THE SURFACE STRUCTURE OF WOOL AND ITS COMPONENTS REVEALED BY METAL SHADOWING

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Summary

Gold shadowing reveals considerable details of surface modifications in damaged wool fibres when viewed by transmitted light in the optical microscope. The method is more rapid and less likely to introduce artefacts than the replica techniques currently employed in electron microscopy. New evidence of the nature of the inner surface of the cuticle is also described.

I. Introduction

A number of electron microscope investigations of surface modifications in damaged wools have been described in which replica techniques were employed (Swerdlow and Seeman 1948; Makinson 1950; Mercer and Roadnight 1950; Elliot and Manogue 1952; Mercer 1953). The direct examination of fibre surfaces in the electron microscope is not possible and little detail is seen by examination with transmitted light in the optical microscope. Dempster and Williams (1946) and Scott and Wyckoff (1949) have stressed the use of gold shadowing as a method of revealing surface texture in the optical microscope and we have recently pointed out the value of this technique in studying the fine histology of wool fibres (Fraser and Rogers 1954*a*). We have now examined wool fibres subjected to a variety of chemical and mechanical treatments and find that details of surface modifications may readily be seen when the wool fibres are gold-shadowed and viewed by transmitted light in the optical microscope.

II. Preparation of Specimens

Selected samples of Merino, Crossbred, and Corriedale wools were solvent-scoured, washed in distilled water, and dried to room humidity. Small staples or individual fibres were then subjected to the following treatments and shadowed with gold at an angle of 35° in the manner already described (Fraser and Rogers 1954*a*).

Stretched wool.—Individual fibres of Corriedale 56's quality were slowly extended 50 per cent. in steam and others 100 per cent. in boiling water with a simple extensometer.

Tryptic digestion.—Corriedale wool (5 g) was incubated for 4 days at 40°C in 500 ml of 1 per cent. crude trypsin (pH 8·5) in the presence of 0·01 per cent. merthiolate as antiseptic, washed in distilled water, and air-dried.

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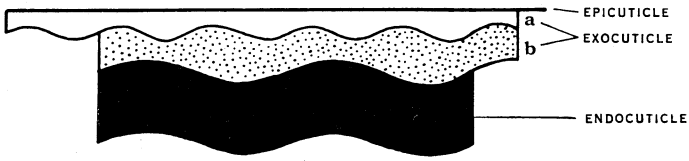
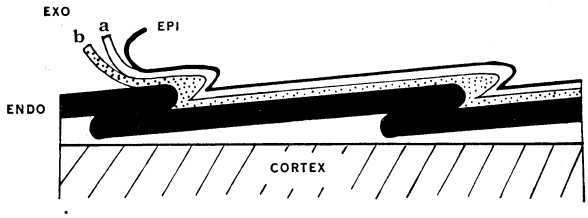
Chlorine and bromine.—Merino 64's quality wool was treated with freshly prepared saturated chlorine or bromine water in the ratio 1 g/100 ml for 15 min, rinsed twice with distilled water, shaken for 15 min with distilled water to remove epicuticle, and finally air-dried.

Sulphuric acid.—Standard commercial top of Merino 64's quality was soaked for 15 min in 10 per cent. w/v sulphuric acid, squeezed out, and dried for 1 hr in a stream of hot air at 100°C.

Cetyl sulphonic acid.—Merino 64's quality wool was incubated with 0·05M cetyl sulphonic acid (pH 2) at 65°C for 6 days, washed, treated with 0·01N ammonium hydroxide for 15 min, and air-dried. This treatment has been shown to disperse the S (alkali-susceptible) component of the bilateral cortex (Fraser, Lindley, and Rogers 1954).



(a)



(b)

Fig. 1.—(*a*) The fine structure of the surface layers of wool in transverse section (Lindberg *et al.* 1949; Lagermalm 1954). (*b*) The distribution of these layers in longitudinal section (Fraser and Rogers 1954*b*).

S-carboxymethyl wool.—Merino 64's quality wool was repeatedly reduced with 0·4M sodium thioglycollate, pH 5·6, and alkylated with 0·5 per cent. iodoacetic acid in buffer at pH 7·2, washed, and dried (O'Donnell 1954).

III. Results

Current theories of the fine structure of the surface layers of wool are summarized in Figure 1 (Lindberg *et al.* 1949; Lagermalm 1954; Fraser and Rogers 1954*b*) and the detail revealed by gold shadowing will be described in relation to these component layers.

