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# BIOLOGICAL ASPECTS OF THE SULPHUR CONTENT OF ROMNEY WOOL

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## SUMMARY

There was a seasonal rhythm in the sulphur content of wool of free-grazing Romney ewes. The sulphur content was lowest when wool production was highest and vice-versa, so that within wide limits it is probable that the availability of sulphur is not a limiting factor in wool production.

The sulphur content of lambs' wool was low, increasing to the two-tooth fleece thereafter remaining constant to 5 years of age. There was considerable variation in the sulphur content both between years and between individual sheep.

There were variations in sulphur content over the fleece which were not due to weathering or medullation. Wool may be severely weathered with no apparent effect on the percentage of sulphur.

The sulphur content of fibres of secondary follicle origin was higher than that of those from primary follicles.

OVER THE PAST DECADE there has been an increasing invasion of traditional wool markets by fabrics made of synthetic fibres. Many of these fabrics are made attractive to the customer by the incorporation of special features such as permanent pleating or "easy-care" properties. These properties may now be incorporated in wool fabrics by chemical treatments which depend for their effect upon the reactivity of the disulphide bonds in the wool.

Barritt and King (1929) found that the sulphur content of a wide range of wools varied from 3.03 to 4.13%. The majority of this sulphur was present as the amino acid cystine which comprises about 12% by weight of the fibre. While the number and high reactivity of the disulphide bonds have a very marked effect on the chemical and physical properties of a wool, few data are available on wool sulphur levels in relation to the soils and plants on which sheep graze, or on the subsequent processing performance of the wool.

## MATERIALS AND METHODS

Unless otherwise stated, all the wool samples tested were obtained from the Department of Agriculture's Invermay Re-

search Station, which is situated at the head of the Taieri plain. The sheep were free grazing throughout the year with some supplementary swedes and hay during the winter months. Pastures were topdressed annually with 2 to 3 cwt of superphosphate per acre during late February or March.

Details of the sheep and sampling positions which were used in these trials are given in the appropriate sections of the results.

#### SULPHUR ANALYSIS

Greasy wool samples were cleaned by extraction with petroleum ether (b.p. 58° C) in a soxhlet, followed by washing in distilled water. Sulphur determinations were carried out on duplicate 0.2 to 0.3 g samples by wet digestion with a nitric-perchloric acid mixture and estimation of the sulphur as barium sulphate using the method of Myers (1959). Sulphur contents were expressed as a percentage of the oven-dry weight of the sample.

Disulphide and thiol sulphur were determined on 0.6 g samples refluxed for 3 hours in an oil bath at 140° C with 20 ml of 5 N hydrochloric acid. The sulphur in the hydrolysate was measured using the phosphotungstic acid reagent of Folin and Marenzi (1929).

#### RESULTS

The results obtained, together with a short review of the literature and data on the samples used are presented in four sections. These are concerned with variations in sulphur content with season and wool production, age, sampling site and weathering, and fibre length and fibre type.

##### 1. SEASON AND WOOL PRODUCTION

Barritt and King (1929) and Bonsma (1931) found considerable variation in the sulphur content along the staple, with a high sulphur percentage in the tip. Bonsma related high sulphur contents to periods when the sheep were on highly nutritious feed. Studies on seasonal changes in wool sulphur are complicated by environmental effects as weathering alters both the physical and chemical properties of the fibre. There is a reduction in the cystine content and an increase in cysteic acid (Louw, 1960).

McMahon and Speakman (1939) reported a decrease in total sulphur as a result of natural weathering, while Barritt and King (1929) found a similar result with wool exposed to ultra-violet light.

Samples were clipped from the same mid-side sampling position on 6 ewes at 4-monthly intervals, on about the 20th of the month, commencing in March 1956 when the ewes were two-tooths. A sample of the full year's growth was taken immediately prior to shearing in November, from the mid-side position on the other side of each ewe.

