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Wool Studies.

III. The Uniformity of a Series of Fibre Thickness Measurements on a Small Sample of Medium Merino Wool.

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INTRODUCTION.

THE fibre diameter of wool, being either directly or indirectly associated with a variety of other characteristics, is required in the majority of problems in wool research. It is essential, therefore, that the procedure of sampling and the technique of preparing wool for diameter measurement should be placed on a fundamentally sound basis. Various characteristic properties of the material however, complicate the establishment of a sound technique of sampling. Wool as such does not readily permit a random selection of individual fibres and any endeavour to select a representative sample of fibres by personal judgment is bound to be biassed. In wool studies sampling is absolutely necessary since the preparation of the whole available material for the measurement of fibre diameter is not only practically impossible in other than very small quantities of wool, but also undesirable because it renders the material useless for further investigations.

The necessity for some adequate system of sampling a quantity of wool is, therefore, a basic consideration, and it is strange, as Wildman (1936) has emphasised, that so little attention has in the past been devoted to this aspect in the assessment of wool characters. The contribution of Fraser Roberts (1930) constitutes about the only comprehensive work in which adequate control of sampling errors was achieved in the course of a series of laboratory determinations of the average fineness of a sample of raw wool. These investigations involved the use of the weight-length method in determining this character and the system of zoning and sampling used by Roberts therefore has more particular reference to the determination of mean fibre length. In the microscopic measurement of fibre diameter, however, the same principles apply and in practice the method of sampling and of slide preparation is that described by Duerden (1929), and in general use in this laboratory. It consists of zoning the original quantity and selecting at random a number of small staples from each zone. From each such staple a small strand of fibres is drawn without selection and these strands are combined to form the ultimate sample which is prepared for measurement. The preparation consists in cutting this sample into small fragments along the entire length of the fibres or else removing small fragments at intervals along the length. These fragments are thoroughly mixed and a suitable portion removed and mounted on a slide for microscopic reading. The final determination of fibre diameter is therefore made after the original quantity of wool has been reduced in four successive stages each one of which constitutes a process of sampling. These stages are in order, (*a*) the removal of staples from the original zones, (*b*) the taking of small strands from each of these staples, (*c*) the mounting on a slide of a portion of the fibre fragments (*d*) the measurement of a limited number of fibre fragments on the slide. The first two stages comprise the manual process of sampling while the remaining stages involve problems of efficient slide preparation. Both aspects are equally important but sampling methods can only be discussed when it is known that the preparation of the slides is such that the sample will be adequately represented when a suitable number of readings are taken. The representativeness of a series of readings from a slide is dependent upon the thoroughness of mixing of the fragments, and the uniformity of their distribution over the slide.

The microscope method has been followed, because of its many advantages over other methods e.g. the diffraction, weight-length and micrometer caliper methods (van Wyk, 1937). It has also been adopted by the International Wool Conference as the standard method for wool fibre thickness determinations.

Scope of the Present Study.

In the past many disturbing differences between successive slides prepared from the same sample of wool and even between repeated measurements of the same slide were frequently experienced. These differences were often of such a significantly high order that the soundness of diameter measurements by this method was regarded with suspicion. It will be appreciated that these inconsistencies, if beyond reasonable control, would completely nullify the value of fibre diameter determinations by this method and vitally affect many aspects of wool research. Hence it was decided to investigate the whole process of slide preparation and the microscopic determination of fibre diameter.

The observed discrepancies between slides and successive readings of the same slide point to an inadequate mixing of the fibre fragments and to a heterogeneity in the distribution of these fragments over the slide. The present investigation is therefore designed specifically to examine these problems in slide preparation.

A group of ten slides was prepared from each of four mixtures of fibre fragments and each slide was traversed systematically so that twenty-five readings were made in each of ten different areas on the slide. The representativeness of slides, depending on the mixing of the cuttings may be estimated from the variance between consecutive slides while the distribution of fragments over a slide may be estimated from the variance between the ten different localities considered. The observations from these ten localities were recorded in as many columns and thus simultaneously formed twenty-five rows of ten observations each. Hence the 250 measurements from a single slide may be considered as constituting a 10 by 25 Latin square, and the variance analysed accordingly.

The four mixtures of fragments mentioned refer to the four methods of cutting which may be employed in preparing a staple of raw wool for the measurement of fibre diameter. These methods of cutting are referred to as treatments A, B, C and D and are described in the following paragraph.

Two observers each made the complete series of observations using different microscopes. To separate personal differences in the readings as between observers from possible differences due to microscopes (however unlikely this may be) the slides of Treatment A were read a second time. For this purpose groups of five slides from this treatment were allotted at random to each of the two microscopes and these were read in turn by both observers on each instrument.

Description of Technique and Procedure.

The material used in this investigation consisted of a single small staple of medium merino wool (about 66's quality number) and approximately 8·0 cms. ($3\frac{1}{4}$ inches) in length. The quantity taken was such as to represent roughly the amount of wool obtainable from four square centimetres of skin surface on a sheep of medium fleece density. The weight of the sample after thorough scouring with repeated changes of benzol, and conditioning in a humidity chamber at 70°F. and 70 per cent. relative humidity was 0·90 gm. The subsequent handling of the material, until the preparation of the slides was complete, was performed in the humidity room under the constant atmospheric conditions specified.

The staple was divided into ten zones by longitudinal partition so that each zone consisted of wool weighing approximately one tenth of the original weight. These zones were identified by serial numbers 1 to 10.

