ELECTRON MICROSCOPE STUDIES OF THE SURFACE STRUCTURE OF WOOL AND OTHER FIBRES

by

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INTRODUCTION

A study of the structure and morphological details of wool fibre surfaces is important both on biological and technological grounds, and, as is well known, both the optical and electron microscopes have contributed to our knowledge of the surface features. Replica methods have been used in both fields[1,2], and although promising results have been obtained using the "reflection" electron microscope[3], surface replication is still the most suitable method for general use in the electron microscopic study of fibre surfaces. Hitherto, except in a few isolated instances, replication has involved the use of polymeric materials for obtaining the cast[4,5,6,7]. In other fields, notably metallurgy, it has been found that materials of low molecular weight (*e.g.* silicon monoxide[8], carbon[9], or certain metals) are capable of giving replicas with better resolution and having improved stability in the electron beam; these advantages are, however, somewhat offset by the comparative fragility of the replicas and the increased complexity of their production process. That such methods have not been used more for fibres is largely due to the difficulty of stripping the replica from the fibre; with polymeric materials a surface cast of a fibre can be made by embedding it in a film of the swollen or softened plastic and pulling it away when the latter has hardened, a technique of little use when the casting material has to be deposited by condensation from the vapour. The only successful low molecular weight replicas of fibre surfaces previously described have been obtained by dissolving the fibre (after coating by evaporation) in a solvent which does not affect the casting material[10]. The difficulty of complete dissolution of keratin fibres renders this technique of limited applicability as far as they are concerned.

In this paper two methods of obtaining low molecular weight replicas of wool and other fibre surfaces will be described; their essential common feature is that the fibre is partly embedded in polystyrene or celluloid. One method gives a direct replica in SiO, and the replicas of wool fibre surfaces were found to give micrographs so rich in detail that the superiority of SiO over, say, polystyrene as a casting material is beyond question. In fact, so extra-ordinary was the amount of detail revealed by these replicas that it became almost imperative to investigate the problem of reproducibility in order to make it quite clear that artifacts were not being produced. The second method of replication was developed with this end in view; it is a two stage method in which the fibre remains embedded *in situ* when the first cast (in

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silver) is stripped from it; the final positive replica is made from the first by evaporating SiO on it, and the silver is then dissolved away in acid. Since the fibre remains undisturbed, a second replica can be taken from the same surface area for comparison with the first. By using this technique it was established that the details seen in the replicas were, indeed, representations of details of the surface texture and not artifacts. Furthermore, an entirely new field was opened up by the possibility of subjecting the once-replicated surface to some treatment before repeating the replication, and thus obtaining pictures of the same surface before and after treatment.

The final step in the development of these techniques was prompted by the difficulty encountered in assessing relative heights or levels in the replica. It is, in fact, often difficult, when observing the scale edge region in a SiO replica of a wool fibre, to decide which side of the edge corresponds to the overlying and which to the underlying scale. We decided, therefore, to make stereoscopic pairs of micrographs from some of the replicas, and the results were so satisfactory that we now regard this as an almost essential procedure in studying fibre surfaces by replication.

EXPERIMENTAL

(a) *The embedding technique*

The fibres to be examined were cleaned by extraction in ether and alcohol and were subjected to as little mechanical action likely to modify the surface as possible. The first step was to embed the fibre in a suitable material, leaving its upper surface exposed. This was done in the following way. The fibre was cemented at one end to the edge of a microscope slide which was covered by a sheet of polystyrene. The sheet was swollen slightly by applying benzene by a camel hair brush to its surface in the region where the fibre was to be embedded. The free end of the fibre was then taken up and the fibre stretched just taut across the swollen sheet; it was then lowered gently, still under tension, until it made contact with the plastic. In this way it was found possible, by properly judging the tackiness of the polystyrene, to ensure that the fibre sank into the plastic so that rather more than half of the fibre surface was embedded and the remaining free surface was uncontaminated by the embedding material. Successful embedding by this technique was found to be a matter of practice; various modifications of the procedure described (for example, mounting the fibres in a small frame, or loading them with small weights in order to control to some extent the tension) are possible and have been successfully used. An alternative procedure was to use celluloid instead of polystyrene, softening it by applying amyl acetate. In practice, a slight personal preference of one of us (N.R.) for this method led to its adoption as the standard technique. In either case the sheet of plastic was about 0.25 mm thick, and the softening was restricted to a surface layer.