Table 1 shows the mean sulphur percentages of these 6 ewes over 4 successive years together with the daily production of clean oven-dry wool per cm<sup>2</sup> for each period. Approximately half of the annual wool production of these ewes was grown in the summer 4-month period from November to March, while wool production was lowest in the late winter-spring period from July to November.

The percentage sulphur was 0.32, 0.21, 0.08 and 0.40% lower in successive years in the high wool producing period (November-March) than in the low producing period (July-November). Only in 1958 was the percentage sulphur of the November-March samples as high as that of the following March-July samples.

TABLE 1: THE MEAN WOOL SULPHUR CONTENT AND WOOL PRODUCTION OF 6 ROMNEY EWES.

<i>Year</i>	<i>Sample Period</i>	<i>Sulphur Percentage</i>	<i>Clean Dry Wool (mg/cm<sup>2</sup>/day)</i>
1956	Nov-Mar	3.07	1.23
	Mar-Jul	3.31	0.68
	Jul-Nov	3.39	0.56
1957	Nov-Mar	3.29	1.20
	Mar-Jul	3.43	0.80
	Jul-Nov	3.50	0.54
1958	Nov-Mar	3.41	1.26
	Mar-Jul	3.37	0.63
	Jul-Nov	3.49	0.50
1959	Nov-Mar	3.04	0.95
	Mar-Jul	3.08	0.53
	Jul-Nov	3.44	0.52
1956	Full year	3.18	0.83
1957	Full year	3.37	0.83
1958	Full year	3.40	0.79
1959	Full year	3.14	0.68

The sulphur percentages of the individual ewes showed that over the 4-year period 2 ewes consistently produced wool of low sulphur content (mean 3.16 and 3.23%) compared with the wool of 2 other ewes (3.30 and 3.36%). While the number of sheep in this group was small, the results did not indicate any effect of pregnancy and lactation on the percentage of sulphur, nor was there any apparent relationship between the percentage of sulphur and the total sulphur in the fleece. The average sulphur content of the ewe wool of about 3.3% is somewhat lower than many reported values.

## 2. AGE

The mean sulphur content of the full year samples of the 6 ewes, given in Table 1, increased from 3.18% in the two-tooth fleece to 3.37 and 3.40% in successive years, decreasing in the 5-year fleece to 3.14%. These variations are no doubt principally due to the effects of season and age.

Lloyd and Marriott (1933) showed that medulla is free from sulphur, so that some variation in sulphur content might be expected as Goot (1945b) found that medullation was lowest in the two-tooth fleece and thereafter remained approximately constant with increasing age. However, any effects of medullation on sulphur content would be small, as even very heavily medullated Scottish Blackface wool contains only about 10% by weight of medulla (Ross and Speakman, 1957).

To reduce differences due to season, 6 ewes of 4 different age groups which had been running together since shearing in November 1958 were sampled on the mid-side position in July 1959. On the same day a group of hoggets which had been grazing similar pasture were also sampled. The results of sulphur analyses on these samples, together with those of 2 groups of lambs sampled on the mid-side some years earlier, are given in Table 2.

TABLE 2: AGE AND SULPHUR CONTENT.

Age at Sampling Years - Months	No.	Sulphur Percentage	C. of V. %
2	12	2.75	3.1
5	12	2.76	3.4
10	6	2.80	3.3
1 — 10	6	2.96	4.6
2 — 10	6	3.00	8.1
3 — 10	6	3.03	2.0
4 — 10	6	3.00	2.6

The sulphur content was lowest in the lamb and hogget samples, and was approximately constant in the 2- to 5-year-old ewes which were grazing together. Similar low sulphur contents were found for a group of 6 lamb (4-6 month) samples obtained from different parts of New Zealand. The sulphur contents of these samples ranged from 2.68 to 3.12% with a mean of 2.88%. Other lambs' wool sulphur contents found in another year at Invermay are given in Table 4, and were also low.

The sulphur content of 3.00% for the adult wool samples appears to be very low, but is consistent with the corresponding 4-monthly sample results given in Table 1.