Treatment A: From each of the 10 zones a small strand of fibres was separated laterally and without selection other than for equality of size. These were combined to form a composite sub-sample equal in weight to one-tenth of the total material and to the original weight of a single zone (i.e. 0·09 gm.). This sub-sample was *cut transversely* into as fine a series of fragments as possible, subjecting the whole sub-sample to this treatment *throughout the length of the fibres* composing it. The fragments were poured off and the wool allowed to dry. The clump of wool fragments thus obtained was used to prepare a series of ten consecutive slides.

Before further treatments were commenced the ten zones into which the original material had been divided were allotted at random to two sections to facilitate the application of these methods of cutting, particularly Treatment D.

Treatment B: The two sections were placed adjacent to each other within the folds of an ordinary sheet of writing paper and cuttings made *transversely*, once in each of *three places* along its length, base, middle and tip. The fragments, which were cut as finely as possible (a little over 1 m.m. long) were mixed thoroughly in ether as before and then allowed to dry.

Treatment C.—In this treatment the two sections were *cut once transversely* about the middle of the staple and therefore adjacent to the central cutting of Treatment B. The fragments were mixed thoroughly and treated as before.

Treatment D.—In this treatment *a single oblique* cutting was made across the base half of one section and the tip half of the other and the fragments treated as above.

The clump of fragments finally obtained from each treatment was divided into ten approximately equal portions and from each portion a slide was prepared. In the preparation of the slide each portion was divided into eight zones. From each zone a suitable quantity of fragments was drawn and carefully shaken out over the slide so that fragments from each zone were contributed to every part of the final preparation. The quantity drawn from each zone was completely used so that the question does not arise that the process of shaking the fragments over the slide tends to favour the extent to which either the coarser or finer fibres are contributed. With suitable care and experience slides can be prepared in this way in which no undue clumping of fragments is evident. Each slide was previously prepared by making a thin smear of the mountant, " Euparal ", over the surface in the manner of a blood film. This was done so that the fragments falling on the slide would be *in situ* during preparation and as the cover slip was being pressed over the mountant. Such a precaution was taken because it had been noticed that when pressure was placed on the cover-slip as it was being set in place over the fluid mountant there was a tendency for fragments to be displaced towards the edges thereby disturbing their original even distribution over the slide. Cover slips measuring 2 in. by $\frac{7}{8}$ in. were used throughout and this constituted the area considered in the measurements. Slides prepared in this way may be retained as permanent preparations.

The readings from each slide were made in five longitudinal traverses, consisting of two series of twenty-five consecutive readings separated by a suitable interval. The readings were recorded individually in ten columns of twenty-five which could alternatively be considered as twenty-five rows of ten. Each slide was measured according to the same system by each observer but no attempt was made to make identical traverses. Certain eliminations were consistently made from the series to be measured. No obviously damaged or distorted fragments were considered nor were any tangled clumps, unless a very clear image presented itself. Crossed fibres were not measured if the point of intersection crossed the central section of the scale unless the image of the uppermost fibre could be very clearly distinguished. No fibre was measured whose image for one reason or another was not clearly defined.

In making a fibre measurement only those fibres, and that point of a fibre which passed across the central divisions of the ocular scale between the 20 and 30 unit lines, were taken for measurement. This procedure which constituted the ultimate sampling process, tended to eliminate personal selection and to bring the requirements nearer to the idea of random selection required by theoretical considerations.

Ordinary microscopes with the usual mechanical stage fittings were used by both observers throughout. The unit of measurement employed was $2 \cdot 5\mu$, at a magnification of $500 \times$ and the setting of the microscope at this level was repeatedly checked against a Leitz stage micrometer.

The systems of recording the actual measurements used by the two observers were found to differ slightly. In both cases the division lines on the ocular were used to represent the means of the class intervals. But in the case of the observer P any observation clearly falling between the lines was classed as an intermediate measurement without any attempt at approximation to one division or the other. Such intermediate readings were allotted alternately to the higher and the lower class when the frequency distribution was subsequently drawn up. In the case of the observer Q, however, the only intermediate measurements recorded were those in which an approximation to one unit or the other could not be made. These intermediate readings were dealt with according to the common system which was employed in constructing the frequency distribution tables.

METHODS OF STATISTICAL ANALYSIS.

The statistical analysis of wool fibre diameter measurements is theoretically complicated by the fact that these measurements are by no means normally distributed. In the second study of this series (Malan, 1937) it was shown that the characteristic distribution of fibre diameter measurements is adequately represented by a $\log_e$ normal curve which is based on the assumption that the logarithms of such measurements are normally distributed. On this basis the normal theory, strictly speaking, is not applicable to the actual measurements but to their logarithms.

It is not intended to discuss in detail the distribution of fibre diameter but only to illustrate the general form by two figures I and II. On these charts are presented the observed frequency histograms and best fitting logarithmic and normal curves. The histograms are those obtained from the second series of readings by the two observers P and Q respectively. Each histogram represents 2,500 measurements. The lack of normality is clearly shown on these charts by comparing the histogram with the normal curve indicated by the broken line. The improved fit of the $\log_e$-normal curve, shown by the continuous line, is equally clear on both charts. These charts represent very well the observed frequency distributions of fibre diameter measurements.