Once the fibre had been embedded it was held taut for a few seconds while the polymer hardened. It was usually found desirable to limit the length of fibre exposed, and when this was so the whole of the fibre except for a selected region about 3 mm long was coated with nitrocellulose from solution. Finally the embedded fibre was stored for some hours to allow the last traces of solvent to evaporate.

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(*b*) *Direct (negative) SiO replicas*

The prepared slide with the fibre suitably embedded was supported in a vacuum shadowing apparatus at a distance of 15 cm above a molybdenum boat made by bending into a canoe-like shape a sheet of the metal 4 cm long, 0.5 cm wide and 0.005 cm thick. A quantity of SiO powder (Viacote), estimated to be enough to give when evaporated a film 400 A thick on the specimen slide, was put into the boat. After the usual processes of evacuation, degassing, *etc.*, had been carried out, the SiO was evaporated, by heating the boat electrically, in two steps (each of about 20 sec duration) with an interval of a few minutes between them. The purpose of this was to prevent any overheating of the specimen which might have been a consequence of the high temperature required for evaporation of the SiO. In this way a satisfactory film could be deposited over the exposed surface of the fibre and the surrounding area of the plastic. The problem was how to remove it for examination. The normal wet and dry stripping techniques were found to be useless, and recourse was had to the somewhat retrograde step of backing the SiO film with formvar, cutting out the assembly of embedded fibre, SiO, and formvar, and dissolving the celluloid embedding matrix in acetone vapour in a reflux boiler. To reduce subsequent manipulation to a minimum the composite assembly was placed formvar side down on a specimen grid and mounted in a specimen holder before exposure to the solvent. It was usually found that when the celluloid had been completely dissolved the fibre had been loosened from the replica and could be removed without damaging it.

The replicas of Lincoln wool fibre surfaces obtained by this method showed an exceptional amount of detail. An example is given in Fig. 1, and from a cursory examination of this it is clear that the picture usually put forward of a wool scale surface as being smooth and relatively featureless is by no means a true one for Lincoln wool, at least. It appears, too, that SiO is, as was anticipated, a far more satisfactory replicating material than polystyrene, although, as can be seen, the replicas had a tendency to crack. Experience gained in a few applications of this technique emphasized another important fact, that wide variations in the type of surface texture are a normal feature of the Lincoln wool fibre surface, and it became increasingly evident that some control technique capable of assessing the reproducibility of the results was essential if reliable deductions are to be drawn from the experiments. The simple one-stage technique was therefore discarded, and attention was turned again to the possibility of stripping a cast from the embedded fibre without, in so doing, disturbing the latter.

(c) *Two stage (positive) Ag-SiO replicas*

After some trials it was found that a relatively thick silver film deposited over the embedded fibre by evaporation could be removed by dry stripping. The procedure adopted was as follows. The fibre, having been embedded by the technique described above, was mounted in the shadowing chamber at a perpendicular distance of 7 cm (silver evaporates at a much lower temperature than SiO) from a conical basket-shaped tungsten filament holding enough silver to produce a film with an estimated thickness of 1 micron on the fibre when completely evaporated. After evacuation the silver was heated at such a rate that it took about 12 min to evaporate completely. The slide was then removed, and after the usual trick of breathing on it had been performed[11] a length of cellulose tape was pressed adhesive side down over the region