### 3. SAMPLING SITE AND WEATHERING

Bonsma and Joubert (1934) studied the variation in sulphur content over the body of both free-grazing and outdoor pen-fed Merino sheep. In both groups they found a gradient in sulphur content which increased from fore to hindquarters paralleling a similar gradient in fibre diameter. The sulphur content might be expected to vary over the fleece owing to:

- (1) Variation in the amount of sulphur-free medulla (Goot, 1945a).
- (2) Variation in the ratio of fibres of primary and secondary follicle origin which may have different sulphur contents (Ripa and Speakman, 1951).
- (3) Weathering effects varying both in degree and distance down the staple depending on the position on the ewe.

Samples of a full year's growth were clipped from 6 positions on 5 full-mouth Romney ewes. Details of the sheep and the sampling positions are given by Myers and Ross (1959) and Ross (1961).

Table 3 shows the mean percentage of sulphur for each of the sampling sites. The sulphur content was highest on the neck and only slightly lower on the back, withers, and side, whereas the shoulder and britch samples contained appreciably less sulphur. While none of these samples contained many medullated fibres, the britch samples would have the most and the shoulder and neck samples the least (Goot 1945a). Thus medullation is not the cause of the low sulphur content of the shoulder samples.

The back and withers wool of these ewes was severely weathered almost down to the skin level, but even so the sulphur percentages were high. A measure of the degree of weathering was obtained by determining the alkali solubility of these samples (Smith and Harris, 1936). Duplicate 0.6 g samples were treated

TABLE 3: SULPHUR AND ALKALI SOLUBILITY VARIATION OVER THE FLEECE OF 5 ROMNEY EWES.

Sampling Site	Sulphur %	Alkali Solubility %	S-S Sulphur (as a % of total sulphur) %	SH Sulphur %
Neck	3.38	8.4	90.8	1.5
Withers	3.35	17.5	86.7	3.1
Back	3.35	20.9	81.3	2.8
Side	3.31	10.5	90.4	1.6
Shoulder	3.22	8.5	86.7	1.9
Britch	3.21	9.7	87.9	1.1

with 0.1 N sodium hydroxide at 65° C for 1 hour. As shown in Table 3, the alkali solubility of the back and withers samples was about double that of wool from the other sampling sites. Either the sulphur content of the virgin withers and back wool was higher than that of the other sampling sites, or weathering does not result in a decrease in the percentage of sulphur.

Also shown in Table 3 are the amounts of cystine and cysteine sulphur expressed as a percentage of the total sulphur. The percentage of disulphide sulphur was somewhat variable both between sheep and positions. There was about 91% of disulphide sulphur in the neck and side samples and only 81% in the back samples, while the withers samples had a similar percentage to the shoulder and britch samples. The weathered back and withers samples had about twice the thiol sulphur content of the other sampling sites.

Further evidence that weathering has little effect upon the percentage of sulphur may be derived from the sulphur contents of the 4-monthly and full year samples given in Table 1. Over the 4 years the mean sulphur contents of the full year samples were only 0.01 to 0.02% lower than the sulphur contents calculated from the 4-monthly samples and the corresponding wool growth rate data. The full year samples were exposed to the effects of weathering for a 12-month period, whereas the November-March samples were exposed for only 4 months and the later growing March-July and July-November samples were to a large extent protected by the longer wool surrounding the clipped area.

#### 4. FIBRE LENGTH AND FIBRE TYPE

Burley and Speakman (1953) and le Roux and Speakman (1957) have shown that, compared with fibres of secondary follicle origin, fibres of primary follicle origin from the same

staple are less plastic, swell less in water and formic acid, contain a greater proportion of less accessible crystalline material (Burley, Nicholls and Speakman, 1955), less tyrosine, and require more work to stretch them by 30%. These results suggest that fibres of primary follicle origin would have a greater number of cystine cross-linkages and consequently a higher sulphur content than fibres from secondary follicles. While such a relationship was found by Ripa and Speakman (1951) no consistent relationship between fibre type and sulphur content was found in subsequent investigations (Burley *et al.*, 1955; le Roux and Speakman, 1957). If such sulphur differences do occur they were too small to be accurately measured by micro-sulphur determinations on the small weights of fibres available in each fibre type group which had been measured for plasticity.