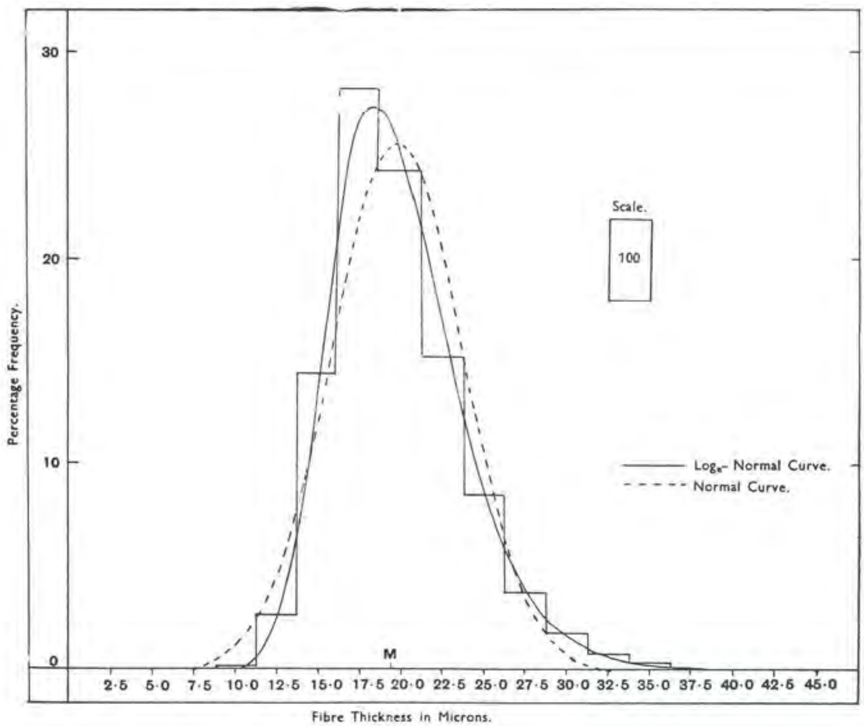

Fig. I.

The application of the normal theory to thickness measurements is more or less in general practice and it was thought advisable to include both the normal and logarithmic analyses of the data under discussion. This is done throughout the paper except for the analysis of variance within slides where the uniformity of distribution of the fragments is considered. Here the group members are rather small and a transformation into logarithmic values by the method of moments becomes too inaccurate.

In the case of the logarithmic analyses the estimates of variance were calculated separately and not by subtracting sums of squares as is often done in the ordinary variance analyses. The reason for this is obvious since the variances (and means) are obtained by transformations of ordinary moments (Malan 1937) and any inaccuracy will seriously affect the difference sum of squares if one or more of the sums of squares is based on a small number of degrees of freedom. In such cases the logarithmic sums of squares were calculated from the logarithms of individual values.

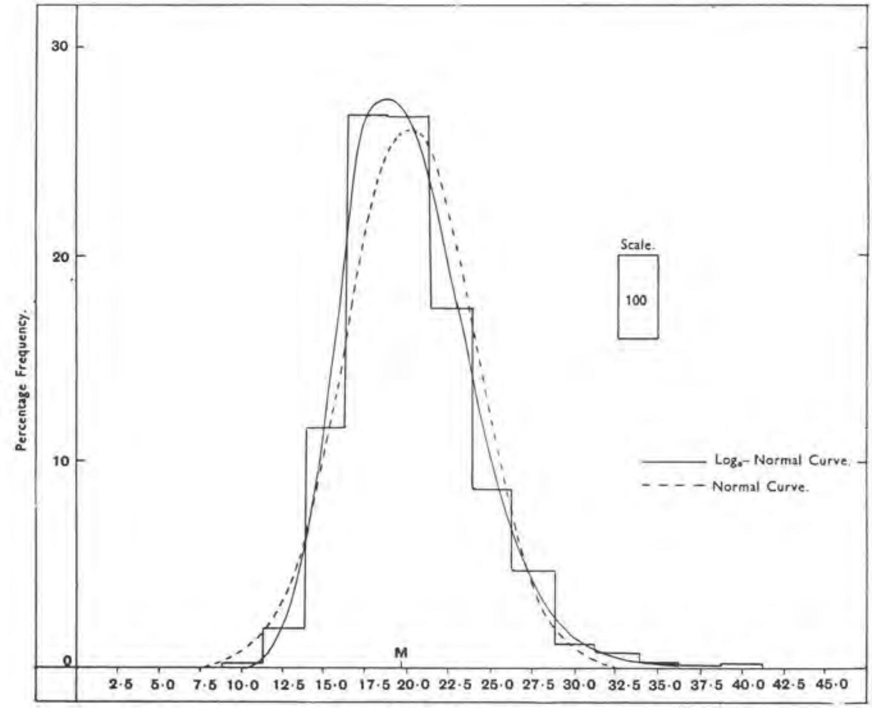

Fig. II.

Presentation of the Data.

(*a*) *Variation within Slides.*

The observations from each slide were analysed as if they constituted a 10 by 25 Latin square. The variance between column means gives an estimate of uniformity in the sense that its significance would indicate a real difference between measurements from different localities on the slide and hence a lack of uniformity in the distribution of fibre fragments. A full table of the analyses of variance is given in Table I. The variance for the two observers, P and Q, are given in adjacent columns for each slide separately. Table I (*a*) contains the results for the slides from Treatment A and similarly I (*b*), (*c*) and (*d*) present the results from each of the other treatments.

An examination of this table is sufficient to illustrate the satisfactory distribution of fibre fragments over the slides. When the row- and column-variances are compared with the corresponding error or remainder variance a significant value is indicated in italics for the 5 per cent. probability and in black type for the 1 per cent. or "highly significant" probability level.

The analysis of variance within each slide contains two independent comparisons of variance, viz. the variance between groups of 25 (columns) and that between groups of 10 (rows) with the remainder variance. Therefore, since there are four treatments with the ten slides each and two observers, the total number of comparisons is 160. As the same slides were measured by both observers there is reason to believe that their results will not be entirely independent. It should be remembered, however, that each slide contains many more fibre fragments than the 250 required for measurement and that no endeavour was made by the observers to measure the same localities on each slide. It is extremely unlikely therefore that the row and column variances will be highly correlated as between observers. Any agreement in this regard between observers should consequently be considered as more evidence that such a result reflects the position on the slide as a whole and not only as describing a particular set of observations from the slide.