(*a*) *Fibre Surfaces*

(i) *Extended Wool.*—Examples of the effect on the cuticle when wool fibres are extended in steam are shown in Plate 1, Figures 2, 3, 5, and 6. No typical damage for a given percentage extension was observed as the strain was localized to some extent. In general, however, the inter-scale tip distance is increased, as compared with unstretched fibres (Plate 1, Figs.1 and 4), owing to either slippage or extension of the cuticle, or both. The appearance of Merino fibres, in which the scale overlap is small, after 100 per cent. extension in steam suggests that the scale substance possesses a long-range extensibility similar to that of the cortex. At 50 per cent. extension (Plate 1, Fig. 2) the scale tips of Corriedale fibres are seen to detach from the underlying scales and protrude from the general surface of the cuticle. This feature is clearly seen in Plate 1, Figure 5, which shows the profile of such a fibre. At 100 per cent. extension (Plate 1, Figs. 3 and 6) a considerable portion of the scale becomes detached, and large flaps protrude from the surface of the fibres.

(ii) *Trypsin-treated Wool.*—It is well known that the cuticle of wool is extensively damaged by the action of crude trypsin, and Plate 2, Figure 1, shows the surface of a fibre in an advanced stage of degradation. Large areas of individual scales appear to have flaked off, giving a chipped appearance. In this particular case the epicuticle seems to have been detached although at other points on the fibre it appeared to be intact, producing a similar appearance to that noted by Mercer (1953).

(iii) *Chlorine and Bromine.*—These reagents cause considerable degradation of the outer layers of the cuticle, and Plate 2, Figure 2, shows the action of chlorine on the scale tips and folds of epicuticle which have dried down to give a "doubling" of the scale edges. In other fibres, where the epicuticle had been detached, the striations of the external scale layers were visible as reported by Swerdlow and Seeman (1948), Elliot and Manogue (1952), and Mercer (1953). The action of bromine on the scale tips is shown in Plate 2, Figure 3, where the epicuticle has been detached, and the corrugated surface of the endocuticle is revealed.

(iv) *Sulphuric Acid.*—The damage that occurs following sulphuric acid treatment is of interest as this is the reagent utilized in carbonizing. Our observations are in general agreement with those of Mercer (1953), who reported very little change in the appearance of the surface after carbonizing. Even excessive carbonizing produces only slight evidence of surface damage, the fibre of Plate 2, Figure 4, is a typical example showing only an exaggerated prominence of the scale tips and occasional chips in the scale edges.

(v) *Cetyl Sulphonic Acid.*—It will be seen from Plate 2, Figure 5, that the cuticle has been extensively damaged by this treatment and it gives the appearance of a thin membrane, presumably the epicuticle, which has dried down on

a much depleted scale structure. The longitudinal furrows are extremely prominent in this case.

(vi) *S-carboxymethyl Wool.*—Although the fibres in this instance have not been exposed to extremes of pH some surface modification has occurred (Plate 2, Fig. 6). The furrows are similar to those of the bromine-treated fibre of Plate 2, Figure 3, and probably reflect the corrugated surface of the underlying endocuticle. It is probable that this feature arose from differential changes in the outer layers of the cuticle during the reduction process.

(*b*) *The Component Layers of the Cuticle*

In addition to its value as a method for detecting surface modifications in damaged wool fibres, gold shadowing provides a method of correlating the fine structural detail observed in the electron microscope with the coarse histological features seen in the optical microscope. New evidence of the nature of the component layers of the cuticle is presented in Plate 3, Figure 1, in which the cuticle has been partially degraded by trypsin, and in Plate 3, Figures 2, 3, and 4, in which wool fibres treated with cetyl sulphonic acid have been extracted with ammonium hydroxide.

The main scale fragment in Plate 3, Figure 1, has a smooth outer surface *a*, similar to that of the fibre in Plate 2, Figure 1, and ridges corresponding presumably to the junctions of overlapping scales, are also visible. The inner surface *b* (Plate 3, Fig. 1) of the smaller scale fragment has a furrowed structure of similar periodicity to the endocuticle as depicted in Figure 1. The furrows terminate in a thickened edge which is probably associated with the ridges on the outer surface of the main scale fragment.

