

SIMULTANEOUS MEASUREMENT OF FINENESS AND YELLOWNESS  
OF WOOL SAMPLES

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ABSTRACT

The effects of fibre diameter and yellowness on the liquid scintillation counting of wool containing [ $^{14}\text{C}$ ]formic acid were studied. The main effect of an increase in diameter was to reduce the scintillation spectrum approximately uniformly over the entire energy range whereas the main effect of yellowness (caused by pre-heating the wool at  $153^\circ\text{C}$ ) was to shift the spectrum towards the lower energies.

Fibre diameter (D) and yellowness index (Y; calculated from measurements of reflectance of light in the red, green and blue regions of the spectrum) were regressed simultaneously on a number of linear and quadratic functions of relative integral counting efficiency (E) and channels ratio (R), using conventional least squares methods. This confirmed that D is closely related to E ( $r = -0.992$ ) but is also slightly correlated with R ( $r = 0.184$ ), and Y is closely related to R ( $r = -0.897$ ) and is slightly correlated with E ( $r = -0.232$ ).

With the calibration equations thus derived, D and Y may be measured simultaneously by liquid scintillation spectrometry with 95% confidence limits of  $\pm 0.45 \mu\text{m}$  and  $\pm 4.5$  units respectively.

## INTRODUCTION

Objective measurements of the fineness (mean fibre diameter) and yellowness of wool samples are becoming increasingly important in the wool industry. Fineness is the main determinant of the quality, and hence the price, of different lots of wool; yellowness restricts the range of colours which may be used in dyeing wool and so may also influence price.

Until recently most measurements of fineness were made after the wool had been processed to form the approximately parallel array of fibres known as "tops", but there is a rapidly increasing demand for the testing of raw wool in order to specify wools objectively before sale. Fineness is usually measured with equipment which operates on the air-flow principle (1,2) : the resistance to flow of a given mass of wool varies with total surface area and hence with fineness. A yellowness index may be calculated from measurements of light reflectance at three wave lengths (3,4).

Another method of measuring fineness has been developed in which a liquid scintillation solution containing [ $^{14}\text{C}$ ]formic acid is added to wool samples and the relative counting efficiencies are subsequently determined after the formic acid has been absorbed by the wool (5,6,7). The counting efficiency is related to diameter, due to the self-absorption of  $\beta$ -radiation within the fibres. The method has been calibrated with a set of standard tops of known fineness, and has been shown to produce results with sufficient accuracy for most commercial and research applications (8), even though this calibration includes small errors due to variable quenching. The most likely cause of quenching in raw wool or tops is yellowness. Some wools acquire a natural yellowness during their growth, while others become yellow during processing due to factors such as heat, ultraviolet light, or alkaline washing (9).

We have examined the possibility that both fineness and yellowness of wool samples could be measured simultaneously by liquid scintillation spectrometry. The use of colour quenching in the study of yellowing of wool fabric has been described (10). We have also obtained further evidence on the conditions in which the same fineness calibration may be used with different liquid scintillation spectrometers.

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### MATERIALS AND METHODS

Wool Samples. - Two groups were studied. Group I comprised six samples of raw wool from individual sheep (7), and six sub-samples of each of these wools which had been heated at 153°C for up to 8 hr to produce a range of yellowness. Each sub-sample (3g) was heated in a "drying pistol" in which the wool was held in a glass cylinder surrounded by the vapour of boiling anisole. Group II comprised eight standard tops (Interwoollabs, Brussels) whose mean diameters were based on measurements in about 70 laboratories, and 19 other tops exhibiting a wide range of yellowness.

The fineness of each sample was measured by the air-flow (1) and sonic (2) methods after calibrating the instruments with the standard tops. The heating described above produced no detectable change in diameter. Yellowness index, expressed as a percentage, was determined with a "Meeco Colormaster Model V" Colorimeter. Samples were prepared by carding 2.5g of clean wool, which was mounted on a white card behind PT300 cellophane and compressed laterally between glass plates. The colorimeter was calibrated to adjust for the effect of the glass and cellophane on the reflectance readings. Readings at three wave lengths were duplicated with the sample rotated 180° between readings and yellowness index was calculated as described by King (4).