Fibres from the different types of follicles commence growth at different times, the last formed fibres, the histerotrichs, appearing above the skin level at the time of birth and for some months thereafter. As the first formed fibres are also the largest and fastest growing and vice versa, the sorting of Romney lambs' wool into groups on the basis of fibre length will give a division related to fibre type. While there may be considerable overlapping of fibre-types in length groups in the centre of the length range found for any wool, the longest group will be composed of fibres largely of primary follicle origin, and the shortest group will be composed of fibres of secondary follicle origin only. By the use of this method sufficiently large samples can be obtained for accurate sulphur determinations.

Mid-side samples from 7 Romney lambs, 5 to 6 months of age were placed with their butts level on a Zweigle comb-sorter, and fibres extracted from the tip-ends in 1 cm length groups. Duplicate sulphur analyses were made on the cleaned wool of each length group. Where necessary adjacent length groups were combined to give sufficient weight of wool.

The sulphur contents of the comb-sorted length groups of the 7 lamb samples are given in Table 4. As would be expected from the results given in Table 2, the sulphur contents which ranged from 2.55 to 2.92% are low compared with wool from adult sheep. In samples 1 to 5 there was a decrease in sulphur content as fibre length increased, while in samples 6 and 7 there was little change in sulphur content with increasing fibre length. That is, the longer fibres of primary follicle origin which are of low plasticity have a lower sulphur content than the shorter fibres from secondary follicles which have a higher plasticity.



TABLE 4: THE SULPHUR CONTENT OF COMB-SORTED LENGTH GROUPS OF 7 ROMNEY LAMB SAMPLES.

Sample	1	2	3	4	5	6	7
Length Group cm	Sulphur Percentage						
0.5-x°	2.92	2.77	2.83	2.92	2.85	2.72	2.57
5.5	—	—	2.81	—	—	—	—
6.5	—	—	2.79	—	—	—	—
7.5	2.84	2.66	2.71	2.83	—	2.71	2.56
8.5	2.81	2.68	2.70	2.80	—	—	2.60
9.5	2.81	2.63	—	2.80	2.80	2.73	2.60
10.5	2.67	2.55	—	2.70	2.78	2.71	2.58
11.5	—	—	—	2.70	2.77	2.71	—
12.5	—	—	—	—	2.73	2.69	—

°The length group 1 cm shorter than the next group measured.

Differences in sulphur content between length groups within a sample may to some extent be due to seasonal changes in sulphur content, weathering, or to differences in the proportions of medullated fibres. If the sulphur content increased in the first few months after birth, then the shorter length groups would be expected to have a higher sulphur content than the longer length groups which contain wool of pre-natal growth. Secondly, the medulla in these samples was confined almost completely to the pre-natal tip. While the results given earlier in this paper indicated that the percentage of sulphur was not decreased by weathering, it is possible that there was a decrease in the total sulphur content of the wool. If this is so, the longer fibre-groups with a long pre-natal tip would be weathered to a much greater degree than the shorter fibre length groups.

To check if these differences were due to changes in the sulphur content of the pre- and post-natal regions of the fibres, staples of lambs 1, 2 and 3 were comb-sorted into length groups. These groups, with the exception of the very short post-natally grown histerotrichs, were cut into tip and butt regions at a level estimated to be about 1 to 2 weeks below the birth point. Some grouping of the tips was necessary to give sufficient weight for sulphur analyses.

Figure 1 shows diagrammatically the sulphur content of the tips and butts of sample 1. Similar gradients in sulphur content were found in samples 2 and 3. In the predominantly pre-natal region, the first formed fibres with the longest tips had a lower sulphur content than the later formed fibres with the shorter tips.

