ELSEVIER

Dyes and Pigments **25** (1994) 255–279

0143-7208/94/$7.00

Fluorescence of Wool Grease

Stephen Collins & R. Stephen Davidson*

The Chemical Laboratory, University of Kent, Canterbury, Kent CT2 7NH, UK

(Received 18 February 1994; accepted 22 March 1994)

ABSTRACT

Wool yolk is highly fluorescent. The amount of wool yolk and intensity of wool yolk fluorescence varies along a Merino wool fibre. The fluorescence intensity of the yolk increases on travelling from tip to the root.

Two components—'grease' and chlorophyll—contribute to the fluorescence of wool grease. The 'grease' fluorescence reveals the presence of a number of chromophores/components. An attempt to separate the chemical constituents and identify them was only partly successful as each of nine fractions obtained by preparatory thin-layer chromatography exhibited similar fluorescence properties. There are two types of chlorophyll present—chlorophyll a *and chlorophyll* b. *Chlorophyll could be detected in all the wool greases examined.*

Artificial UV irradiation gave rise to a number of complex photochemical reactions including the destruction of chlorophyll. After an initial decrease in fluorescence intensity (for unknown reasons), there is a change of non-fluorescing visible-absorbing species to fluorescing UV-absorbing species before a final change in the latter to the former. The relative size of the changes was different for two different wool greases examined.

In-situ studies using blue light as the irradiation source suggest that it also causes a (slower) decrease in fluorescence intensity, but proportionally faster for the 'grease' component than for the chlorophyll component.

1 INTRODUCTION

A large amount of research has been undertaken in order to identify the components of wool wax.[1–5]

* To whom correspondence should be addressed.

The nomenclature of Truter[1] is adhered to, as given below:

'*Yolk* comprises all the physiological products of the fleece except the fibre. It consists of two main fractions, the wool lipid and the suint.

Wool wax, occasionally referred to as the wool lipid, is the lipid material in the fleece that *can* be extracted with the usual fat solvents. It refers specifically to the lipid excretion of the sheep's skin. . .

Suint is the water-soluble material contained in the fleece. . .

Wool grease, after wool has been scoured, i.e. washed, the wool lipids remain suspended in the wash-waters through the agency of the detergent. This emulsion also contains suint. Attempts to recover the lipid usually give products contaminated with detergent and suint. For this reason the product is called wool grease as distinct from wool wax. . .

Lanolin is wool grease that has been subjected to a combination of refining processes designed to lighten its colour, improve its odour and to reduce the free acid content. . .'

Although it has been known for many years that wool wax fluoresces, there are very few reports of investigations of this phenomenon.[6–10] Croner[6] in 1926 appears to have been the first to describe the fluorescence of lanolin.

Using UV irradiation Croner found that lanolin emitted a weak bluish fluorescence. However, Hirst[7] in 1927, while describing the fluorescence of some wools, found that clipped Merino greasy gave a yellow colour with bluish-white tips, but that the 'extracted fats, & c.' are themselves non-fluorescent.

van Raalte[8] in 1928 investigated the luminescence of a number of oils and fats including 'sheep fat'. He observed that most, including sheep fat, did not exhibit luminescence in the crude state. Treatment with sulphuric acid or fuller's earth induced luminescence while decolorisation with norite had no effect. It was decided that a substance inhibiting luminescence was removed during these refining processes. The substance was assumed to be a sterol as it could be precipitated by digitonin. In many respects the substance gave reactions attributed to vitamins. van Raalte also left some fuller's earth refined sheep fat in a test tube in sunlight and after several weeks did not observe any luminescence. Carrière[9] in 1928 disagreed with van Raalte's statement that only refined oils are fluorescent. Carrière said that all oils and fats are fluorescent, with some oils showing a greater absorption of UV than others. He attributed van Raalte's results to using an incorrect observation technique.

It was not until 1970 that the next account of the luminescence of

'wool fat' occurred.[10] By examining sheep wool samples before and after fat removal a strong depression of the luminescence after fat removal was observed, suggesting that the fat was responsible for the luminescence. The proteins in the wool showed no luminescence (which is very strange when one considers the extensive reports of the fluorescence of wool in the literature[7,11–15]). Mezentsev[10] found that not all the incident irradiation reached the wool fibre: some was absorbed and reemitted as luminescence. Thus, the intensity and radiation spectrum reaching the wool was essentially different from the incident irradiation. A dispersion analysis showed that the luminescence depended on fat colour and not on breed of sheep.

This paper presents an examination of the fluorescence of the wool yolk along a wool fibre, together with a detailed examination of the fluorescence of wool grease. The latter involved recording fluorescence spectra, attempting to identify the components of wool grease causing the fluorescence and observing the effect of irradiation upon the fluorescence intensity.

2 EXPERIMENTAL

2.1 Materials

For fluorimetric studies, spectroscopic grade cyclohexane (BDH) and dichloromethane (Aldrich) were used. Chlorophyll (from *Anacystis nidulans* algae: free of chlorophyll *b*) was obtained from Sigma Chemical Co. A raw Merino wool (average diameter 23·7 μm) was supplied by the International Wool Secretariat, Ilkley, UK. Centrifugal wool grease Merino's quality (Merino wool grease) came from Heydemann-Shaw, and Croda wool grease came from Croda Ltd. Six New Zealand wool greases containing chlorophyll contamination (W1–W6) were supplied by Dr D. A. Rankin, WRONZ, New Zealand. On receipt, the wool greases were homogenised, by melting, stirring and leaving to cool, before any analysis took place. The wool greases are described in Table 1. W1 was obtained from scouring a 'dag wool line', i.e. a wool blend containing a large amount of wool contaminated with dags (small balls or lumps of faeces-encrusted wool shorn from the crutches of sheep). W2, W4 and W6 are from the top of their respective drums, which had undergone appreciable oxidation, resulting in them being considerably lighter in colour than their parent wool greases (W1, W3 and W5, respectively). The oxidised wool greases are considerably tackier and harder than their parent wool greases.

TABLE 1
Description of New Zealand Wool Greases

Code name	*Colour of grease*	*Dr Rankin's assessment of chlorophyll contamination*	*Sample obtained from*
W1	Dark brown	Heavy	Bulk of a small drum
W2	Greeny wax		Top of a small drum
W3	Light brown	Lesser	Bulk of a small drum
W4	Golden yellow wax		Top of a small drum
W5	Greeny wax	A typical New Zealand grease of good colour and quality	Bulk of a small drum
W6	Waxy		Top of a small drum

2.2 Variation in fluorescence intensity along fibres

Merino fibres were used without being cleaned. Snippets were obtained from the staples of tip ends, middle portions and root ends. Cross-sections were prepared using a Hardy microtome,[16] and photomicrographs were obtained using both UV and violet excitation modes of a Vickers M17 fluorescence microscope using a 25/0·5 objective.[12] Camera exposure times of 18 s were used with Kodachrome 64ASA Professional (Daylight) colour transparency film (Kodak).

2.3 Spectral characterisation

The fluorescence intensities of the wool greases W1–W6 and Merino wool grease were obtained using a Perkin-Elmer MPF-4 fluorescence spectrophotometer. The wool greases were dissolved in cyclohexane and solutions made up to give an optical density of 0·10 at 350 nm, before being flushed with argon for 10 min (Merino wool grease, 20 min). The samples were sequentially excited at 300, 350, 400, 450 and 500 nm and for each excitation wavelength the fluorescence emission spectra were recorded.