On the basis of 160 different comparisons significance entirely due to random sampling should be shown by a number of comparisons not greatly different from 8. Of the significant values, about two should be highly significant on the above assumptions. The actual position revealed by Table I is 12 significant values of which 3 are highly significant. The increased number of significant values is hardly indicative of a serious degree of heterogeneity in the fibre distributions within slides. No undue increase in variation between either columns or rows are shown by 29 of the 40 slides. Of the remaining eleven where significance is shown, there is one case in which rows were effected and two others where the variance between columns was less than the "error" variance. This is evidently due to chance. In the case of the other eight slides the increased variation between columns was only shown by one or other of the two observers, except for one slide (No. 10) of Treatment D where the estimate of variance between columns is significantly greater than the "error" variance for both observers.

In Table I is also given a column which combines the degrees of freedom from all the slides for each treatment and observer separately. The respective degrees of freedom in this column are in fact ten times the corresponding degrees of freedom for individual slides. These numbers are beyond the available tables and the estimates of variance are compared by calculating their standard errors and the standard errors of their differences. Only in the case of Treatment D is there, for both observers, a significantly increased estimate of variance between columns as compared with the error variance. These differences between the estimates of variance are for observer P, $6 \cdot 288 \pm 2 \cdot 2905$; i.e. approximately $2 \cdot 75$ times its standard error, and for observer Q, $5 \cdot 083 \pm 2 \cdot 407$, i.e. $2 \cdot 1$ times its standard error.

It may therefore be concluded that the preparation of some of the slides of Treatment D was less efficient than may be expected in the sense that the fragments were not uniformly distributed over the slide. For the other treatments the distribution of fragments was on the whole quite satisfactory. The lack of uniformity in the spread of fragments in Treatment D may indicate that the oblique method of cutting demands special care when slides are being prepared. It was in fact more difficult in this treatment to cut fragments as short as was possible with the other treatments and this in itself may explain the defect noticed.

(*b*) *Representativeness of Slides.*

The readings from the ten slides prepared from each of the four treatments were used to determine the variation between slides prepared consecutively from the same mixture of fragments. This variation indicates the uniformity of such a mixture. A separate analysis of variance between and within slides was made for each treatment and observer, the results being given in Table II (*a*) and (*b*) for the ordinary and logarithmic values respectively. The greatest difference in the variances between slides and the corresponding variances within slides is shown in the readings of observer Q for Treatment D where the estimates differ by a quantity about $1 \cdot 86$ times its standard error. This result which is the same for the ordinary and the logarithmic figures, is insignificant, and the results in general therefore illustrate the reasonable agreement between slides from the same treatment.

The arithmetical and geometrical mean diameters, as estimated from each slide, for observers separately, are presented in Table III (*a*) and (*b*) respectively. In treatment D, slide 4, the mean value obtained by the observer Q is rather lower than the others but this may be a chance effect since the variations between all slides is not significantly greater than the variation within slides. It should, however, be noted that the variance between slides is reduced by ignoring slide 4 to a value approximately equal to the estimated variance within slides. In any case the slide means for a particular treatment and observer are in satisfactory agreement amongst themselves.

TABLE II.

Analysis of Variance between and within Slides.

(a) Ordinary values.

Variance due to.	D.F.	A.		B.		C.		D.	
		P.	Q.	P.	Q.	P.	Q.	P.	Q.
Between slides	9	8·390	21·057	20·372	14·720	18·694	8·863	10·413	29·614
Within Slides	2,490	14·712	16·005	15·058	14·599	13·320	13·617	15·441	15·859
TOTAL	2,499	14·689	16·023	15·077	14·600	13·339	13·600	15·423	15·909

(b) Logarithmic values.

Variance.	D.F.	A.		B.		C.		D.	
		P.	Q.	P.	Q.	P.	Q.	P.	Q.
Between Slides	9	0·02065	0·04749	0·05391	0·03309	0·02709	0·02158	0·01932	0·06964
Within Slides	2,490	0·03699	0·03832	0·03794	0·03569	0·03042	0·02990	0·03724	0·03745
TOTAL	2,499	0·03709	0·03809	0·03809	0·03571	0·03053	0·02977	0·03813	0·03760

TABLE I.

Variance within Slides (separately).