Preparation of Samples for Scintillation Counting. - Each test specimen of wool (75mg; 88% dry matter) was placed in a glass ampoule (3ml) to which was added 3ml of toluene containing 3g ButylPBD/l, 0.2g dimethylPOPOP/l, 50 µl Triton X-100/l, and 50µl [<sup>14</sup>C]formic acid/l. About 0.2 µCi <sup>14</sup>C was added to each ampoule in most experiments. Ampoules containing no wool but with extra Triton X-100 (60µl) (7) and with 3ml of the above scintillation solution were prepared with each batch to provide solution standards. After adding the solution, the ampoules were immediately sealed and heated at 70°C for 5 hr. For assay, each ampoule was placed inside a 20ml glass vial which was then filled with water to improve light collection (6).

A small proportion of the radioactive material was not absorbed by the wool but remained in solution. In order to determine the true counting rates attributable to the <sup>14</sup>C within the wool, some ampoules in each batch were opened and a measured volume of the solution withdrawn to enable the proportion of the added <sup>14</sup>C remaining in solution

to be measured.

Radioassays. - Two Packard Model 3375 Liquid Scintillation Spectrometers and a Philips Model PW4510 Liquid Scintillation Analyser were used. The sample changers were held at 3° (3375s) and 16°C (PW4510). One channel in each instrument was operated in the integral mode, with a counting efficiency of about 94% for an unquenched <sup>14</sup>C standard solution (6). A second channel was set with its lower level raised to exclude about 40% of the pulses from an ampoule containing unheated wool from Group I. The upper levels were not used as the background in both channels was negligible compared to the sample count rates. The ratio of the counting rate in the second channel to that in the first was used to monitor quenching. These values, multiplied by 1000, were used as the "channels ratios". The integral counting rates were corrected by subtracting the counting rate due to <sup>14</sup>C labelled compounds left in solution and the corrected counting rates of the wool samples were divided by the corrected mean rate of the solution standards to give relative counting efficiencies.

The spectra of representative samples were determined in a multichannel analyser whose detector, preamplifiers, high voltage, coincidence circuitry and sum amplifier were those of a standard Philips PW4510 instrument. The conventional measuring channels, however, were replaced by a special amplifier/discriminator unit. This contained a linear pulse amplifier and 24 discriminator circuits, connected to various stages of the amplifier. The bias levels of the discriminators were arranged so that the instrument behaved as a 23 channel pulse-height analyser, all channels having the same relative width of 1.414 : 1. A pulse-height range from 2 millivolts to 5.793 volts was covered in this way. Pulses below 2 millivolts were registered in channel 0, and those larger than 5.793 volts were registered in channel 24.

Statistical Methods and Calibrations. - Using conventional least squares methods, fibre diameter (D) and yellowness index (Y) were regressed simultaneously on a number of linear and quadratic functions of relative integral counting efficiency (E) and channels ratio (R). The most complex model fitted was in the form of two simultaneous quadratic equations in which, for the *i*<sup>th</sup> sample,

$$D_i = a + bE_i + cE_i^2 + dR_i + eR_i^2 + \text{residual} )$$

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$$Y_i = a' + b'E_i + c'E_i^2 + d'R_i + e'R_i^2 + \text{residual} \dots 1$$

and  $a, b, \dots, e, a', \dots, e'$  = regression coefficients.

A number of less complex models (sub-sets of equations 1) were also fitted. The model with the smallest residual mean square was chosen as the calibration equation.

This technique of calibration corresponds to the "inverse" rather than the "classical" method of Krutchkoff (11), who showed that the former is superior. The distinction is of little importance in the present case because, although  $D_i$  and  $Y_i$  are the controlled variables they are not estimated without error. The inverse method had the additional advantage that the resultant equations estimated  $D_i$  and  $Y_i$  directly, and confidence limits for the estimates could be obtained from conventional multiple regression formulae (12), while the classical method required solving simultaneous quadratic equations.

### RESULTS

Yellowness Index. - When first measured by the colorimetric method, the yellowness indices of the unheated wools in Group I ranged from 19.9 to 23.8%, the coarser wools having the higher values, while the heated wools gave values of up to 61.4%. During the following six months the indices of the unheated wools increased slightly (to 20.6-24.4%) while those of the heated wools decreased by up to 7 units.