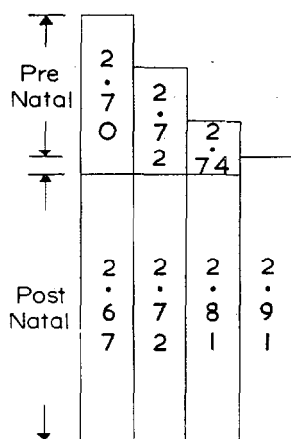


Fig. 1: The sulphur content of a sample of a Romney lamb's wool, in which the comb-sorted fibre length bundles were cut a little below the birth level.

This decrease may be due to medullation which is higher in the tips of the first formed fibres. The wool in the post-natal region would be grown over a similar time, as the very short histerotrichs were not included in the shortest group. Post-natally the fibres were almost completely free of medullation, and would largely have been protected from any weathering effects. There was a marked increase in the sulphur content of the post-natal region in the later formed fibres of secondary follicle origin. It was between the butt ends of fibres of different fibre types that Speakman and co-workers found differences in the chemical and physical properties reviewed earlier. The sulphur contents reported in this paper are, however, the reverse of what might have been expected from their results.

The relationship between fibre length and sulphur content was also measured on one woolly hogget and on 4 mature Romney wool samples of unknown origin. These samples were all free of medulla. The short fibres, 1 to 5 cm, of the hogget sample had a high sulphur content of 2.88%, decreasing to 2.79% for the combined fibres from 6 to 16 cm and to 2.74% for the fibres longer than 20 cm. The mature Romney samples also showed a small decrease with increasing fibre length. The difference in sulphur content between the longest and shortest groups was 0.09, 0.05, 0.05, and 0.01% for the 4 samples.

#### DISCUSSION

A definite seasonal rhythm was found in the sulphur content of the wool of free-grazing New Zealand Romney ewes. The sulphur content was lowest when wool production was highest

and vice versa. The low sulphur content associated with high wool production suggests that within wide limits availability of sulphur is probably not a limiting factor in wool production. This may be the reason why, in the numerous trials in which elemental sulphur, sulphate, cystine, cysteine, or methionine were fed to sheep, no increase in wool growth was found. The low sulphur content of the summer grown wool is probably an indication of a lower sulphur intake by the sheep in relation to wool production during this period, although the proportion utilized during this high wool growth period may, however, be higher.

Bonsma (1931) found high sulphur contents in the tips of staples which he related to periods when the sheep were grazing on highly nutritious feed. He found little variation in the sulphur content along the staple in 2 sheep which were fed on a constant diet throughout the year.

If the results given in Table 1 are considered in terms of the sulphur content of the tips and butts of staples, then the tip wool contains on the average 0.25% less sulphur than the butt wool. Such differences have usually been interpreted to mean that the sulphur content of the tip wool has decreased owing to weathering. The majority of sheep at Invermay are pre-lamb-shorn. If these ewes had been shorn in July 1958 and 1959, instead of in November, the sulphur content of the tip wool would have been about 0.4% higher than that of the butt wool. The distribution of sulphur-free medulla is not constant along a staple, but is usually higher in the tip. This would result in a slightly lower sulphur content. Considerably more information than the sulphur content of the tip and the butt of a staple is therefore necessary before any difference in the percentage of sulphur can be attributed to weathering.

The percentage of sulphur in severely weathered back and withers samples, which had alkali solubilities twice those of samples taken from other regions of the fleece, was on the average only 0.03% lower than the neck samples, and 0.04, 0.13 and 0.14% higher than the sulphur contents of the side, shoulder and britch samples respectively. This range of sulphur contents over the fleece of 0.2%, which is not due either to weathering or to medullation, is sufficiently large as to necessitate care in sampling. Either the sulphur content was higher in the back and withers samples before weathering, or weathering does not decrease the percentage of sulphur. Weathering does, however, result in a decrease in the cystine content and an increase in

the percentage of cysteine and other sulphur containing compounds. Changes in the total sulphur and the percentage of sulphur will depend upon the amount of sulphur and non-sulphur containing decomposition products leached from the fibre as a result of weathering.