Variance.	Slide.	1.		2.		3.		4.		5.		6.		7.		8.		9.		10.		D.F.	Total.	
	D.F.	P.	Q.	P.	Q.	P.	Q.	P.	Q.	P.	Q.	P.	Q.	P.	Q.	P.	Q.	P.	Q.	P.	Q.		P.	Q.
(a) Treatment A.																								
Between Columns	9	9·914	11·525	23·169	*26·414*	15·803	12·069	3·081	23·803	10·933	13·400	9·525	*33·248*	12·125	23·444	18·056	12·503	26·169	18·567	21·669	30·359	90	15·344	20·533
Between Rows	24	21·452	9·496	19·214	11·963	9·296	13·021	13·389	13·483	15·589	9·223	21·056	11·973	11·823	14·219	12·239	16·214	13·933	*30·431*	12·079	19·921	240	15·386	14·899
Remainder	216	14·023	16·363	15·247	12·953	14·646	17·252	15·369	17·657	14·909	12·329	15·248	15·418	13·056	17·640	15·353	16·948	14·456	14·649	13·799	18·183	2,160	14·611	15·939
TOTAL	249	14·589	15·434	15·916	13·344	14·172	16·657	15·324	17·477	14·831	12·069	15·601	15·730	12·904	17·520	15·151	16·717	14·829	16·312	13·801	18·790	2,490	17·712	16·005
(b) Treatment B.																								
Between Columns	9	**2·489**	9·669	25·011	16·192	19·277	17·323	11·123	13·233	17·289	27·733	9·248	19·044	7·789	**28·225**	12·136	15·392	28·652	*5·302*	25·902	22·136	90	15·891	17·425
Between Rows	24	21·256	19·798	9·702	12·713	11·927	18·308	13·213	13·754	13·499	12·963	14·973	15·579	10·369	11·704	18·723	12·298	14·558	19·161	13·594	12·702	240	14·181	14·898
Remainder	216	15·226	13·414	14·299	15·752	13·439	13·237	14·629	12·064	17·801	17·629	17·760	14·323	12·054	11·871	16·384	13·858	16·341	17·519	13·270	14·816	2,160	15·120	14·448
TOTAL	249	15·347	13·894	14·243	15·475	13·504	13·873	14·366	12·269	17·368	17·544	17·184	14·614	11·738	12·446	16·456	13·763	16·614	17·236	13·758	14·876	2,490	15·058	14·599
(c) Treatment C.																								
Between Columns	9	17·636	6·025	5·767	15·000	20·389	9·456	16·114	15·108	6·711	9·604	7·333	13·289	14·789	14·581	14·081	**37·718**	9·625	15·558	10·167	*31·847*	90	12·261	16·895
Between Rows	24	10·483	12·713	15·923	12·656	12·396	12·025	14·329	19·702	11·892	15·052	13·489	15·744	14·473	14·036	15·744	14·168	18·073	11·121	10·364	8·776	240	13·717	14·058
Remainder	216	12·358	12·449	13·018	13·814	11·848	12·048	11·820	16·029	17·533	12·383	15·742	12·166	15·443	14·158	11·112	13·828	12·374	11·728	11·955	15·710	2,160	13·320	13·431
TOTAL	249	12·368	12·242	13·036	13·745	12·209	11·953	12·217	16·374	16·598	12·540	15·221	12·552	15·326	14·161	11·665	14·205	12·824	11·807	11·737	16·589	2,490	13·320	13·617
(d) Treatment D.																								
Between Columns	9	9·042	8·469	11·289	16·656	*37·281*	22·767	*30·933*	19·748	15·794	31·122	28·337	22·044	10·614	17·111	13·323	21·736	21·614	22·247	*33·788*	*26·192*	90	**21·261**	*20·809*
Between Rows	24	15·625	12·296	10·728	11·058	10·256	11·256	21·381	8·463	20·964	24·108	20·579	12·683	17·444	13·281	12·611	16·692	22·652	18·244	22·536	23·879	240	17·477	15·196
Remainder	216	15·083	14·835	11·396	14·422	17·101	15·377	15·453	14·819	13·497	15·949	15·728	20·922	13·826	15·971	14·868	13·053	17·708	17·392	15·069	14·525	2,160	14·973	15·726
TOTAL	249	14·912	14·361	11·328	14·178	17·171	15·247	16·584	14·393	14·325	17·284	16·652	20·168	14·058	15·752	14·594	13·717	18·326	17·649	16·464	15·848	2,490	15·441	15·859

TABLE III.

Mean Values in μ for Treatments A, B, C and D and Observers P and Q.

(a) Arithmetical Means.

Slide.	A.		B.		C.		D.	
	P.	Q.	P.	Q.	P.	Q.	P.	Q.
1..........	19·79	20·39	20·12	20·37	20·81	21·39	19·85	20·61
2..........	19·73	20·29	19·44	20·11	21·08	21·10	19·74	20·28
3..........	19·49	20·10	20·00	19·82	20·30	21·16	20·27	20·12
4..........	19·89	20·41	19·46	19·64	20·87	21·19	19·82	19·51
5..........	19·72	19·64	20·04	20·16	20·72	21·10	19·82	20·66
6..........	19·81	19·91	19·49	20·38	20·80	21·06	20·12	20·68
7..........	19·35	20·00	19·36	19·83	20·66	21·49	19·73	20·40
8..........	19·60	20·53	19·49	19·87	20·81	21·37	19·88	20·25
9..........	19·93	20·06	19·68	20·09	20·55	20·93	20·27	20·31
10..........	19·83	20·51	19·85	20·09	20·60	20·95	19·96	20·49
Mean........	19·71	20·18	19·69	20·04	20·72	21·17	19·95	20·33

(b) Geometrical Means.

Slide.	A.		B.		C.		D.	
	P.	Q.	P.	Q.	P.	Q.	P.	Q.
1..........	19·44	20·03	19·76	20·05	20·53	21·12	19·50	20·28
2..........	19·35	19·98	19·10	19·75	20·79	20·79	19·47	19·95
3..........	19·15	19·71	19·68	19·49	20·02	20·90	19·87	19·77
4..........	19·53	20·01	19·11	19·35	20·60	20·83	19·43	19·16
5..........	19·37	19·35	19·63	19·75	20·34	20·82	19·48	20·27
6..........	19·44	19·55	19·08	20·04	20·46	20·78	19·73	20·22
7..........	19·04	19·59	19·08	19·54	20·31	21·18	19·40	20·04
8..........	19·24	20·15	19·09	19·55	20·55	21·06	19·54	19·93
9..........	19·58	19·68	19·28	19·69	20·26	20·67	19·84	19·90
10..........	19·50	20·08	19·53	19·74	20·33	20·58	19·57	20·13
Mean........	19·36	19·81	19·33	19·69	20·39	20·87	19·58	19·96

The analyses of the ordinary and logarithmic variances do not differ materially and, in fact agree very closely on the results of the significance tests. When the two types of variance are considered it should be realised that the ordinary standard deviation is measured in units of observation while the corresponding logarithmic coefficient is a measure of relative variability. When this coefficient is multiplied by two it has been termed the coefficient of relative variability and is, to some extent comparable with the ordinary coefficient of variability. These data are given in Table IV (*a*) and (*b*) respectively.