Eight representative wool samples (2.5g) with yellowness indices ranging from 22 to 38% were immersed in a liquid scintillation solution (100ml each) of the same composition as described above except that unlabelled formic acid was used. After heating at 70°C for 5 hr (7) the wool was washed with ethanol and light petroleum, conditioned, and yellowness index measured again. All values decreased by about 1.6 units, and the correlation between values before and after immersion was 0.988.

The yellowness indices of the wools in Group II ranged from 21 to 50%. This covers the range of natural yellowness observed in Merino wool produced in Australia (Jackson, unpublished).

Liquid Scintillation Spectrometry. - The effects on the scintillation spectrum due to the presence of wools varying in fineness and yellowness were first studied. The major effect of increasing diameter was to reduce the spectrum approximately uniformly over the whole energy range

(Fig. 1) whereas the major effect of yellowness was to shift the spectrum towards the lower energies (Fig. 2). Thus, it is legitimate to use a channels ratio method to correct the relative integral counting efficiency, and hence diameter, for the effect of yellowness.

An examination of the relationships between D, Y, E, and R was carried out with six test specimens of each wool sample, prepared by the procedure described and assayed twice with a counting time of two minutes per sample. The measurements of Y by means of the colorimetric method were made within two weeks of conducting the radioassays.

The proportion of added  $^{14}\text{C}$  remaining in solution varied slightly, being larger with the finer wools and with the wools which had been pre-heated for the longest times. However, as the range of values (0.7-1.4%) was small, the mean (1.04%) was used as the correction for all samples.

The data for the wools of Group I were subjected to regression analysis to provide calibration equations. The correlations confirmed the results in Figures 1 and 2 - diameter is closely related to integral counting efficiency ( $r = -0.992$ ) but is also slightly correlated with channels ratio ( $r = 0.184$ ); yellowness index is closely related to channels ratio ( $r = -0.897$ ) and is slightly correlated with integral counting efficiency ( $r = -0.232$ ).

The "goodness of fit" of model 1 and its various sub-models is shown by the residual mean squares in the analyses of variance (Table I). It is clear that the full model is not necessary for either fibre diameter or yellowness index, but a different sub-model is appropriate for each. For diameter the equation with the smallest residual ( $0.04284 \mu\text{m}^2$ ) was:

$$\hat{D}_i = 202.36 - 4.381 E + 0.02445 E^2 + 0.00614 R \dots\dots 2$$

where  $\hat{D}_i$  = the estimated mean fibre diameter of the  $i^{\text{th}}$  sample.

For yellowness index the equation with the smallest residual ( $3.962\% ^2$ ) was:

$$\hat{Y}_i = -88.2 - 0.560 E + 0.818R - 0.000947R^2 \dots\dots 3$$

where  $\hat{Y}_i$  = the estimated yellowness index of the  $i^{\text{th}}$  sample.

For fibre diameter the correlation between  $\hat{D}_i$  and  $\hat{D}_i$  (multiple correlation coefficient) for the calibration

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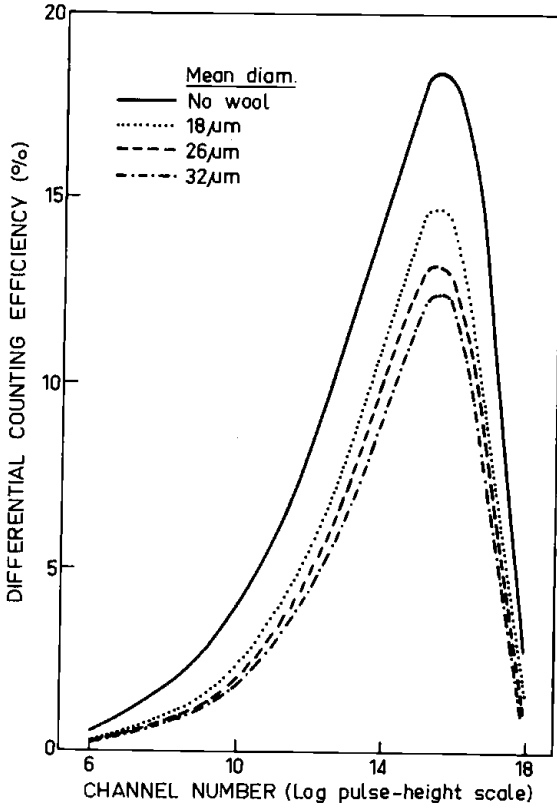


Figure 1. Effect of an increase in fibre diameter on scintillation spectrum in radioassay of [ $^{14}\text{C}$ ]formic acid in wool (75 mg in 3 ml ampoules). The yellowness indices of these wools were in the range 20-23%.