The sulphur content of Romney lambs' wool is low, 2.5 to 2.9%, compared with that of adult sheep, 3.0 to 3.6%, running on the same property. The sulphur content increased in the hogget fleece and was steady in ewes from 2 to 5 years of age. The percentage of sulphur in adult fleeces in different years, however, may vary considerably. There was an average of approximately 100 g of sulphur in the fleeces of mature Romney ewes.

The marked difference in sulphur content between lambs' and adult wool may be the basic reason for the differences in properties of these wools and the different end uses to which they are put.

In the small groups of sheep which have been tested, the range of sulphur contents of mid-side samples has been of the order of 0.2 to 0.3%. Over the 4 years of the 4-monthly sampling trial some ewes produced wool with consistently low sulphur contents compared with other ewes. This individuality of sulphur content may be a function of appetite or efficiency of utilization although there was no relationship between percentage of sulphur and total wool production or total sulphur in the wool produced by these ewes annually. It would be of interest to determine the heritability of the sulphur content of wool. Such a project would provide material on which to study the importance of sulphur on the physical and chemical characteristics of the fibres and on the processing performance of the wool.

Contrary to expectations based on the physical and chemical properties of individual fibres, the sulphur content of fibres of primary follicle origin was lower than that of fibres derived from secondary follicles. The distribution of disulphide cross-linkages between the crystalline and amorphous regions of the fibre, rather than the total sulphur content, may be the principal factor governing these physical and chemical properties. The fibre mass, produced in a given time by a primary follicle, will be greater than that produced by a secondary follicle, so that per unit of time the same, or even a greater amount of total sulphur may be incorporated in the fibre by a primary than by a secondary follicle. Sulphur studies therefore may be useful as a research tool in the study of follicle competition and follicle efficiency.

The percentage of sulphur in a wool at any given time will depend principally upon:

- (1) The sulphur content of the feed supply.
- (2) The amount of feed ingested.
- (3) The proportion of sulphur utilized.
- (4) The rate of wool production.
- (5) The age of the sheep.
- (6) The individuality of the sheep.
- (7) The sampling site.
- (8) The degree of medullation.
- (9) The fibre type composition of the sample.
- (10) The effects of weathering.

Melville and Sears (1953) found seasonal variations in the nitrogen, potassium, calcium, and phosphorus content of pastures. Similar variations will no doubt occur in the sulphur content. Walker, Adams and Orchiston (1955) found differences in the sulphur contents of grasses and clovers and as the proportions of these in the pasture vary through the season, so will the over-all sulphur content.

While the vital role played by the cystine cross-links in controlling the chemical and physical properties of a wool is unquestioned, nevertheless, the form in which all the sulphur occurs in the fibre, and its distribution between crystalline and amorphous regions is not fully known; nor has the chemical breakdown of cystine by weathering been fully investigated. Such information is necessary before a full understanding of the role of sulphur in wool production and processing can be made. Some preliminary studies on New Zealand Corriedale and Romney wool by Sidey (1931), indicated a positive correlation between laboratory assessed spinning quality and sulphur content.

The variations in sulphur content no doubt largely reflect variations in the cystine content, while there are probably similar variations in the other amino acids of which wool is composed. Considerable care is therefore necessary in the interpretation of the results of investigations into the physical and chemical structure of New Zealand Romney Crossbred wool.

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## DISCUSSION

Q: *Dr Ross has said that a deficiency of sulphur in feed is not reflected in sulphur content of wool. Is the reverse likely to happen?*

A: I stated that within wide limits sulphur does not appear to be a limiting factor in wool production. Nevertheless, I would expect that a prolonged deficiency of sulphur in the feed would be reflected in the sulphur content of the wool, particularly over the period when wool growth is at a maximum. The percentage of sulphur in the wool of sheep fed upon a diet rich in sulphur may be both high and relatively constant throughout the year.

Q: *Is it at all possible that sulphates could occur in wool fibre?*

A: While there is no sulphate in the newly formed wool fibre, some sulphate of external origin may be found at later stages.