UV/visible absorption spectra of W1–W6 were obtained using a Philips PU8720 UV/vis scanning spectrophotometer. Infrared spectra of W1–W6 were obtained using a Perkin-Elmer 983G instrument after having spread the grease between a pair of sodium chloride discs.

2.4 Attempts to isolate the species responsible for fluorescence

Preliminary investigations were carried out using thin-layer chromatography (TLC) (silica sheets layer thickness 0·2 mm, DE Plastik folien Kieselgel 60

F_{254} (Merck)). Merino wool grease was dissolved in chloroform, and TLC was performed using hexane/ethyl acetate in a variety of ratios. The ratio 4:1 was found to provide a reasonable separation. Quantitative investigations were then carried out using preparatory TLC plates (20 cm × 20 cm, silica, obtained from Mr T. Gilby, City University, London, UK). Merino wool grease was dissolved in chloroform and applied to the plates prior to elution with hexane/ethyl acetate (4:1). Two plates were run: on the first 98·9 mg of wool grease were used and on the second 107·1 mg. After elution, the appropriate fractions from the two plates were combined. The fractions were located using a 254 nm TLC lamp. The fractions were not completely separated from one another so overlapping portions were discarded. The fractions were removed, and washed with dichloromethane/ethyl acetate (1:1, 3 × *c.* 15 ml) using a sintered glass frit, and the resulting solutions were evaporated under reduced pressure. This was followed by dissolving in dichloromethane, transfer to a weighed sample bottle, evaporation of the solvent using a stream of nitrogen, and the sample bottle being reweighed.

Fluorescence intensities of the fractions and Merino wool grease were measured using a MPF-4 fluorescence spectrophotometer. The samples were dissolved in dichloromethane and made up to give an optical density of 0·10 at 350 nm (but without correction for solvent absorbance). UV/visible absorption spectra of these solutions were recorded. The samples were sequentially excited at 300, 350, 400 and 450 nm and for each excitation wavelength the fluorescence emission spectra were recorded. Some samples were also excited at 500 nm.

2.5 Effect of Blacklight (UV) irradiation

W1 and Merino wool grease were each placed on a glass plate (*c.* 20 cm × 5 cm), which had been warmed, and were spread evenly over most of the plate using a spatula. The greases were then irradiated under an array of Blacklight fluorescent tubes (Philips, 3 × 18 W, 600 mm) (having a maximum emission at *c.* 365 nm)[14] and were periodically sampled. The fluorescence intensities of the samples were recorded using a MPF-4 fluorescence spectrophotometer. The samples were dissolved in cyclohexane and made up to give an optical density of 0·10 at 350 nm and their UV/visible absorption spectral recorded. The samples were flushed with argon for 10 min prior to fluorescence spectra being recorded. The samples were excited at 350 nm and the fluorescence emission between 360 and 620 nm was recorded and taken as indicative of the fluorescence arising from the 'grease'. The samples were also excited at 400 nm and the fluorescence emission between 630 and 710 nm was recorded and

taken as indicative of the fluorescence arising from chlorophyll in the wool grease.

2.6 Effect of in-situ irradiation

For this purpose a modified LAB16 fluorescence microscope[17] (Carl Zeiss, Oberkochen, Germany) was used. The microscope's high pressure mercury lamp (50 W) acted simultaneously as the excitation and irradiation sources. Fluorescence emission spectra were recorded with a monochromator, grating 1200 grooves/mm (Bausch & Lomb, Rochester, NY, USA). Fluorescence intensities were determined with a photomultiplier tube R446 (Hamamatsu, Japan), connected to a Tektronix digitiser TD20T (Beaverton, OR, USA) and/or via an 8 bit A/D interface to a microcomputer C64 (Commodore, Braunschweig, Germany).

W1 was placed on a glass slide and irradiated *in situ* on the LAB16 microscope, equipped with a 16/0·40 Neofluar objective, for 3 min and then for an additional 5 min. The fluorescence spectrum of the sample was measured initially, after 3 min irradiation and again after 8 min irradiation. The investigation was performed twice—once using UV excitation mode (i.e. filter system G365/FT395/LP420) and once using visible excitation mode (i.e. filter system BP450-490/FT510/LP520).

Croda wool grease was placed on a glass slide and irradiated *in situ* on the LAB16 microscope, equipped with a 40/0·75 Neofluar objective, for 30 s using an argon ion laser, model LH 1232KS (Toshiba, Japan), wavelength 488 nm, output power 10 mW. The diameter of the laser beam impinging on the sample was 4 μm. The fluorescence intensity at various wavelengths was measured before and after irradiation using the argon ion laser (pulse length 1 μs) and the visible mode of the microscope (modified) (i.e. only filters FT510/LP520).

3 RESULTS AND DISCUSSION

3.1 Variation in fluorescence along fibres

Cross-sections of tip, middle and root portions of uncleaned Merino staples were prepared and fluorescence micrographs were produced using both UV and violet excitation modes. The most intense fluorescence was obtained using the violet excitation mode and, therefore, only these results are reproduced in Figs 1–3. The cross-section of the root portions (Fig. 1) shows practically zero emission from the fibres, along with an intense fluorescence from the wool yolk surrounding the fibres. This

Fig. 1. Fluorescence micrograph of a cross-section of the root region of an uncleaned Merino wool staple (violet excitation, magnification × 300).

Fig. 2. Fluorescence micrograph of a cross-section of the middle region of an uncleaned Merino wool staple (violet excitation, magnification × 300).

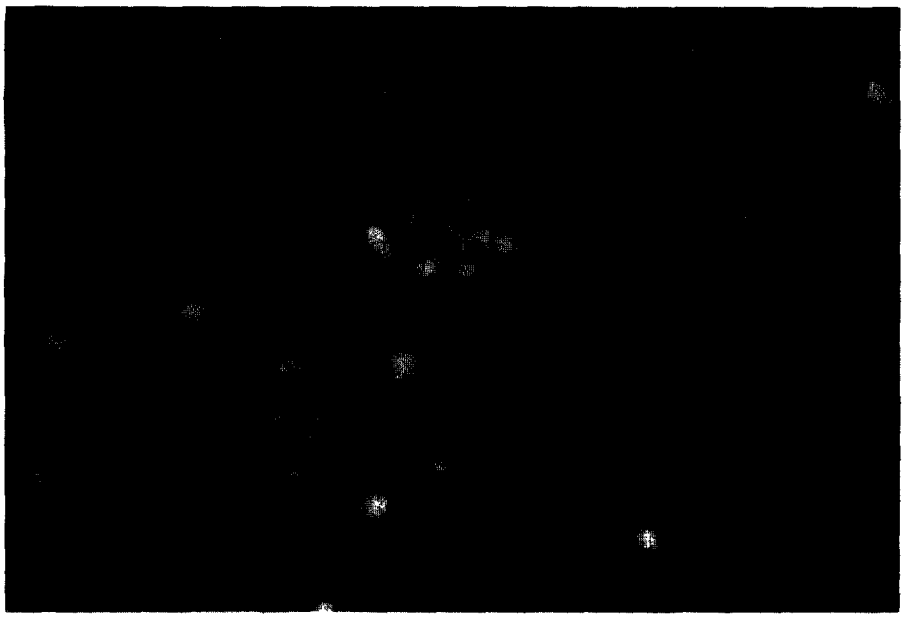

Fig. 3. Fluorescence micrograph of a cross-section of the tip region of an uncleaned Merino wool staple (violet excitation, magnification × 300).

effect is not uniform as there appears to be intense yolk fluorescence from some of the fibres. This is probably due to the scalpel used in preparing the cross-section[16] inadvertently spreading the yolk as it cut through the fibres. Some spreading of the yolk is also observed in the cross-section of the middle portions (Fig. 2). The fibres have a weak fluorescence. However, the wool yolk has a bright fluorescence, but less than the root portions. In the cross-section of the tip portions (Fig. 3) only a medium fluorescence from the fibres can be seen. These observations agree with the previous reports[12,13] that the natural visible fluorescence of Merino fibres varies along the length of the fibre, from highly fluorescent tips to barely fluorescent roots.