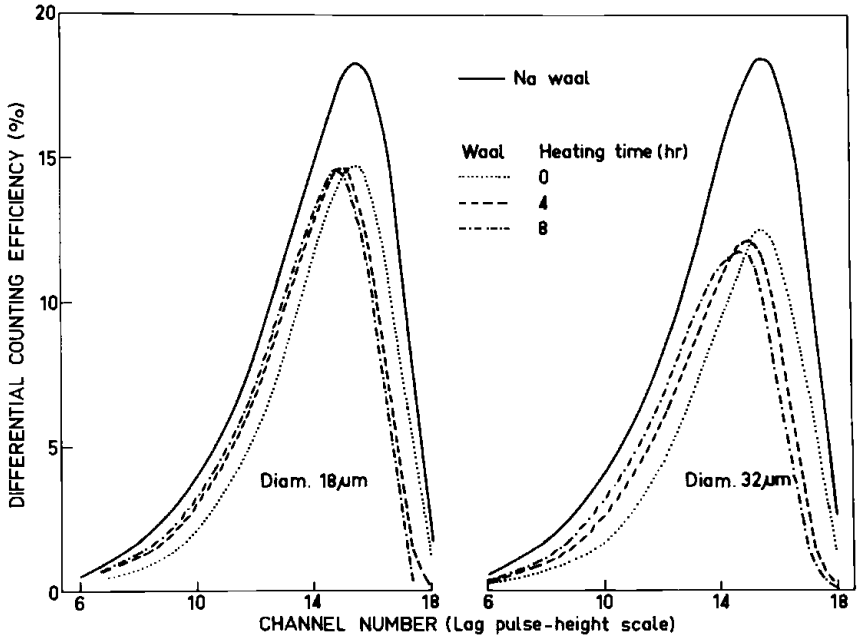


Figure 2. Effect of an increase in yellowness, due to pre-heating wool at 153°C, on scintillation spectrum in radioassay of [<sup>14</sup>C]formic acid in wool (75 mg in 3 ml ampoules).

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set of data was 0.995 (Fig. 3). For yellowness index the corresponding correlation between  $Y_i$  and  $\hat{Y}_i$  was 0.916 (Fig. 4).

The precision of an estimate of diameter ( $\hat{D}_i$ ) and yellowness ( $\hat{Y}_i$ ) from a set of  $E_i$  and  $R_i$  values, using equations 2 and 3, is given in Table II. The estimates are slightly more precise at intermediate  $E_i$  and  $R_i$  values. The confidence intervals in Table II are appropriate when the same number of ampoules (six, each assayed twice) as in the calibration experiment are averaged to obtain  $E_i$  and  $R_i$  before using equations 2 and 3. If a multiple of that number were used, say  $N$  times as many, an approximate confidence interval would be that in Table II divided by  $\sqrt{N}$ . An exact confidence interval could be obtained (as were those in Table II) from formulae given by Draper and Smith (12).

An excellent correlation ( $r = 0.999$ ) between observed and predicted values was obtained when equation 2 was used to calculate  $\hat{D}_i$  for the wools of Group II (Fig. 3) which were not used in deriving the calibration equations. For the standard tops the maximum difference was  $0.4\mu\text{m}$  for the sample of nominal diameter  $34.9\mu\text{m}$ . For the other tops the maximum difference was  $0.5\mu\text{m}$  for diameters below  $35\mu\text{m}$  and  $1.5\mu\text{m}$  for those above  $35\mu\text{m}$ . The larger differences for the coarse wool were probably due to differential effects of medullation (13) on measurements by the two methods.