Further evidence of a smooth chemically resistant outer layer of the cuticle is seen in Plate 3, Figure 2, although there is some evidence of longitudinal striations in this case. Plate 3, Figure 3, shows the appearance of the inner surface of a cuticle fragment from the same preparation. Various stages of degradation are present in this fragment; at the thickest part *a* the inner surface is comparatively smooth and featureless and appears to be continuous across the inner edge of the adjacent scale. At *b* this layer presents a pock-marked appearance and between *b* and *c* longitudinal furrows similar to those of Plate 3, Figure 1, are seen. The furrowed layer between *b* and *c* is continuous across the inner scale edge but ends abruptly to reveal a thinner striated layer at *c*.

Further evidence of the fact that the inner scale edges project from the inner surface of the cuticle is seen in Plate 3, Figure 4, which depicts a cortical cell isolated from the *H* segment of the bilateral cortex of merino wool by controlled hydrolysis with cetyl sulphonic acid (Fraser, Lindley, and Rogers 1954). The transverse depressions revealed by the shadowing probably correspond to the impressions left by the inner scale edges, that is to say, the cell was located on the periphery of the cortex with its uppermost surface in contact with the inner layer of the cuticle.

IV. Discussion

(*a*) *Fibre Surfaces*

It appears, from the specimens that we have prepared, that the examination of gold-shadowed wool fibres by transmitted light in the optical microscope provides a simple and rapid method of assessing surface modification. The resolution of the optical microscope is limited to about 0·3 μ but structural detail near this limit is readily visible owing to the high contrast obtained. Although the resolving power of the electron microscope is very much greater, the replica technique employed in the preparation of specimens is both tedious and liable to produce artefacts.

The depth of focus of a high numerical aperture, short-focus objective is extremely small and it is difficult therefore to record photographically the details of curved fibre surfaces. This is particularly noticeable in fine wools where the radius of curvature is about 10 μ. This does not detract from the value of the method, however, as with visual observation the objective may be racked up and down in the usual manner.

(*b*) *The Component Layers of the Cuticle*

Present-day knowledge of the component layers of the cuticle, summarized in Figure 1, is based upon the examination of scale fragments from grossly degraded fibres in the electron microscope (Mercer and Rees 1946*a*, 1946*b*). A major difficulty, however, in building up a comprehensive picture of the struc-

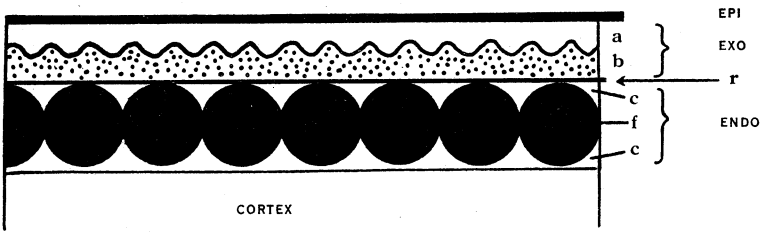


Fig. 2.—An alternative view of the nature of the component layers in the cuticle of wool seen in transverse section (diagrammatic). *epi,* epicuticle; *exo,* exocuticle; *end,* endocuticle; *r,* resistant outer layer of endocuticle; *f,* fibrillar endocuticle component isolated by enzymic or acid hydrolysis; *c,* endocuticle cementing material.

ture of the cuticle has been the lack of a suitable microscopical method for investigating the relationship between the structural details observed in the electron microscope and the coarser features seen in the optical microscope. This difficulty originates not so much from the limited resolving power of the optical microscope as the low contrast in the comparatively transparent wool fibre components. It is clear from the photomicrographs of Plate 3, however, that this difficulty may be overcome by the use of gold shadowing, as many details of the fine structure of the cuticle, normally only visible in the electron microscope, then become visible in the optical microscope. The transparency

of the wool fibre in the optical microscope is now an advantage as specimen thickness is not severely limited as in electron microscopy and some correlation of fine and coarse histology is possible.

According to Figure 1(*a*) the outer surface of the endocuticle is corrugated, or furrowed, and the furrows run parallel to the fibre axis. This feature is seen in Plate 2, Figure 3, where the exocuticle and epicuticle have been removed by treatment with saturated bromine water (Fraser and Rogers 1954*b*). In fibres partially degraded with trypsin (Plate 2, Fig. 1; Plate 3, Fig. 1) and with cetyl sulphonic acid (Plate 3, Fig. 2) the outer surface of the cuticle appears comparatively smooth. This may be due to the epicuticle masking the nature of the underlying structures, or to the continuous outer layer of the endocuticle which is revealed after oxidation with peracetic acid and extraction with ammonium hydroxide (Fraser and Rogers 1954*c*).