The observations of Hirst[7] confirm the findings of Fig. 1 and raise the question as to why there is no wool yolk fluorescence from the tip portions. There are a number of possible explanations. Firstly, the yolk could have become nonfluorescent as a result of the natural weathering process. However, studies on the effect of UV and blue light irradiation on wool grease (see later) revealed that, although the natural fluorescence decreased greatly over a long period of time, it did not reach zero in the time investigated. Secondly, the yolk could have been removed by the natural weathering process. It is known that weathering of wool fat/lanolin leads to an increase in water soluble substances[18,19] which could have

been lost from the fleece by rain before the sheep was sheared. It is hard to imagine that this would account for the difference on its own. Thirdly, and more likely, the explanation could be a combination of the first two coupled with the sensitivity of the film (or in the case of Hirst, the eye) i.e. as the yolk weathered over a long period of time some of the yolk was lost from the fibre and the fluorescence of the remaining yolk decreased to such a point that it did not register on the film (or eye).

Fluorescence micrographs (not shown) were also obtained of longitudinal portions of uncleaned Merino fibres. These revealed that the wool yolk was present in the form of patches and 'blobs' rather than being uniformly spread over the fibre.

The possibility exists that some of the fluorescence of the yolk arises from the presence of biodeteriogens. Spores of these bacteria and fungi are universal, only needing suitable conditions to develop.[20] Wool stained by the action of biodeteriogens sometimes produces fluorescence when the material is examined under UV light.[7,21–24] Not only can biodeteriogens be fluorescent themselves,[24,25] but they are also capable of producing fluorescent pigments.[26] Not all sheep are affected in the same way. They divide into two groups—immune and susceptible sheep.[27] (Selective breeding is the best way to reduce the incidence of susceptible sheep.[20,27]) It has been suggested that in susceptible fleeces, warmth and moisture may induce wax breakdown through detergent action of suint.[20] Additionally, ester-splitting of wool wax in fleece-rot and mycotic dermatitis has also been suggested.[28] However, some samples of wool wax show a high inhibitory effect on the actinomycete *Dermatophilus congolensis* (the organism responsible for the disease in sheep known as 'lumpy wool').[29] The activity of the wax was attributed to the two saturated fatty acids (+)10-methyldodecanoic acid and 12-methyltridecanoic acid, which were obtained after saponification, and which were found to have high inhibitory activity against Gram positive bacteria.

Significant microbial spoilage may be readily apparent due to severe pigmentation and fibre tendering; while a charcteristic fusty odour is normally associated with fungal growth.[20] None of these attributes were apparent with the wool fleece used to prepare the fluorescence micrographs of Figs 1–3. Additionally, it has been reported that direct microscopic examination may reveal extensive fibre damage due to bacterial activity (normally identified by the characteristic production of cortical cells) or fungal growth on the surface or within the fibre.[20] Similarly, these properties were not readily apparent with the wool fleece used. Consequently, biodeteriogens did not contribute significantly, if at all, to the fluorescence of the yolk in Figs 1–3.

Yolk consists of two main fractions: wool wax and suint.[1] It is not

apparent from the fluorescence micrographs whether the fluorescence is restricted to only one of the fractions. In order to investigate the fluorescence properties of wool wax an attempt was made to recover it from Merino wool but this was not particularly successful as it proved difficult to remove the dirt from the wax. Consequently, the rest of the studies involve the use of commercial wool greases. (The fluorescence properties of suint were not investigated, but this could be usefully carried out in the future.)

3.2 Spectral characterisation

Although it has been known for a long time that wool grease fluoresces,[6] the fluorescence spectrum of wool grease has not been reported. Figure 4 shows the fluorescence spectra of a commercial Merino wool grease for a number of excitation wavelengths. (Figures 4 and 5 have been redrawn from the originals by taking measurements every 5 nm and have been corrected to remove the Raman peaks arising from the solvent, cyclohexane.) The presence of a number of emission maxima (see also Table 2) indicates the presence of more than one chromophore in the wool grease and has resemblances to the situation reported for wool.[15,30]

Figure 5 shows the fluorescence spectra of W1 for a number of excitation wavelengths. (W1 is a New Zealand wool grease containing heavy

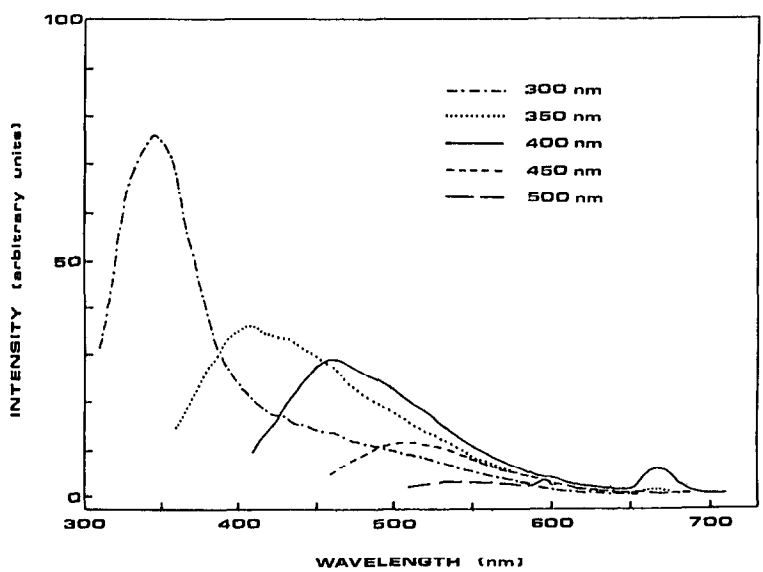

Fig. 4. Fluorescence spectra of Merino wool grease for a number of excitation wavelengths.