In general there was also a good correlation between the observed and predicted yellowness indices for the wool tops. However, the values of some of the yellower tops, as calculated from equation 3, were several units higher than the observed values (Fig. 4), showing that the calibration made only with heat-yellowed wools did not apply strictly to all of the tops.

In several experiments carried out over a period of a year, ampoules containing sub-samples of the wools from Group I were prepared using the procedure described. Throughout this period the samples were assayed on two of the counters (3375s) and the channels ratios were measured with the gain and discriminator settings originally chosen. The relative integral counting efficiencies remained constant, but the channels ratios varied significantly unless the instruments were carefully normalized on each occasion (Packard Instrument Manual). However, the ranking of samples on the basis of channels ratio remained the same ( $r = 0.98-0.99$ ) when the results from different experiments or instruments were compared.

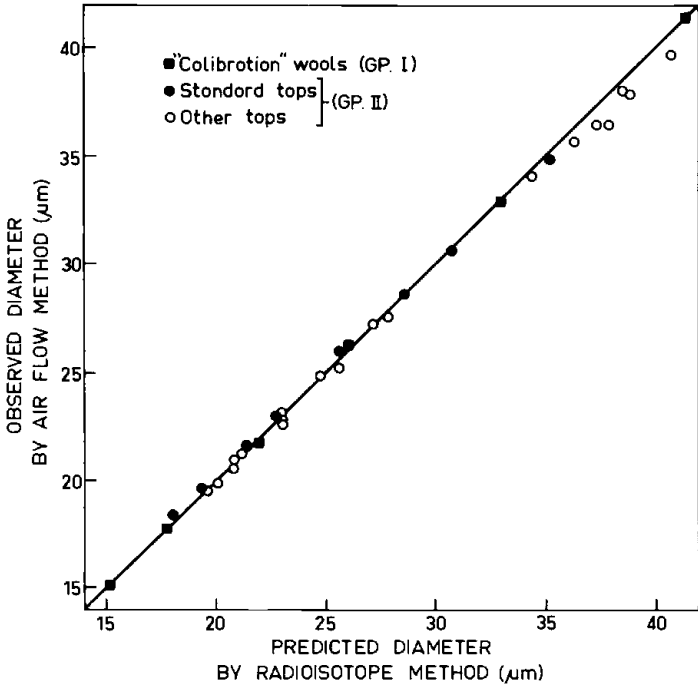


Figure 3. Comparison of mean fibre diameters predicted from calibration equation 2 (based on results for wools of Group I) with values obtained by air-flow (standard tops) and sonic (other wools) methods.

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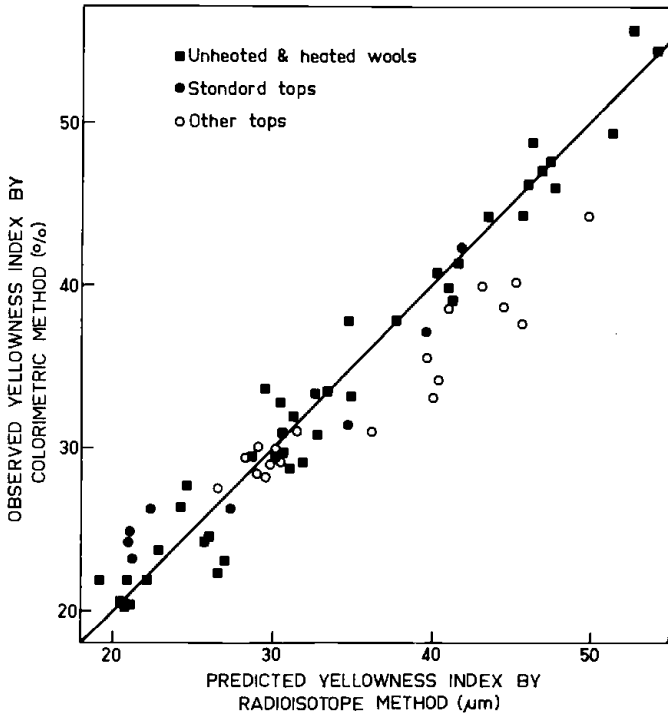


Figure 4. Comparison of yellowness index values predicted from calibration equation 3 (which was based on results for wools of Group I) with those obtained by measurements of reflectance in the colorimeter.