The nature of the inner surface of the enzyme-resistant component of the endocuticle, previously unknown, is clearly seen in Plate 3, Figures 1 and 3, to be similar to that of the outer surface. The thickness of this component of the endocuticle, measured radially from the centre of the fibre, is depicted by Lindberg *et al.* (1949) as constant, but it seems more probable that it may be formed by the coalescence of fibrils *c.* 0·6 μ in diameter. In the intact fibre this component appears to be embedded in a material having less resistance to enzymic or acid hydrolysis (Fig. 2), as the inner surface of the cuticle in both trypsin and cetyl sulphonic acid preparations has a smooth texture where least damage has occurred, e.g. area *a* of Plate 3, Figure 3. In addition, it appears that the inner scale edges project from the inner surface of the cuticle, and this is confirmed by the impression retained in the cortical cell of Plate 3, Figure 4, which, it is presumed, was originally located on the periphery of the cortex with its uppermost surface in contact with the inner surface of the cuticle.

V. Acknowledgments

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VI. References

Barnes, R. B., Burton, C. J., and Scott, R. G. (1945).—*J. Appl. Phys.* **16**: 730.
Dempster, W. T., and Williams, R. C. (1946).—*Anat. Rec.* **96**: 27.
Elliot, R. L., and Manogue, B. (1952).—*J. Soc. Dy. Col.* **68**: 12.
Fraser, R. D. B., Lindley, H., and Rogers, G. E. (1954).—*Biochem Biophys. Acta* **13**: 295.
Fraser, R. D. B., and Rogers, G. E. (1954*a*).—*Biochem. Biophys. Acta* **15**: 146.
Fraser, R. D. B., and Rogers, G. E. (1954*b*).—*Biochem. Biophys. Acta* (in press).
Fraser, R. D. B., and Rogers, G. E. (1954*c*).—*Text. Res. J.* (in press).
Lagermalm, G. (1954).—*Text. Res. J.* **24**: 17.
Lindberg, J., Mercer, E. H., Philip, B., and Gralén, N. (1949).—*Text. Res. J.* **19**: 673.
Makinson, K. Rachel (1950).—*Text. Res. J.* **20**: 22.
Mercer, E. H. (1953).—*Aust. J. Appl. Sci.* **4**: 107.
Mercer, E. H., and Rees, A. L. G. (1946*a*).—*Nature* **157**: 589.
Mercer, E. H., and Rees, A. L. G. (1946*b*).—*Aust. J. Exp. Biol. Med. Sci.* **24**: 147.

MERCER, E. H., and ROADNIGHT, L. (1950).—*Text. J. Aust.* **25**: 440.
O'DONNELL, I. J. (1954).—*Text. Res. J.* **24**: 1058.
SCOTT, D. B., and WYCKOFF, R. W. G. (1949).—*Amer. J. Clin. Path.* **19**: 63.
SWERDLOW, M., and SEEMAN, G. S. (1948).—*Text. Res. J.* **18**: 536.

EXPLANATION OF PLATES 1-3

PLATE 1

Fibre surfaces gold-shadowed from the direction of the tip at an angle of 35°. Magnification × 1000, negative prints.

Fig. 1.—Untreated fibre, Corriedale 56's quality.
Fig. 2.—Corriedale 56's quality fibre extended 50 per cent. in steam.
Fig. 3.—Corriedale 56's quality fibre extended 100 per cent. in boiling water.
Fig. 4.—Profile of untreated Corriedale fibre.
Fig. 5.—Profile of Corriedale fibre extended 50 per cent. in steam.
Fig. 6.—Profile of Corriedale fibre extended 100 per cent. in boiling water.