TABLE 2
Fluorescence Emission Maxima and Relative Fluorescence Intensities of Three Wool Greases after Excitation at a Number of Wavelengths

Excitation wavelength (nm)	*Fluorescence emission maxima (nm) and fluorescence intensities*[a]		
	Merino	*W1*	*W2*
300	345(76)	330(213), 345(206)	355(97)
350	405(36)	385(86), 405(75), 672(69)	405(63), 665(4)
400	460(29), 665(6)	455–465(31), 672(283)	460(29), 665(17)
450	510(12)	510–525(13), 660(30)	510–520(9), 660(2)
500	530–560(3)	540–560(4), 672(27)	535–560(2), 665(1)

[a] Intensities measured at peak height and expressed in arbitrary units.

chlorophyll contamination, the sample being obtained from the bulk of a small drum.) Again, the presence of a number of emission maxima (see also Table 2) indicates the presence of more than one chromophore in the wool grease. Very noticeably for this wool grease is a peak at 672 nm and this is attributed to chlorophyll. Chlorophyll *a* in toluene was found to fluoresce with an emission maximum at 670 nm, indicating that the emission peak at 672 nm in wool grease spectra is due to chlorophyll *a*. Chlorophyll in the grease could have arisen from dung contamination[31,32] or possibly by direct contact of the fleece with plants. Higher plants contain chlorophylls *a* and *b* (in the ratio of 3:1).[33] In diethyl ether,

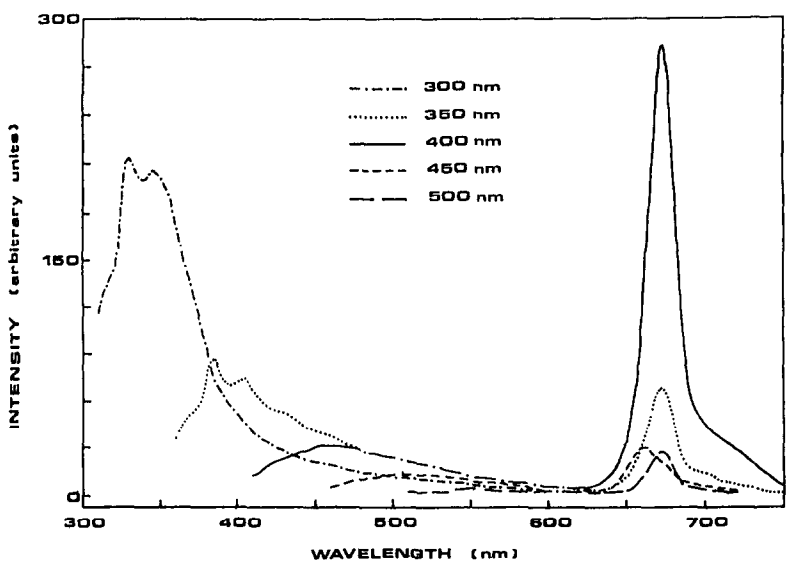

Fig. 5. Fluorescence spectra of W1 for a number of excitation wavelengths.

chlorophyll *a* fluoresces with a maximum at 669 nm while chlorophyll *b* fluoresces with a maximum at lower wavelength, 647 nm.[34] The fluorescence emission peak at 660 nm in wool grease would seem to be due to chlorophyll *b*.

The fluorescence spectra of W2 for a number of excitation wavelengths were also recorded (not shown). (W2 is a New Zealand wool grease which was obtained from the same drum as W1, but from the top rather than from the bulk of the drum.) These spectra are similar to those for the Merino and W1 wool greases with the presence of a number of (similar) emission maxima (Table 2). A feature of this grease was the much reduced intensity of the chlorophyll emission peaks, along with the reduced intensity of the 'grease' peaks, especially those produced by excitation at 300 nm. Closer examination of the fluorescence spectra of Merino wool grease (Fig. 4) reveals the presence of a small amount of chlorophyll *a*. The intensities of the spectra from the Merino wool grease are similar to those from W2.

The fluorescence spectra of W3 and W4 (being samples from the bulk and top, respectively, of a small drum of a New Zealand wool grease containing lesser chlorophyll contamination) as well as of W5 and W6 (being samples from the bulk and top, respectively, of a small drum of a typical New Zealand wool grease) were obtained (not shown). Like W2 these spectra are similar to those for the Merino and W1 wool greases with the presence of a number of (similar) emission maxima. The results of the Merino and W1–W6 wool grease samples are briefly summarised in Table 3. The fluorescence intensity at 350 nm is an approximate

TABLE 3
Fluorescence Intensities of a Number of Wool Greases after Excitation at a Number of Wavelengths

Wool grease	*Fluorescence Intensity at*[a]	
	350nm[b]	*670nm*[c]
Merino	75	6
W1	201	282
W2	97	17
W3	105	56
W4	40	5
W5	171	89
W6	64	14

[a] Arbitrary units.
[b] Peak height at 350 nm after exciting 300 nm.
[c] Peak height at 670 nm after exciting at 400 nm.

measure of the amount of 'grease' fluorescence, while the fluorescence intensity at 670 nm is an approximate measure of chlorophyll fluorescence. It is clear that all the wool greases have at least some chlorophyll in them. For the New Zealand wool greases the top of the drums (W2, W4 and W6) have a lower level of 'grease' fluorescence, together with a much lower level of chlorophyll fluorescence than the bulk of the drums (W1, W3 and W5, respectively).

The infrared spectra (not shown) of the wool greases from the top of the drums (W2, W4 and W6) showed (relative to their parent wool greases: W1, W3 and W5, respectively) an increase in absorption in the range 1400–1000 cm^{-1} and a large increase in absorption at 1676 and 1550 cm^{-1}. It is known that weathering causes an increase in absorption at 1670/1680 cm^{-1}, corresponding to α,β-unsaturated ketones.[35–38] This agrees with the supplier's observation that the wool greases at the top of the drums had undergone appreciable oxidation, resulting in them being considerably lighter, tackier and harder than their parent wool greases. The decrease in chlorophyll fluorescence on weathering of the wool greases (top of the drums relative to the bulk) is not surprising as chlorophyll is known to be light sensitive.[33,39]

Thus, it seems clear that a new measure of weathering of wool grease is the decrease in the level of fluorescence intensity, and this suggests that the Merino wool grease examined had already undergone weathering. However, care has to be taken on this last point. The Merino wool grease presumably came from Australian sheep while wool greases W1–W6 came from New Zealand and the climatic conditions may be different, resulting in a different amount of chlorophyll contamination in the first place. Although, even if this is correct, it does not, *per se*, account for the lower level of 'grease' fluorescence which the Merino wool grease exhibited.

3.3 Attempts to isolate the species responsible for fluorescence

To assist in the identification of the grease components commercial Merino wool grease was separated into a number of fractions using preparative TLC. Nine fractions (identified by using a 254 nm TLC lamp) were recovered (Table 4). From applying 206·0 mg of wool grease, 124·5 mg (60%) was recovered in these nine fractions, overlapping portions having been discarded (and some having been spilt in the recovery process). The fractions varied in qualitative fluoresence intensity (as just mentioned) and in colour, indicating a number of constituents. By way of comparison the colour of unseparated Merino wool grease is yellowy brown.

TABLE 4
Various Pieces of Information Concerning the Fractions Obtained from the TLC Separation of Merino Wool Grease

Fraction number	R_F[a]	*Fluorescence intensity*[b]	*Mass (mg)*	*Colour*
1	0·94	Medium	35·4[c]	Pale yellow waxy
2	0·84	Strong	3·5[c]	Solid[d]
3	0·80	Medium	14·1	Light brown
4	0·73	Medium	22·5	Brown
5	0·61	Weak	11·0	Golden yellow waxy
6	0·42	Weak	4·2	Yellowy/brown
7	—[e]	—[f]	11·0	Golden yellow waxy
8	0·12	Medium	5·4	Dark brown
9	0	Strong	17·4	Dark brown

[a] Ratio of the distance travelled by the solute to the distance travelled by the solvent.
[b] As observed using a 254 nm TLC lamp.
[c] Amount remaining after some having been spilt.
[d] Too small an amount to see the colour.
[e] All on the TLC plates between fractions 6 and 8.
[f] Nonfluorescent.

The UV/visible absorption spectra of the fractions reveal that most have a very broad UV absorption like the parent wool grease (Table 5). This indicates that the fractions are still mixtures. Two of the fractions (nos 2 and 9) exhibit a shoulder in their UV absorbance indicating slightly more specificity, while only one fraction (no. 8) has a definite peak and yet even this is superimposed on to a broad UV absorbance.

