of areas of high fluorescence in the form of regular dots or granules. These areas of high fluorescence could be randomly distributed or related to some aspect of the fibre structure, e.g. a particular morphological component.

Wool was separated into its morphological components, cuticle, cortex, and cell-membrane complex (cmc). This was carried out by using untreated wool tops as well as tops that had been bleached (oxidatively, reductively, and fully). Amino-acid analyses of the components were obtained, and a summary of these, indicating the cysteic acid and cystine content, is shown in Table 2. This indicates that, compared with the untreated wool tops' components, the reductively bleached tops' components generally have a variable-sized increase in cysteic acid content, whereas the oxidatively bleached tops' components show a large increase in cysteic acid content (and the fully bleached tops' components show an even higher cysteic acid content). Since cysteic acid is non-quenching,[9] then it is to be expected that the amount of cysteic acid will correlate with the fluorescence intensity.

On comparing the different components, the cortex and cmc have about the same amount of cysteic acid, whereas the cuticle has a larger amount. This suggests that the cuticle should be the most fluorescent component. However, it is important to consider the quenching effect of cystine. Whereas the amount of cystine generally varies only slightly between bleaching treatments, it varies greatly between the different components. Hence the cuticle samples, which have the largest amount of non-quenching cysteic acid, also have the largest amount of quenching cystine, so the two effects may 'cancel' each other out.

Infra-red spectra (not shown) of the morphological components were obtained by using FTIR with a diffuse-reflectance attachment. Cysteic acid is known[29,30] to have a characteristic absorption at 1040–1045 cm^{-1}. Surprisingly, this technique was not very sensitive and so proved of little use. For example, the cysteic acid absorption peak was clearly visible in the cortex of oxidatively bleached wool but absent from the cortex of untreated wool.

TABLE 2
Selected Amino-Acid Compositions of Morphological Components of Variously Bleached Wool Tops

Morphological components	*Amino-acid composition (mol 100 mol^{-1})*	
	Cysteic acid	*Cystine*
Cuticle		
Untreated	1·099	9·124
Oxidatively bleached	3·401	8·662
Reductively bleached	1·511	9·031
Fully bleached	2·745	8·049
Cortex		
Untreated	0·190	6·144
Oxidatively bleached	1·376	5·395
Reductively bleached	1·199	5·487
Fully bleached	1·516	5·740
Cmc		
Untreated[a]	0·559	4·231
Oxidatively bleached	1·091	1·612
Reductively bleached	0·262	3·452
Fully bleached	1·338	1·962

[a] The protein content of this sample was quite high and makes the result suspect.

Fluorescence spectra of the different morphological components were obtained by using UV excitation. Figure 7 shows that the spectra of cortex obtained from untreated wool tops has a broad maximum at 440–460 nm. This broad peak arises from the large bandwidth used (20 nm) because of the very weak intensity of the signal. The fluorescence spectra of the cortex material obtained from fully bleached and oxidatively bleached wool tops are similar (Fig. 7). Indeed, the fluorescence spectra of all the samples, both for the different morphological components and for the different chemical treatments used, are similar (not shown). In the measurements, the signals were so weak (as well as the samples being inhomogeneous) that it was only possible to measure the spectra of samples once a brighter-than-average sample had been picked. Visual qualitative comparison suggested that the fluorescence of the bleached-wool components was more intense than the untreated wool components and that the fluorescence intensity of the cortex material was greater than that of the cuticle or cmc components.