The integral counting efficiency of  $^{14}\text{C}$  in solution was slightly different in the three instruments studied. Because of this the relative counting efficiencies of the wool samples differed slightly, but significantly, in the three instruments, being lowest in the instrument with the highest sensitivity. However, when the lower discriminator setting of the most sensitive instrument was raised so that the counting efficiency of the solution standards was the same in each instrument, the relative counting efficiencies of the [ $^{14}\text{C}$ ]formic acid-wool samples were also the same, within experimental error, in the three instruments (Table III). This is due to the fact that proportionally more counts are lost with the solution standards than with the wools when the lower discriminator is raised. Apparently small differences in the average photocathode sensitivity of the photomultiplier pair in different instruments can be adequately compensated by varying the lower level of the measuring channel.

#### DISCUSSION

We draw three main conclusions from the results. Firstly, the fineness of wool samples can be measured by liquid scintillation spectrometry with good accuracy irrespective of yellowness, at least over the range of yellowness index observed in Australian Merino wools and in the standard tops. Secondly, a simultaneously measured channels ratio may be used to predict yellowness index provided other causes of quenching (pigmented wool, vegetable matter) are excluded from the samples and provided a standardized procedure is used in which the water content of the wool and the composition of the scintillation solution are kept constant. Thirdly, the evidence suggests that the same calibration equations can be used with different liquid scintillation spectrometers.

In measuring channels ratios, care is needed in checking for drifts in the liquid scintillation spectrometer. A set of unquenched  $^{14}\text{C}$  standards and of standards containing various amounts of an appropriate yellow dye would enable the desired counting conditions to be set in a given instrument from time to time or in different instruments. Wool is not suitable as a standard for colour measurements because of the changes which can occur in its yellowness.

We have mainly studied the quenching effect produced by pre-heating wool. Electron microscopic evidence (14)

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shows that wool can withstand the amount of heat which we applied, without undergoing any detectable changes in its physical shape and characteristics except for the discoloration produced. Further research is needed on the degree of correlation of quenching with yellowness caused by other agents and on the correspondence between the measuring conditions, with respect to colour, of the scintillation counter and the colorimeter. Several points can be mentioned:

(i) Type of illuminant. The colorimeter used is a tristimulus instrument - that is, it illuminates the sample in turn with broad wavelength bands in the red, green, and blue regions. The yellowness index used in this study is a combination of the reflectances of the sample using all three illuminants - in fact it is (red-blue)/green. In the scintillation ampoule the "illuminant" is the scintillations of the primary scintillator as modified by the secondary scintillator or wavelength shifter - that is, there is only one illuminant instead of three and its spectrum is in the blue region. The channels ratio is therefore closely related to blue reflectance. However, for a wide sample of Australian raw wool (Jackson, unpublished), blue reflectance is very highly correlated with yellowness index, so that channels ratio can be used as a predictor of yellowness for raw wool, but not as a general chromatic measure such as might be required for pigmented raw wools or dyed tops.

(ii) Type of observer. The phototubes of the scintillation counter also differ from that of the "Colormaster", being sensitive mainly to pulse energies in the range corresponding to the blue region of the spectrum.

(iii) Geometry of sample viewing conditions. The "Colormaster" colorimeter illuminates the sample surface at 45 degrees and views the scattered light at 90 degrees. The sample is usually mounted in air. The sample in the scintillation counter ampoule is illuminated from all points within the ampoule and is viewed from a fixed position. The sample is immersed in toluene (refractive index 1.496). Since the refractive index of toluene is much closer to that of wool (1.548) than to that of air, the quenching effect could be much more a measure of transmission than reflection. The arrangement and packing of fibres is known to be important in the colorimetric measurements, but have not yet been investigated for the scintillation technique.

The scintillation counting method described in this

paper was developed primarily for measuring the fineness of small wool samples. A more accurate prediction of yellowness index could probably be obtained by changing the procedure, for example by using a much larger sample (and a larger ampoule), and accepting a larger quench correction and hence a less accurate prediction of diameter. Alternatively a separate technique, based on liquid scintillation counting of some other  $^{14}\text{C}$ -labelled compound or another radionuclide in the presence of wool, could perhaps be developed for the prediction of yellowness index alone. However, in view of the much greater importance of diameter, the former approach of simultaneous measurement of both properties remains more attractive.