Fluorescence spectra of Merino wool grease, as well as of the nine fractions, were recorded for a number of excitation wavelengths (not shown). These spectra are similar to the 'grease' component of the spectra already reported (Figs 4 and 5) with the presence of a number of emission maxima (Table 5). (The values in Table 5 have been corrected to remove gross Raman effects only, notably the peak at 330 nm on excitation at 300 nm, and do not set out to cover the chlorophyll part of the spectra.) Each fraction is clearly still a mixture although there are some differences between the fractions in their relative emission intensities at the different exciting wavelengths, and also some (small) differences in their emission maxima. The latter observation may not be significant given that many peaks are broad.

Wool wax is a complex mixture of esters of high molecular mass, containing small amounts of free acids and free alcohols.[1] The isolation of the wax esters is so difficult that it is seldom attempted. The usual method of investigation is to hydrolyse the wax and examine the resultant

TABLE 5
UV/Visible Absorption Peaks as well as Approximate Fluorescence Emission Maxima and Relative Fluorescence Intensities after Excitation, at a Number of Wavelengths, of Merino Wool Grease and Fractions Obtained from it

Fraction number	*UV/vis absorption*	*Approximate fluorescence emission maxima (nm) and intensities for given excitation wavelengths (nm)*[a]				
		300	*350*	*400*	*450*	*500*
Merino	V. broad UV	355(21)	410(27)	460(33)	510(18)	—[b]
1	V. broad UV	340(159)	380(63)	430(42)	515(15)	—[b]
2	V. broad UV[c]	350(49)	390(47)	455(41)	520(70)	540(47)
3	V. broad UV	350(123)	395(87)	435(48)	520(24)	Broad(3)
4	V. broad UV	345(64)	395(47)	460(43)	505(34)	550(9)
5	V. broad UV	350(69)	405(66)	450(63)	510(29)	560(12)
6	V. broad UV	345(117)	400(108)	440(57)	515(27)	—[b]
7	V. broad UV	340(60)	405(58)	445(60)	500(30)	540(11)
8	Broad[d]	350(23)	395(35)	460(55)	515(63)	535(59)
9	Broad[e]	360(17)	425(38)	455(71)	510(69)	535(57)

[a] Intensities measured at peak height and expressed in arbitrary units.
[b] Not recorded.
[c] Small shoulder at 320 nm.
[d] Broad with peak at 274 nm.
[e] Broad with shoulder at 260 nm.

acids and alcohols separately; 138 saturated acids (plus 42 unsaturated acids) have been identified,[3] together with over 70 alcohols,[4] and it has been calculated that wool wax must contain at least 8000 mono- and di-esters, in addition to polyesters.[1] This contrasts with only about 50 esters having been identified so far.[40–42] Consequently, it is not surprising that the preparative TLC described has failed to isolate individual components present in the wool grease. This work has, however, shown that the phenomenon of components in wool grease to fluoresce is widespread. Further work on the isolation of wool grease components to establish which components fluoresce and to find out their identity could usefully be carried out.

3.4 Effect of Blacklight (UV) irradiation

The effect of UV irradiation on the natural fluorescence of wool grease was investigated by irradiating two types of wool grease with Blacklight fluorescent lamps. Merino wool grease (Fig. 6) and W1 (Fig. 7) were used. In each case the fluorescence arising from excitation at 350 nm was taken as indicative of the 'grease' fluorescence while the fluorescence

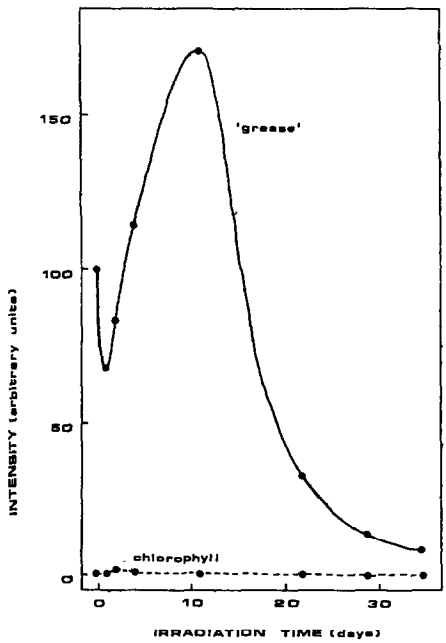

Fig. 6. Variation in fluorescence intensity of Merino wool grease with time of Blacklight (UV) irradiation (excitation at 350 nm = 'grease', excitation at 400 nm = chlorophyll).

arising from excitation at 400 nm was taken as indicative of chlorophyll fluorescence (cf. Fig. 5). For each wool grease the intensities of the 'grease' and chlorophyll fluorescence are relative to the initial intensity of the 'grease' fluorescence (at day 0).

The fluorescence of the 'grease' component of Merino wool grease (Fig. 6) first decreased slightly, then increased greatly (to a higher level than that initially observed) before decreasing substantially. The variation in chlorophyll fluorescence is likely to be connected to experimental error as the amount present was so small. The fluorescence of the 'grease' component of W1 (Fig. 7) showed a similar pattern to that of the Merino wool grease, but the magnitudes of the changes were different. The intensity decreased greatly, then increased slightly (but to a lower level than that initially observed) before decreasing further. The chlorophyll fluorescence was very intense initially, but very rapidly decreased. This is in accord with the knowledge that chlorophyll is light sensitive.[33,39]

The values of the fluorescence intensity of the 'grease' component of Merino wool grease and W1 are restated in Table 6 along with the emission maxima of the samples. Most of the peaks are broad. With the Merino wool grease the fluorescence maximum is initially at *c.* 400 nm, then quickly increases to *c.* 430 nm (at day 1) where it remains for a while

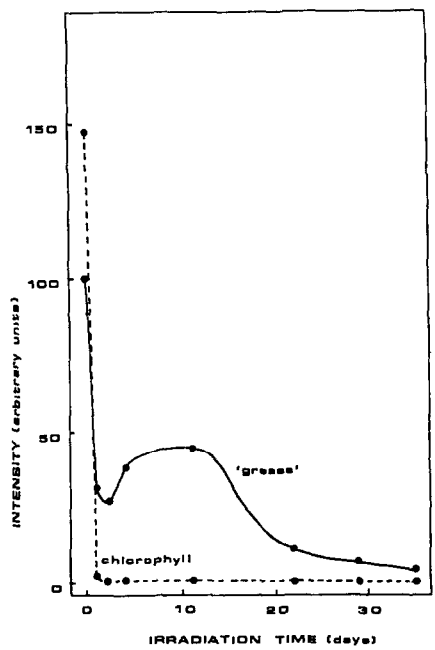

Fig. 7. Variation in fluorescence intensity of W1 with time of Blacklight (UV) irradiation (excitation at 350 nm = 'grease', excitation at 400 nm = chlorophyll).

before decreasing (after day 11) to *c.* 400 nm at the end (day 35). With W1 the fluorescence maximum is initially at 405 nm then increases (but not so quickly) to 430 nm (at day 4) before decreasing to *c.* 400 nm at the end (at day 35). By comparing the fluorescence intensities and emission maxima it is clear that there is no correlation between the two, for both the Merino wool grease and W1. It is apparent from these results that complex photochemical reactions are occurring. A number of processes could be occurring either individually or together or following one another, including:

(1) the change of a nonfluorescent species to a fluorescent species;
(2) the change of a fluorescent species to a nonfluorescent species;
(3) the change of one type of fluorescent species to another type of fluorescent species;
(4) the change of one type of nonfluorescent species to another type of nonfluorescent species.