Subsequently, an attempt was made to quantify the fluorescence

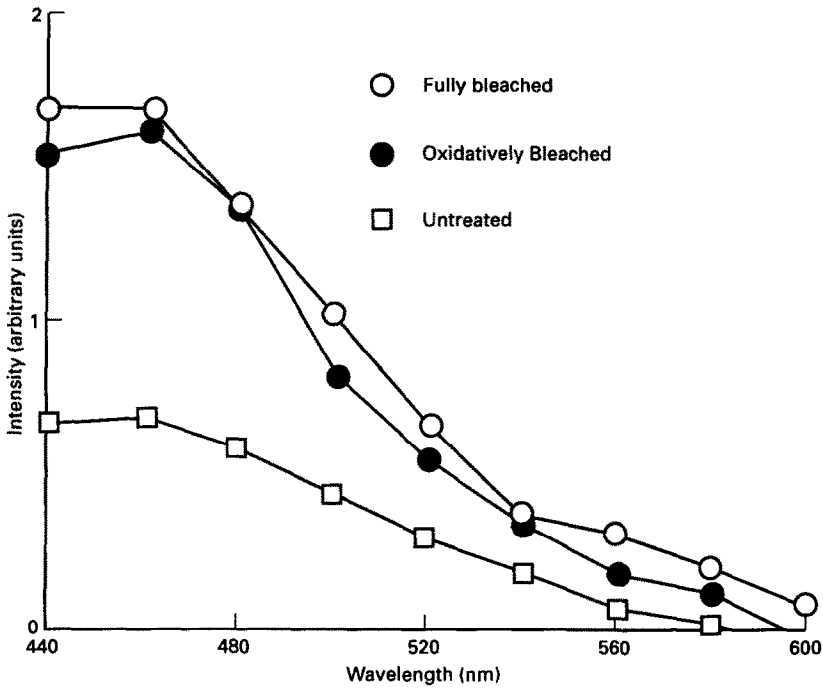

Fig. 7. Fluorescence spectra of cortex from differently bleached wool tops (UV excitation).

intensities of the different morphological components by using 488-nm laser excitation (Table 3). This was frustrated by difficulties in preparing uniform samples and uniformly filling the field of view in the microscope. Consequently, the results shown in Table 3 exhibit considerable scatter, the only meaningful deduction being that the cortex is the most fluorescent of the three morphological components. Since the cuticle surrounds the cortex in the wool fibre, it would be expected that the cuticle would suffer more weathering and so be more fluorescent. The increased cysteic acid content of the cuticle (Table 2) does indeed show that it suffers more weathering. Yet the cuticle also has a larger amount of cystine, which acts as a quencher. Evidence has thus been provided in support of the proposal earlier that the two effects might 'cancel' each other out. However, further work needs to be done, e.g. investigating the related role of damage to tryptophan (which results in the production of visible-fluorescing degradation products[9,10,12]) before the situation is fully understood.

It has not been possible to correlate the areas of high fluorescence in a wool-fibre cross-section with any of the particular morphological components investigated. Further work could usefully be directed towards investigating other morphological components, e.g. cortical cells or

TABLE 3
Fluorescence Intensities of Morphological Components of Variously Bleached Wool Tops (488 nm excitation, Fluorescence Monitored at 550 nm)

Morphological component	*Fluorescence intensity*							
	Untreated		*Oxidatively bleached*		*Reductively bleached*		*Fully bleached*	
	Mean[a]	*s.d.*[b]	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*
Cuticle	17·3	4·97	11·2	7·68	25·7	14·68	9·7	3·62
Cortex	29·3	5·59	40·9	6·95	47·5	13·66	35·0	6·40
Cmc	40·3	36·15	10·7	5·42	22·8	23·81	32·1	29·21

[a] Arbitrary units.
[b] Standard deviation.

macrofibrils. However, useful qualitative results were obtained from the components studied: cuticle, cortex, and cmc. The use of tops for obtaining the morphological components probably caused at least some of the scatter observed in the fluorescence intensities. Tops naturally contain intact fibres with a wide range of fluorescent intensities.[9] Any effect due to different bleaching treatments or different morphological components is thus superimposed upon the natural 'tip-root effect'. Being able to examine only middle portions of wool fibres, as recently used for observing the effect of UV irradiation,[11] would help overcome this problem.

4 CONCLUSION

The use of microspectrofluorimetry has shown that the natural visible fluorescence of merino fibres varies along the length of the fibre from highly fluorescent tips to barely fluorescent roots. The increase in fluorescence is due to an increase in the (same) emitting-species, rather than the production of new types of fluorescent species.

A comparison of wool fabrics subjected to various treatments revealed that all had the same fluorescence spectra, indicating that the same emitting-species is responsible. Compared with untreated fabric, oxidatively bleached fabric showed an increase in fluorescence intensity, whereas laser-blue-light-irradiated fabric and UV-radiation-irradiated fabric (irradiated *in situ* on the microscope) both showed a decrease. This last, unexpected, result was established as arising from the influence of the embedding medium used. The use of immersion oil or glycerol caused the fluorescence intensity of the wool to decrease. It was also confirmed that 365 nm radiation causes photoyellowing.

Qualitative results were obtained for the morphological components: cuticle, cortex, and cmc. Bleaching treatments were found to increase fluorescence intensity. Components from bleached and unbleached wools exhibited the same fluorescent-emission spectra, revealing the same emitting species as being responsible. The cuticle is the most-weathered component, but, owing to the large amount of cystine it contains, it is not the most fluorescent. The cortex is the most fluorescent.

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