Although the prediction of yellowness index by this method is less accurate than that obtained with the colorimeter, the results so far obtained show that it is good enough for use in genetic selection of sheep or for allocating different lots of wool to one of several yellowness classes. At present these are the two main applications of yellowness measurement in the wool industry.

#### ACKNOWLEDGEMENTS

We wish to thank Mr W.H. Clarke for the measurements of fineness by the sonic method; and Miss B. Royal, Miss E. McKay, and Mrs L. Phillips for assistance with the yellowness index measurements, statistical calculations, and radioassays respectively.

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TABLE I

Analyses of variance of fibre diameter and yellowness index for Model 1 and various sub-models, fitted to the calibration set of data

Fibre Diameter			Yellowness Index		
Source <sup>†</sup>	D.F.	M.S.	Source <sup>†</sup>	D.F.	M.S.
R(E)	1	3363.9**	R(R)	1	3363.9**
resid.	40	1.372	resid.	40	20.393
R(E <sup>2</sup>  E)	1	50.501**	R(E R)	1	575.18**
resid.	39	0.1121	resid.	39	6.167
R(R E, E <sup>2</sup> )	1	2.743**	R(R <sup>2</sup>  R,E)	1	89.798**
resid.	38	0.04284	resid.	38	3.962
R(R <sup>2</sup>  E, E <sup>2</sup> , R)	1	0.0404 <sup>N.S.</sup>	R(E <sup>2</sup>  R, R <sup>2</sup> , E)	1	0.116 <sup>N.S.</sup>
resid.	37	0.04290	resid.	37	4.066

<sup>†</sup> The notation is exemplified by the following:

R(E) = reduction due to regression on E

R(E<sup>2</sup>|R, R<sup>2</sup>, E) = reduction due to regression on E<sup>2</sup> given regressions on R, R<sup>2</sup>, and E.

\*\* Significant at 1% level.

N.S. Not significant.

TABLE II

Precision of predicted fibre diameter ( $\hat{D}_i$ ) and yellowness index ( $\hat{Y}_i$ ) expressed as the 95% confidence interval for an estimate of  $\hat{D}_i$  or  $\hat{Y}_i$  from a single set of  $E_i$  and  $R_i$  values (mean of 6 ampoules) using equations 2 and 3.

$E_i$	$R_i$	$\hat{D}_i$	Precision for $\hat{D}_i$	$\hat{Y}_i$	Precision for $\hat{Y}_i$
55	465	38.6	$\pm 0.47$	56.8	$\pm 4.9$
55	565	39.2	$\pm 0.44$	41.2	$\pm 4.3$
55	615	39.5	$\pm 0.44$	26.3	$\pm 4.3$
65	465	24.0	$\pm 0.46$	51.2	$\pm 4.9$
65	565	24.6	$\pm 0.43$	35.6	$\pm 4.1$
65	615	24.9	$\pm 0.44$	20.7	$\pm 4.2$
75	465	14.3	$\pm 0.47$	45.6	$\pm 5.0$
75	565	14.9	$\pm 0.44$	30.0	$\pm 4.2$
75	615	15.2	$\pm 0.45$	15.1	$\pm 4.3$

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TABLE III

Comparison of integral counting rates on three liquid scintillation counters

Solution Standards	Mean integral counting rate*(counts/min)			
	A 808520	B 795780	C 793920	D 795880
Wool Samples Mean diameter ( $\mu\text{m}$ )	Relative counting efficiency* (%)			
	A	B	C	D
15.1	75.0	75.5	75.5	75.4
17.8	71.5	72.0	72.1	72.0
21.8	67.9	68.2	68.3	68.1
26.2	63.9	64.3	64.3	64.3
32.9	58.9	59.3	59.4	59.3
41.4	53.8	54.0	53.9	53.8

\* A = Philips Model PW4510; attenuator 0.0, discriminators, 40- $\infty$

B = Philips Model PW4510; attenuator 0.0, discriminators, 320- $\infty$

C,D = Two Packard Model 3375; gain 100%, discriminators, 20- $\infty$

In this experiment each ampoule contained about 0.38  $\mu\text{Ci}^{14}\text{C}$ .