Figures 6 and 7 clearly show that the first two processes are happening and the results shown in Table 6 suggest that the third process is also happening. However, the same effect may also be produced by a combination of the first two processes if the fluorescent species produced in

TABLE 6
Variation in Fluorescence Intensity and Emission Maxima for Merino Wool Grease and W1 upon Blacklight (UV) Irradiation.

Time (days)	*Merino wool grease*[a]		*W1*[a]	
	Fluorescence intensity[b]	*Emission maxima (nm)*	*Fluorescence intensity*[b]	*Emission maxima (nm)*
0	100·0	390–410	100·0	405
1	67·2	420–440	31·6	410–420
2	84·2	410–450	26·8	410–440
4	115·0	420–440	37·7	430
11	171·3	410–450	44·1	410–430
22	33·0	380–430	10·7	400–440
29	14·5	380–430	7·1	380–440
35	9·0	380–420	3·9	380–420

[a] Excitation at 350 nm.
[b] Fluorescence measured between 360 and 620 and expressed in arbitrary units (initial value = 100).

the second process has a different fluorescence maximum to the one destroyed in the first process. It is not possible to tell from these (fluorescence) results whether or not the fourth process is occurring.

The Merino wool grease and W1 also exhibit differences. It is possible that the large amount of chlorophyll present in W1 is sensitising reactions; alternatively, the greases may give rise to very different reactions. Thus, it was observed that over the 35 days of Blacklight irradiation the Merino wool grease went from being soft and yellowy brown in colour to being hard and pale yellow. Over the same period of time, W1 went from being soft and dark green–brown to being hard and pale yellow.

The UV/visible absorption spectra of the Merino wool grease recorded over the period of irradiation are shown in Fig. 8. (Both this figure and the corresponding figure for W1, Fig. 9, are of the solutions used for the fluorescence measurements. Consequently, they were all made to the same optical density (0·10) at 350 nm.) For the 'visible' part of the spectrum (>350 nm) the absorbance decreased for 4 days irradiation, then increased after 11 days (but still less than that initially) and carried on increasing to reach a maximum at 29/35 days (much higher than that initially). For the 'UV' part of the spectrum (< 350 nm) the absorbance increased slowly for 11 days irradiation, then underwent large decreases to reach a minimum at 35 days.

Figure 9 shows the UV/visible absorption spectra of W1 over the period

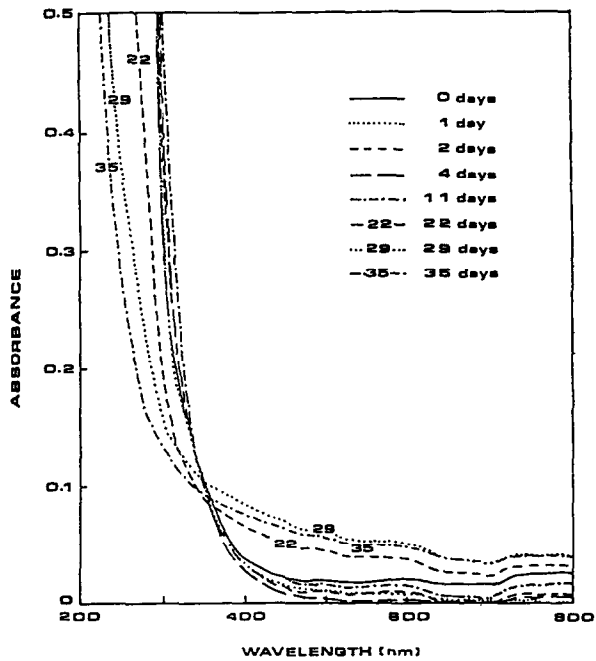

Fig. 8. Variation in UV/visible absorbance of Merino wool grease with time of Black-light (UV) irradiation (each sample made to give an optical density of 0·10 at 350 nm).

of irradiation. For the 'visible' part of the spectrum the absorbance from the chlorophyll present (having peaks at 411 and 665 nm) rapidly disappeared after 1 day of irradiation, but, apart from this, the spectra showed little difference for 11 days whereupon the absorbance increased to reach a maximum at 29/35 days. For the 'UV' part of the spectrum (<350 nm) the absorbance increased slowly for 11 days irradiation, then underwent large decreases to reach a minimum at 35 days.

There is good general agreement between Merino wool grease and W1 concerning the changes in the UV/visible absorption spectra. By comparing these with the fluorescence results in Table 6 some further understanding of the photochemical processes occurring can be gained. For the Merino wool grease: from day 0 to day 1 it is unclear what is happening; from day 1 to day 11 there is a change of nonfluorescing visible-absorbing species to fluorescing UV-absorbing species, while after day 11 there is a change of fluorescing UV-absorbing species to nonfluorescing visible-absorbing species. For W1 a similar pattern emerges: from day 0 to day 2 it is unclear what is happening (apart from the destruction of chlorophyll); from day 2 to day 11 there is a change of nonfluorescing visible-absorbing species to fluorescing UV-absorbing

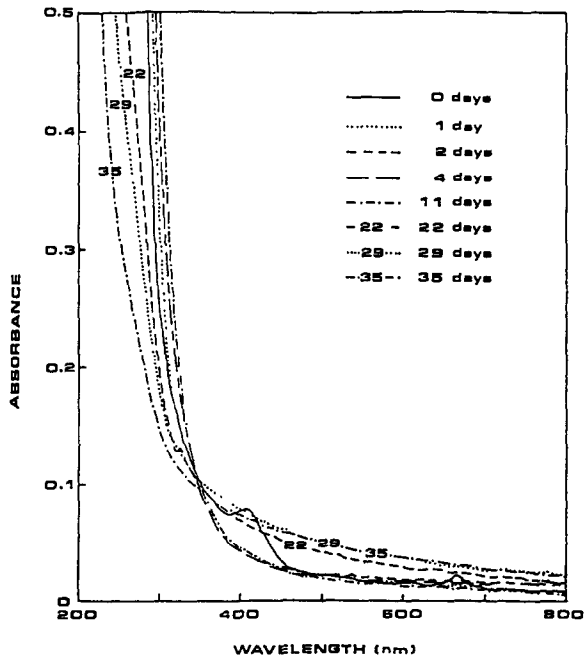

Fig. 9. Variation in UV/visible absorbance of W1 with time of Blacklight (UV) irradiation (each sample made to give an optical density of 0·10 at 350 nm).

species, while after day 11 there is a change of fluorescing UV-absorbing species to nonfluorescing visible-absorbing species.

Anderson and Wood examined wool grease recovered by the centrifugation of wool scour liquors,[36] and compared unrecovered/recovered wool grease with tip/base waxes obtained by double-shearing a selected sheep. They found that the unrecovered grease correlated with tip wax, exhibiting an increase in UV absorbance in the region 220–280 nm (using 0·03% (w/v) grease/wax solution in cyclohexane), which is characteristic of autooxidised wool wax components.[36] The 'problem' with Anderson and Wood's results is that they only provide a 'snapshot' of weathering, which is a continuous process. Whilst the results presented here have monitored the effect of UV irradiation over time this does not exactly replicate the effect of weathering, which includes the combined effects of moisture and all of the sunlight spectrum (visible light as well as UV). The differences in the fluorescence results between Merino wool grease and W1 are interesting and unaccounted for. They could be due to the presence of impurities, e.g. chlorophyll or metal ions,[43] or simply to a different 'grease' composition.