TABLE IV.

Coefficients of Variability.

(*a*) *Ordinary Values.*

Slide.	A.		B.		C.		D.	
	P.	Q.	P.	Q.	P.	Q.	P.	Q.
1..........	18·95	18·94	19·14	17·95	16·54	16·01	19·11	18·05
2..........	19·93	17·65	19·05	19·23	16·78	17·24	16·70	18·22
3..........	18·93	19·98	18·02	18·44	16·83	15·98	20·13	19·07
4..........	19·48	20·18	19·12	17·45	16·39	18·97	20·22	19·09
5..........	19·18	17·30	20·48	20·47	19·35	16·42	18·74	19·82
6..........	19·60	19·38	20·94	18·47	18·43	16·47	20·57	21·43
7..........	18·19	20·62	17·30	17·41	18·63	17·18	18·65	19·13
8..........	19·52	19·60	20·48	18·31	16·04	17·31	18·87	17·94
9..........	18·98	19·81	20·89	20·35	17·07	16·05	20·83	20·37
10..........	18·38	21·55	18·33	18·86	16·26	19·72	20·01	19·11
Mean........	19·10	19·61	19·37	18·73	17·28	17·08	19·36	19·30

(*b*) *Logarithmic values.*

Slide.	A.		B.		C.		D.	
	P.	Q.	P.	Q.	P.	Q.	P.	Q.
1..........	18·79	18·77	18·97	17·81	16·43	15·91	18·94	17·91
2..........	19·69	17·51	18·89	19·06	16·66	17·11	16·54	18·07
3..........	18·79	19·79	17·87	18·28	16·72	15·88	19·93	18·90
4..........	19·16	19·97	18·95	16·64	16·28	18·63	20·02	18·92
5..........	19·00	17·18	20·27	20·25	19·17	16·32	18·58	19·63
6..........	19·42	19·21	20·72	18·27	18·28	16·36	19·77	21·19
7..........	18·04	20·40	17·17	17·29	18·46	17·06	18·49	18·96
8..........	19·33	19·42	20·27	18·16	15·94	17·18	18·71	17·80
9..........	18·17	19·62	20·18	20·14	16·95	15·95	20·60	20·17
10..........	18·04	20·62	18·18	18·70	16·15	18·96	19·81	18·94
Mean........	18·84	19·31	19·18	18·49	17·14	17·26	18·91	19·08

(*c*) *Difference between Treatments.*

It was made clear in the description of the experimental layout that the difference in the treatments of the fibres before the actual preparation of the slides constituted a difference in the fibre populations sampled. The treatment means, although belonging to the same small staple do not, therefore represent identical fragment populations. This fact is further considered and illustrated by Table V which presents the analysis of variance between treatments, between slides, within treatments, and within slides for observers and methods of analysis separately.

Analysis of Variance between Treatments.

(a) Ordinary Values.

Variance.	D.F.	Observers.	
		P.	Q.
Between Treatments	3	580·024	649·436
Within Treatments	36	14·468	18·564
Between Slides	39	—	—
Within Slides	9,960	14·633	15·020
TOTAL	9,999	—	—

(b) Logarithmic Values.

Variance.	D.F.	P.	Q.
Between Treatments	3	0·65213	0·70051
Within Treatments	36	0·03024	0·04295
Between Slides	39	—	—
Within Slides	9,960	0·03565	0·03534
TOTAL	9,999	—	—

The estimates of variance between treatment means is considerably higher than the other two estimates and there can be no doubt about the existence of real differences between them. These mean values are given at the bottom of the columns in Table IV *(a)* and *(b)* each being the result obtained from 2,500 observations.

The individual differences between the arithmetical and logarithmic values of the treatments means are further analysed in Table VI *(a)* and *(b)* respectively. Significant differences are printed in italics while black type denotes that the difference is highly significant. Thus it is seen that the means for Treatment C are highly significantly greater than those for the other treatments. The differences between the values for Treatments A and B are insignificant. The means of Treatment D occupy an intermediate position being less than C and greater than A and B. In the case of observer Q the difference between D and A is not quite significant.

The difference between the treatment means is adequately explained by variations in diameter along the length of the fibres composing the original staple. The relatively high value for Treatment C is probably due to the presence of a region of greater average

TABLE VI.

Differences between Treatment Means (in μ).

(a) Arithmetical Means.

		C.	D.	A.	B.
C.	P................	—	**0·769**	**1·001**	**1·002**
	Q................	—	**0·843**	**0·990**	**1·391**
D.	P................	—	—	*0·232*	*0·253*
	Q................	—	—	0·147	**0·295**
A.	P................	—	—	—	0·021
	Q................	—	—	—	0·148

(b) Geometrical Means.

		C.	D.	A.	B.
C.	P................	—	**0·81**	**1·03**	**1·06**
	Q................	—	**0·91**	**1·06**	**1·18**
D.	P................	—	—	*0·22*	*0·25*
	Q................	—	—	0·15	*0·27*
A.	P................	—	—	—	0·03
	Q................	—	—	—	0·12

thickness towards the middle of the staple, since this treatment represents a single transverse cut. The lack of a real difference between the values for A and B indicates that the changes in diameter along the length of the staple were proportionately represented by the three transverse cuts of Treatment B. Under normal conditions no real difference between A and D as regards mean fibre diameter was expected and the slightly higher mean for Treatment D is probably due to the removal of relatively narrow zones from the tip and base of the staple during the earlier treatments. These portions would ordinarily be included but in the present study this could not be done for treatment D owing to the exigencies of the special handling of the material during the process of preparation described earlier in this paper.