PLATE 2

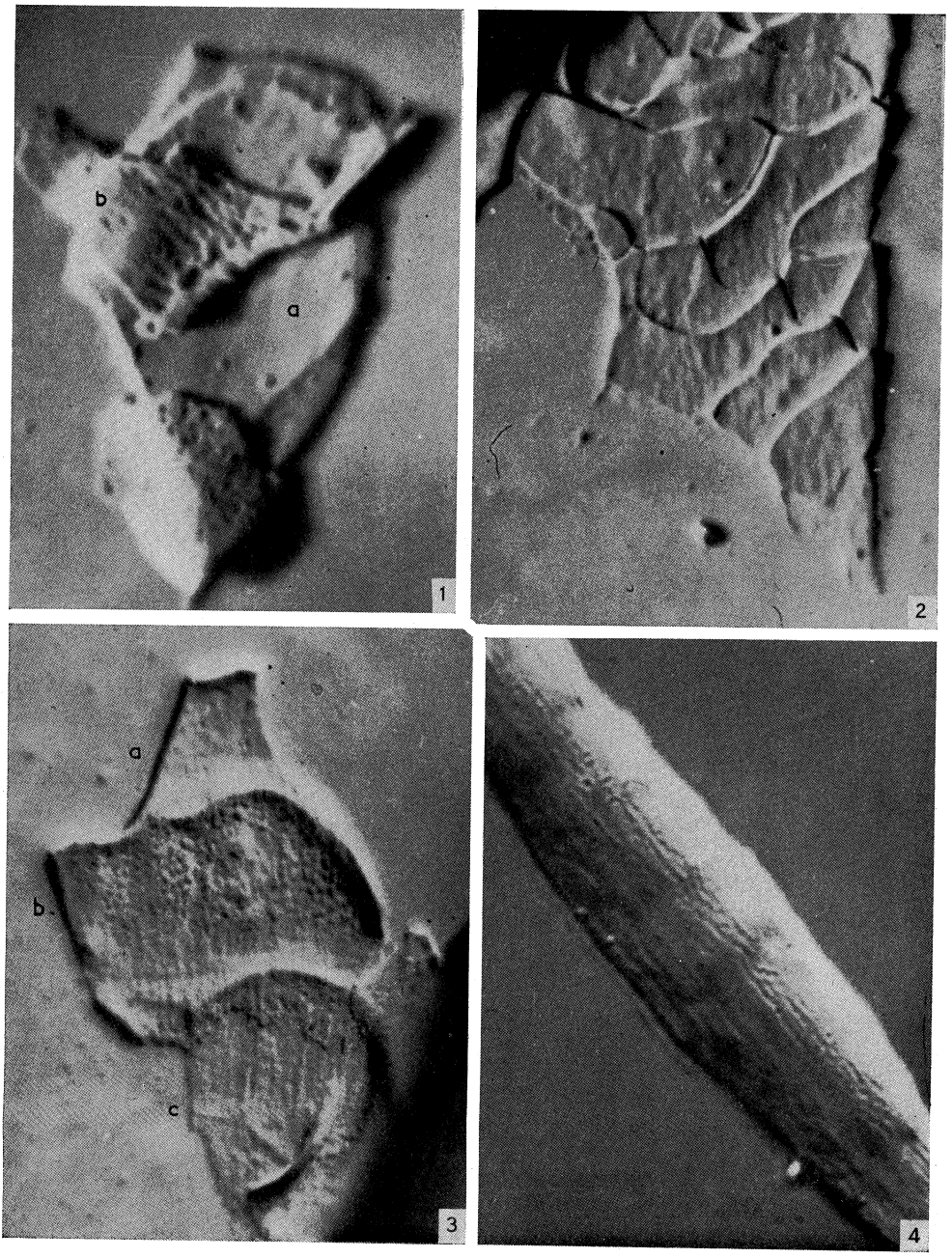
Fibre surfaces gold-shadowed from the direction of the tip at an angle of 35°. Magnification × 1000, negative prints.

Fig. 1.—Corriedale 56's fibre partially digested with crude trypsin.
Fig. 2.—Merino 64's fibre treated with chlorine (epicuticle intact).
Fig. 3.—Merino 64's fibre treated with bromine (epicuticle and exocuticle detached).
Fig. 4.—Merino fibre treated with 10 per cent. sulphuric acid.
Fig. 5.—Merino 64's fibre treated with cetyl sulphonic acid and ammonia.
Fig. 6.—Merino 64's fibre reduced with thioglycollic acid and oxidized with iodoacetic acid.

PLATE 3

Fig. 1.—Scale fragment isolated from trypsin-digested wool showing smooth nature of outer surface (*a*), and corrugated inner surface (*b*) of overlying fragment. Magnification × 2600, negative print.
Fig. 2.—Outer surface of scale fragment isolated from wool treated with cetyl sulphonic acid and extracted with ammonia. Magnification × 1280, negative print.
Fig. 3.—Inner surface of scale fragment from the same preparation as Plate 3, Figure 2, showing (*a*) continuous inner cuticle layer; (*b*) partial dispersion of inner cuticle layers; (*c*) complete dispersion of inner cuticle layers. Magnification × 1280, negative print.
Fig. 4.—Cortical cell from the *H* segment of the bilateral cortex from the same preparation as Plate 3, Figure 2. Note transverse depressions left by inner surface of cuticle. Magnification × 2000, positive print.

STRUCTURE OF WOOL



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