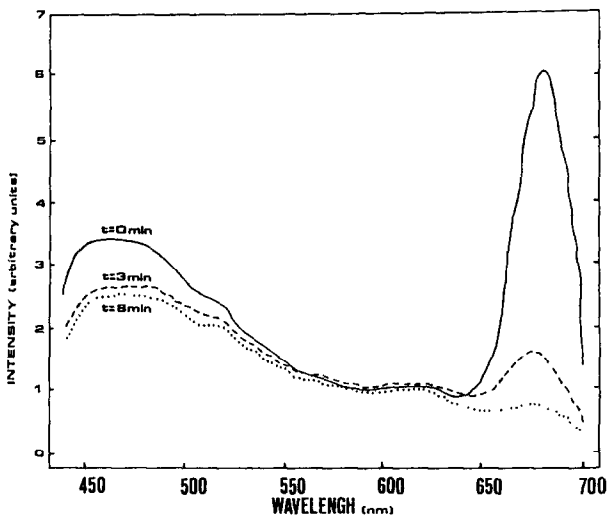

Fig. 10. Variation in fluorescence spectra of W1 during in-situ irradiation with UV light (UV excitation).

3.5 Effects of in-situ irradiation

In-situ irradiations of the wool greases using a microscope were carried out. No embedding medium was used. Three investigations were carried out, using: W1 and UV light, W1 and visible light as well as Croda wool grease and 488 nm laser light.

For the first investigation, W1 was irradiated *in situ* with UV light (broad band 365 nm) and fluorescence spectra recorded at various intervals (Fig. 10). (Both Figs 10 and 11 have not been corrected for the background fluorescence (not shown) arising from the optics, etc., which represents most of the nonchlorophyll part of the spectra at $t = 8$ min.) Fluorescence from both the 'grease' and chlorophyll components (cf. Fig. 5) can be seen and both are observed to decrease rapidly upon UV irradiation.

In the second investigation, W1 was irradiated *in situ* with visible light (450–490 nm) and fluorescence spectra were recorded at various time intervals (Fig. 11). Again, fluorescence from both the 'grease' and chlorophyll components (cf. Fig. 5) can be seen and both are observed to decrease upon visible irradiation, although the rate is noticeably slower than when using UV light (Fig. 10).

In the third investigation, Croda wool grease was irradited *in situ* with 488 nm (blue) laser irradiation with fluorescence spectra being recorded

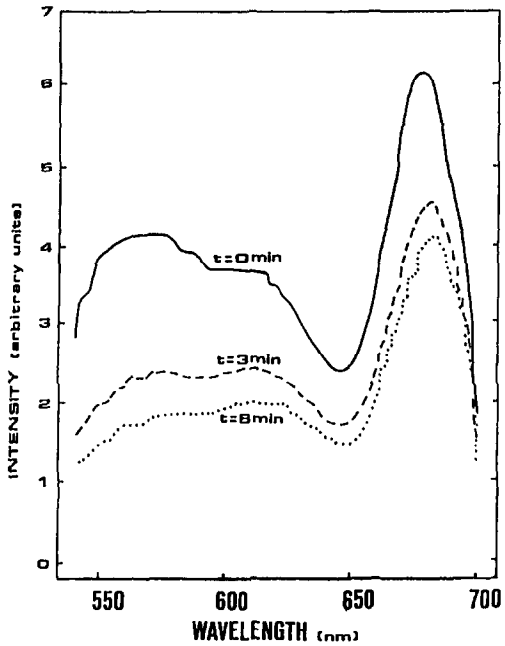

Fig. 11. Variation in fluorescence spectra of W1 during in-situ irradiation with visible light (visible excitation).

before and after (Fig. 12). (The spectra have not been corrected for the small amount of background fluorescence present (35 arbitrary units).) The initial spectrum shows fluorescence from 'grease' and chlorophyll components (cf. Fig. 5). Although the amount of chlorophyll fluorescence is small, it does nevertheless demonstrate the widespread presence of chorophyll in wool greases. Upon irradiation the fluorescence from both the 'grease' and chlorophyll components is diminished, particularly the 'grease' component.

However, there is a problem with all the in-situ results presented here in that they are only 'snapshots' and so might unduly simplify a complex situation. Owing to the higher light intensities involved, different processes may be occurring than those which happen in nature (and in non in-situ irradiations such as reported earlier).

For W1 and UV light (Fig. 10) the 'grease' and chlorophyll components decrease approximately at proportionally the same rate unlike Blacklight (UV) irradiation (Fig. 7) where the chlorophyll component decreased at a much faster rate. In comparison with W1 and visible light (Fig. 11) the 'grease' component decreases quicker than the chlorophyll component (although both do so at a slower rate than with UV light).

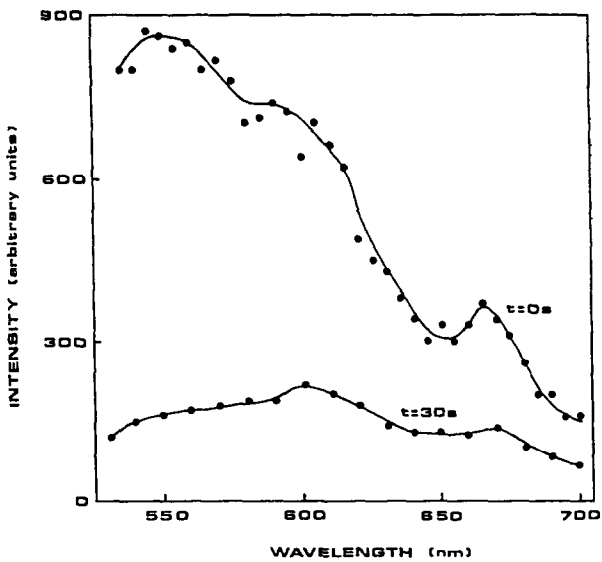

Fig. 12. Fluorescence spectra of Croda wool grease before and after 30 s in-situ 488 nm laser irradiation (488 nm excitation).

This is reiterated by the Croda wool grease with 488 nm laser irradiation where the proportional decrease of the 'grease' component is more than the chlorophyll component. However, care has to be taken as it was observed with blue light irradiation of wool[14] that intense laser irradiation (488 nm) could cause effects different from those observed with blue (Northlight) irradiation.

The only previous report of the effect of light on the fluorescence of wool grease was by van Raalte.[8] He left some fuller's earth refined sheep fat, which exhibited luminescence, in a test tube in sunlight and after several weeks did not observe any luminescence. Since the test tube was probably made out of glass it would have filtered out some of the UV portion of sunlight.[14] Hence, the photodegradation of the sheep fat must have been stimulated by mainly visible irradiation. The present results agree with van Raalte's and show that visible irradiation causes a decrease in the intensity of the fluorescence of wool grease.

It is interesting to compare the effect of light irradiation on wool and wool grease. For wool, UV irradiation causes an increase in fluorescence intensity while blue light (not laser) causes no change.[14] However, for wool grease, both UV and blue light irradiation cause a decrease. Clearly, the species responsible for the fluorescence of both wool and wool grease cannot be identical, although there may be constituents common to both.