It is interesting to note from Table IV that the variance coefficients for Treatment C are less than the others. This corresponds to the fact that variations along the length of the staple were excluded by this treatment. Taking 17·2 per cent. and 19·0 per cent. as the coefficients of relative variability for Treatment C and the average for the other three respectively, it is found that the coefficient of relative variability *within* fibres is approximately 2·55 per cent. Within treatments the coefficients of variability estimated from different slides are in good agreement.

The Difference between Observers.

By considering the treatment means in Table III there is also, apart from the difference between treatments, an obvious difference between the corresponding means for the two observers. These differences are shown in Table VII (*a*) and (*b*). The treatment means for observer Q are consistently higher than those for P with an average difference between the arithmetical means of $0 \cdot 413 \pm 0 \cdot 0244$ μ which is approximately 17 times its standard error. For the geometrical means the average difference is $0 \cdot 42$ μ, while the average difference between the natural logarithms of the geometrical means is $0 \cdot 0216 \pm 0 \cdot 002664$, i.e. about $8 \cdot 1$ times its standard error. (The standard errors are obtained from the variance within slides in Table V by the formula,

$$\text{S.E of difference} = \sqrt{\frac{S_P^2}{n_1} + \frac{S_Q^2}{n_2}}$$

where S_P^2 and S_Q^2 are the respective variances for the two observers P and Q and $n_1 = n_2 = 10{,}000$).

Since different microscopes were used by the two observers it was decided to include some further observations in which the personal and microscopic differences were separated, however unlikely a microscopic difference appeared. For this purpose the ten slides of Treatment A were chosen and divided into two random groups of five. One group was alotted to each microscope and all the slides were read by each observer on the respective instruments. The observed mean values, both arithmetical and geometrical are shown in Table VIII, (*a*) and (*b*) respectively, where the two microscopes are denoted by M_1 and M_2. An analysis of variance is given in Table IX (*a*) and (*b*) for the ordinary and logarithmic values respectively. There is obviously no indication of a difference between microscopes for either of the two observers. The difference between observers remained unaltered and in fact, was remarkably constant throughout all the observations.

TABLE VII.

Mean differences between Observers.

(*a*) *Arithmetical means.*

Observer.	Treatment.				Mean.
	A.	B.	C.	D.	
P................	19·71	19·69	20·72	19·95	20·02
Q................	20·18	20·04	21·17	20·33	20·43
Difference.........	0·47	0·35	0·45	0·38	0·41

(*b*) *Geometrical Means.*

Observer.	Treatment.				Mean.
	A.	B.	C.	D.	
P................	19·36	19·33	20·39	19·58	19·66
Q................	19·81	19·69	20·87	19·96	20·08
Difference.........	0·45	0·36	0·48	0·38	0·42
Ratio Q/P........	1·0232	1·0186	1·0235	1·0194	1·0219

TABLE VIII. (All values are in μ).

Slide means for the two microscopes and observers.
(Treatment A. New Series.)

(*a*) *Arithmetical means.*

Microscope.	Slide.	Observer.		
		P.	Q.	Q.–P.
M_1..........................	A_1.........	19·53	20·06	—
	A_3.........	19·45	19·60	—
	A_8.........	20·07	20·09	—
	A_9.........	19·88	19·76	—
	A_{10}.........	19·69	20·64	—
	Mean........	19·72	20·03	0·31
M_2..........................	A_2.........	19·70	20·16	—
	A_4.........	19·85	20·53	—
	A_5.........	19·86	20·13	—
	A_6.........	19·74	19·88	—
	A_7.........	20·29	20·12	—
	Mean........	19·89	20·16	0·27
	General Mean	19·81	20·10	0·29
M_1—M_2.....................	—	−0·17	−0·13	—

(b) Geometrical Means.

Microscope.	Slide.	Observer.		
		P.	Q.	Q.–P.
M_1	A_1	19·17	19·75	—
	A_3	19·51	19·27	—
	A_8	19·68	19·78	—
	A_9	19·45	19·45	—
	A_{10}	19·35	20·27	—
	Mean	19·50	19·78	0·28
M_2	A_2	19·33	19·82	—
	A_4	19·43	20·19	—
	A_5	19·53	19·64	—
	A_6	19·34	19·52	—
	A_7	19·90	19·77	—
	Mean	19·41	19·75	0·34
	General Mean	19·44	19·76	0·32
M_1—M_2	—	0·07	0·05	—

TABLE IX. *Analysis of Variance.*
Comparison of Microscopes. (Treatment A. New Series.)
(a) Ordinary Values.

Variance.	D.F.	Mean Squares.	
		P.	Q.
Between Microscopes	1	16·800	11·900
Within Microscopes	8	14·970	26·568
Between Slides	9	15·173	24·938
Within Slides	2,490	15·417	14·566
TOTAL	2,499	15·471	14·601

(b) Logarithmic Values.

Variance.	D.F.	P.	Q.
Between Microscopes	1	0·00931	0·01095
Within Microscopes	8	0·02994	0·06683
Between Slides	9	0·02765	0·06062
Within Slides	2,490	0·03850	0·03399
TOTAL	2,499	0·03726	0·03439

Discussion.

In considering the methods of cutting employed in this investigation there is much to recommend Treatments C and D on statistical grounds. In these methods every fragment represents a different fibre whereas in Treatments A and B several fragments from the same fibre may be included in the same set of readings. This possibility is greater in A than in B since only three fragments per fibre could be included by the latter method, whereas in the former as many fragments as there were cuttings over the whole length of the fibres could be included. In view of the variations in thickness along the length of fibres it can hardly be determined how many cuttings per fibre and at which places would adequately represent the average fibre diameter of the sample. These objections are eliminated in Treatment D which contains fragments ranging over the whole length of the staple and at the same time only one fragment per fibre. Treatment D however requires great care in the spreading of the wool in an even layer thus to ensure that more or less the same number of fibres are cut at each point along the diagonal. By an uneven spread the number of fibres cut at different distances from the base will vary and the fragments will not properly represent the variations in thickness along the length of the fibre.

Treatment C presents the average diameter of the fibres at a particular stage of growth only and does not allow for variation in thickness along the length of the staple. This method will therefore give a smaller coefficient of variability, as the data shows, but the mean diameter will depend on the position of the transverse cut. For comparative purpose this method is the most useful provided there is no doubt about the position of the transverse line along which the sample is to be cut. Furthermore in view of the constant relation between the standard deviation and the mean fibre diameter for a particular sheep the genetical coefficient of variability is best obtained by Treatment C. For the determination of this coefficient or variability the particular line of cutting is probably unimportant.

The data reveals a rather less satisfactory distribution of fragments in the case of slides from Treatment D which probably indicates that it is more difficult to obtain uniform mixtures when fragments are obtained by oblique cuttings. In this treatment it was certainly more difficult to secure cuttings as equal in length and as short as in the other treatments and good care should be taken in this respect. Length of fragment and evenness of length are probably the two most important factors in the preparation of a good mixture. The distribution of fragments over the slides of the other treatments was fairly satisfactory. Similarly the agreement between slides from the same mixture of fibre fragment was within the limits of random sampling in that the variation between such slides was in no case greater than the variation within slides.

The personal difference between observers was the only feature in the process of slide measurement which proved to be more serious than was originally anticipated.

The one observer obtained consistently lower mean values than the other. This feature is very disconcerting since it suggests that diameter measurements may only be regarded as strictly comparable when taken by the same observer. Even though the observed difference was an extreme one in our experience, the possibility of its existence in other cases introduces an element of doubt in all comparisons where different observers are concerned. Observers from the same institute may be standardised but it is hardly possible to consider the standardisation of observers from different institutes and countries.

The methods of analysis based on the assumptions of normal and logarithmic distribution of fibre diameter measurements did not materially affect the results. The comparisons of variances seem to agree as regards significance when a probability level of 5 per cent. is taken. When the probability deviates considerably from the 5 per cent. level there appears to be a large difference between the two corresponding values of the respective analyses but this does not alter the conclusions since significance is judged by only considering the critical levels of 5 per cent. and 1 per cent. probability. It is suggested that the 5 per cent. probability level should be used for significance tests when the normal theory is applied. This level apparently agrees with the same level in the logarithmic analysis and there can be no doubt that the latter provides a more correct hypothetical distribution function for fibre diameter measurements.

It is to be noted that certain differences exist between the methods of slide preparation which we have adopted in this study and those advocated by Wildman and Daniels (1937). With us the fluid used for mixing was ether whereas Wildman uses cedar wood oil which is also his mountant. Ether may contain such impurities as water and alcohol which might affect the results by causing swelling of the fibres. While this point most certainly requires further investigation it does not affect the present study since the cuttings from the samples were mixed in the same sample of ether. Preliminary investigations with various samples of ether including some which were completely dry as well as others containing known volumes of water have not so far revealed any significant effect due to this factor. Another less important difference between Wildman's method and ours was in the final mountant used which in our case was " Euparal ". The permanency of such a slide permits check measurements to be made when desired, since storage for long periods is possible.

This study has indicated that for samples of minimal size, (that is about 1 gram in weight) the measurement of a single well-prepared slide provides a satisfactory estimate of the mean fibre diameter. However, during the routine preparation of slides, cases inevitably occur where the sample it not adequately represented. In view of this we recommend the making of duplicate slides from each sample. Such slides will serve as a check on each other when required in doubtful cases.

As a check on the uniformity of dispersion of the fibre fragments on the slide we have found it useful to record the readings as successive groups of twenty-five, and to include the variance between such group means in the analysis of variance. Unsatisfactory slides may frequently be detected as a result of this procedure.

The present study is considered as a useful investigation preliminary to wider studies on the problems of sampling wool either in bulk or on the living animal.

SUMMARY AND CONCLUSIONS.

1. A series of fibre diameter determinations was made on a small staple of medium Merino wool, for the purpose of examining the representativeness of such measurements.

2. Four different methods of preparing the material were adopted and ten slides made from each.

3. Two observers measured 250 fibre fragments on each of the forty slides thus obtained.

4. The advantages of each method of treatment are separately discussed.

5. An unexplained but highly consistent difference in the measurements made by the two observers was noted and is regarded as requiring further examination.

6. For each observer separately the results as regards variation both between and within slides for each treatment showed the consistency required by statistical theory.

7. Statistical analysis of the results according to both the normal and logarithmic theories of distribution showed good agreement at the 1 per cent. and 5 per cent. levels of probability. Best agreement was demonstrated at the 5 per cent. level.

8. It was concluded that the measurement of a single well prepared slide will provide an adequate estimate of the mean fibre diameter of a wool sample of the size examined in this study. It is recommended however that in routine analyses permanent duplicate slides be prepared and that provision be made in recording the results for calculating the variance between successive groups of readings within the slides.

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