ACKNOWLEDGEMENTS

This work was supported by the Wool Foundation, SERC and the Australian Wool Corporation. We thank Drs P. H. Greaves (IWS) and M. E. C. Hilchenbach for assistance with fluorescence microscopy; Dr D. A. Rankin (WRONZ) and Mr B. Robinson (IWS) for providing wool greases; and Dr H. J. Hageman, Mrs B. Huband, Dr A. O'Donnell, Mrs C. J. Wang, Dr Y. Xue, Mr J. Q. Zhao and Dr H. S. Zhang for assistance in translating papers.

REFERENCES

1. Truter, E. V., *Wool Wax*. Cleaver-Hume Press, London, 1956, pp. 1–60.
2. Stewart, R. G. & Story, L. F., *Technical Papers. Vol 4: Wool Grease*. WRONZ, Christchurch, 1980.
3. Motiuk, K., Wool wax acids: a review. *J. Am. Oil Chem. Soc.*, **56** (1979) 91–7.
4. Motiuk, K., Wool wax alcohols: a review. *J. Am. Oil Chem. Soc.*, **56** (1979) 651–8.
5. Motiuk, K., Wool wax hydrocarbons: a review. *J. Am. Oil Chem. Soc.*, **57** (1980) 145–6.
6. Croner, F., Über die fluorescenz von ölen im ultravioletten licht. *Z. Angew. Chem.*, **39** (1926) 1032.
7. Hirst, H. R., Ultra-violet radiation as an aid to textile analysis. *J. Text. Inst.*, **18** (1927) 369–75 .
8. van Raalte, A., De luminescentie van oliën en vetten. *Chem. Weekblad*, **25** (1928) 544–6.
9. Carrière, J. F., De fluorescentie van oliën en vetten. *Chem. Weekblad*, **25** (1928) 632–4.
10. Mezentsev, E. G., The physiological role of the luminescence of wool fat. *Dokl. Vses. Akad. Sel'skokhoz, Nauk*, (1) (1970) 33–6.
11. Smith, G. J. & Melhuish, W. H., Fluorescence and phosphorescence of wool keratin excited by UV-A radiation. *Text. Res. J.*, **55** (1985) 304–7.
12. Collins, S., Davidson, R. S., Greaves, P. H., Healey, M. & Lewis, D. M., The natural fluorescence of wool. *J. Soc. Dyers Col.*, **104** (1988) 348–52.
13. Schäfer, K., The natural fluorescence of wool. *J. Soc. Dyers Col.*, **107** (1991) 206–11.
14. Collins, S. & Davidson, R. S., Aspects of the photobleaching and photo-yellowing of wool. *J. Soc. Dyers Col.*, **109** (1993) 202–9.
15. Melhuish, W. H. & Smith, G. J., Photobleachable fluorescent species in wool keratin. *J. Soc. Dyers Col.*, **109** (1993) 163–5.
16. Wildman, A. B., *The Microscopy of Animal Textile Fibres*. WIRA, Leeds, 1954, pp. 26–32.
17. Davidson, R. S. & Hilchenbach, M. M., The use of fluorescent probes in immunochemistry. *Photochem. Photobiol.*, **52** (1990) 431–8.
18. Sandell, E., Fat oxidation in pharmaceutical practice. *Svensk Farm. Tid.*, **54** (1950) 473–91, 501–13, 525–33.
19. Clark, E. W. & Kitchen, G. F., Autoxidation, and its inhibition, in anhydrous lanolin. *J . Pharm. Pharmacol.*, **13** (1961) 172–83.
20. McCarthy, B. J. & Greaves, P. H., Mildew—causes, detection methods and prevention. *Wool Sci. Rev.*, **65** (1985) 27–48.

21. Anon., The mildewing of wool: causes and prevention. *Wool Sci. Rev.*, **6** (1950) 31–42.
22. Garner, W., *Textile Laboratory Manual*, 3rd edn. Heywood, London, 1967, Vol. 6, p. 153.
23. Lewis, J., Microbial biodeterioration. In *Economic Microbioloy*, ed. A. H. Rose. Academic Press, London, 1981, pp. 81–130.
24. Greaves, P.H. & McCarthy, B.J., A microsopical study of severe biodeterioration in a textile floor covering: a case history. *J. Text. Inst.*, **82** (1991) 291–5.
25. McCarthy, B.J. & Greaves, P.H., Auto-fluorescence from standard textile biodeteriogens. *Melliand Textilber.*, **71** (1990) 913–14, E415–16.
26. Fraser, I. E. B. & Mulcock, A. P., Pigment production by a strain of pseudomonas aeruginosa isolated from the fleece of sheep. *J. New Zealand Assoc. Bacteriologists*, **11** (1956) 2–7.
27. Fraser, I. E. B. & Truter, E. V., Bacterial discoloration of wool. *J. Text. Inst.*, **51** (1960) T857–62, T873–5.
28. Goodrich, B. S. & Lipson, M., *Aust. Vet. J.*, **54** (1978) 43.
29. Goodrich, B. S. & Roberts, D. S., Antimicrobial factors in wool wax. *Aust. J. Chem.*, **24** (1971) 153–9.
30. Jones, S. K. R., The photoyellowing of wool. PhD Thesis, City University, London, UK, 1986.
31. Hansen, E. C., Bauer, J., Benischeck, J. I., Leonard, E. A. & Wollner, H. J., Chlorophyll contamination of wool and its effect on the lightfastness of dyed shades. *Am. Dyestuffs Rep.*, **47** (1958) 118–24.
32. Stewart, R. G., Light-fugitive yellow in wool. *Proc. Int. Wool Text. Res. Conf. Paris*, **2** (1965) 143–53.
33. Granick, S. & Govindjee, Chlorophyll. In *McGraw-Hill Encyclopedia of Science and Technologv*, 6th edn. McGraw-Hill, New York, 1987, Vol. 3, pp. 556–9.
34. Govindjee, & Papageorgiou, G., Fluorescent compounds (plants). In *McGraw-Hill Encyclopedia of Science and Technoloy*, 6th edn. McGraw-Hill, New York, 1987, Vol. 7, pp. 206–10.
35. Horn, D.H.S., Wool wax. VIII—The composition of the unsaponifiable material. *J. Sci. Food Agric.*, **9** (1958) 632–8.
36. Anderson, C. A. & Wood G. F., Fractionation of wool wax in the centrifugal recovery process. *Nature*, **193** (1962) 742–4.
37. Yao, Y., Zhou, A., Yang, H., Zhuang, Y., Li, F. & Zhu, P., Effects of weathering on wool wax composition and properties. *Fangzhi Xuebao (J. China Text. Eng. Assoc.)*, **3** (1982) 581–7.
38. Horn, D. H. S. & Ilse, D., The autoxidation of wool wax. *Chem. and Ind.*, (1956) 524–5.
39. Jen, J. J. & Mackinney, G., On the photodecomposition of chlorophyll *in vitro*—1. Reaction rates. *Photochem. Photobiol.*, **11** (1970) 297–302.
40. Tiedt, J. & Truter, E. V., The constitution of some wool wax esters. *Chem. and Ind.*, (1952) 403.
41. Crabtree, H. E. & Truter, E. V., Constitution of some steroid esters of wool wax. *J. Sci. Food Agric.*, **25** (1974) 1441–9.
42. Sydykov, Z. S., Segal, G. M. & Koshoev, K. K., Isolation of cholesterol isononatriacontanoate from sheep wool wax. *Khim. Prir. Soedin.*, (6) (1977) 820–1.
43. Janecke, H. & Senft, G., Oxidation sensitivity of lanolin. *Pharmazie*, **12** (1957) 550–60.