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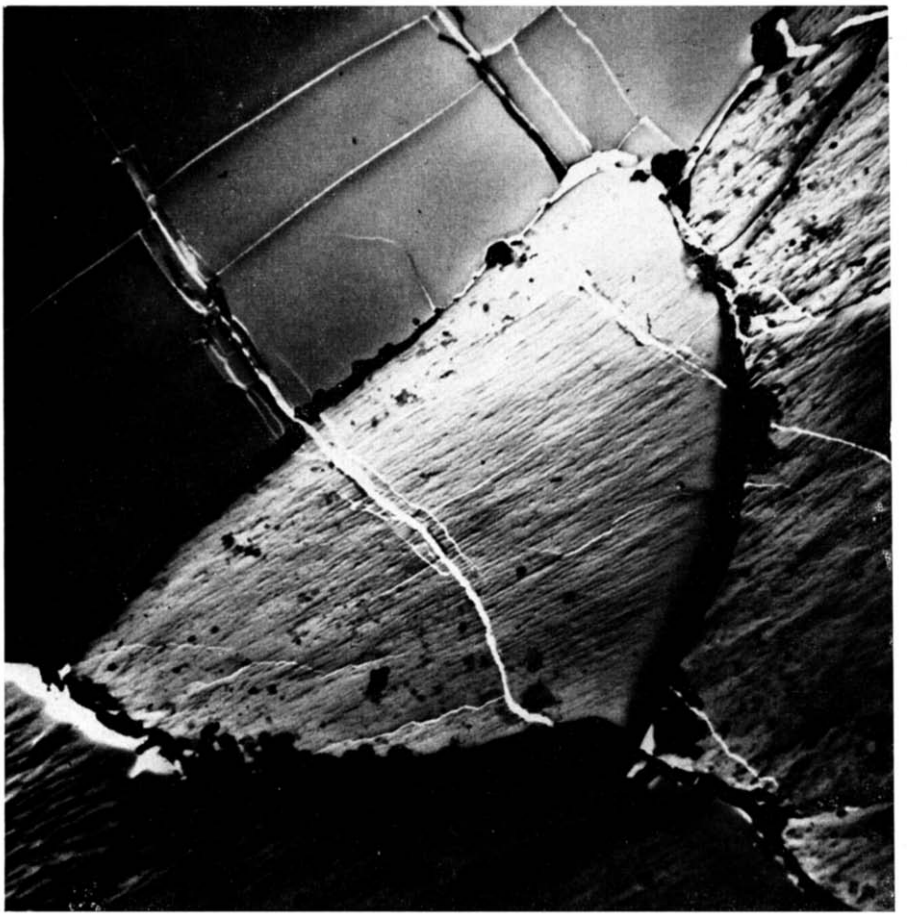

Fig. 1. Lincoln wool fibre, untreated. Direct (negative) surface replica (SiO). Photographic print from original micrograph. × 10,000.

containing the silver coated fibre. After the silver film had been scored along the edges of the tape, one end of the latter was gripped in tweezers and slowly lifted away from the slide, carrying the silver with it and leaving the embedded fibre behind. The tape was placed on a glass support, silver side up, and coated with SiO to an estimated depth of 400 A by the technique described above. This coating was subsequently backed by a thin formvar film, as it was found that the SiO was otherwise too fragile to withstand the manipulation necessary to remove the silver from it. The final stages in the process involved first dissolving the adhesive on the cellulose tape by petroleum ether, then washing the Ag-SiO-formvar repeatedly in fresh solvent, and finally dissolving the silver in dilute (1:3) nitric acid and washing the residual SiO-formvar replica in distilled water.

In quality, the replicas thus obtained appeared to be little inferior to those given by the one-stage technique. A check on the graininess of the silver was made by evaporating some to cover a glass surface and coating this film with SiO; the composite film was then stripped from the glass and the silver dissolved to leave a SiO

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replica of its surface. The grain in this was almost imperceptible at magnifications up to 10,000 times.

The first application of the two-stage technique was to test the reproducibility of the replication of a single scale on the surface of a Lincoln wool fibre. To do this the whole process was repeated with the same fibre, and the final replicas were mounted on grids for examination in the electron microscope. One difficulty which arose, even when the length of the replicated region was restricted to the diameter of the specimen grid, was that of positioning the replicas on the conventional square-mesh grids so that corresponding scales could be located. This difficulty was finally overcome by using special grids, made by the photographic process developed by CHALLICE[12], with a hexagonal array of circular openings surrounding a larger central hole. This made it much easier to locate a selected scale region in both replicas. Until experience had been gained it was found to be advantageous, having first roughly checked the positions of the replicas on the grids by examination in the optical microscope, and thus ensured that corresponding regions lay in the grid openings, to make a mosaic of a fair length of the replica and do the final matching up on the micrographs. With experience, however, this tedious procedure was found to be unnecessary, and the scale could be picked out in the two replicas by visual examination of the microscope screen. When making comparisons of this kind the instrument settings were, of course, kept the same. An example of the reproducibility is given in Fig. 2; in fact, (b) here is the seventh replica which was taken from this particular fibre, (a) being the first, so that it is amply evident that replication by this method can give reproducible results and has little effect on the surface texture of untreated wool fibres. There is, indeed, some slight disturbance at one point of the scale edge; but remembering the drastic nature of this test, the results must be judged eminently satisfactory.

With this point established, the next step was to show how small modifications

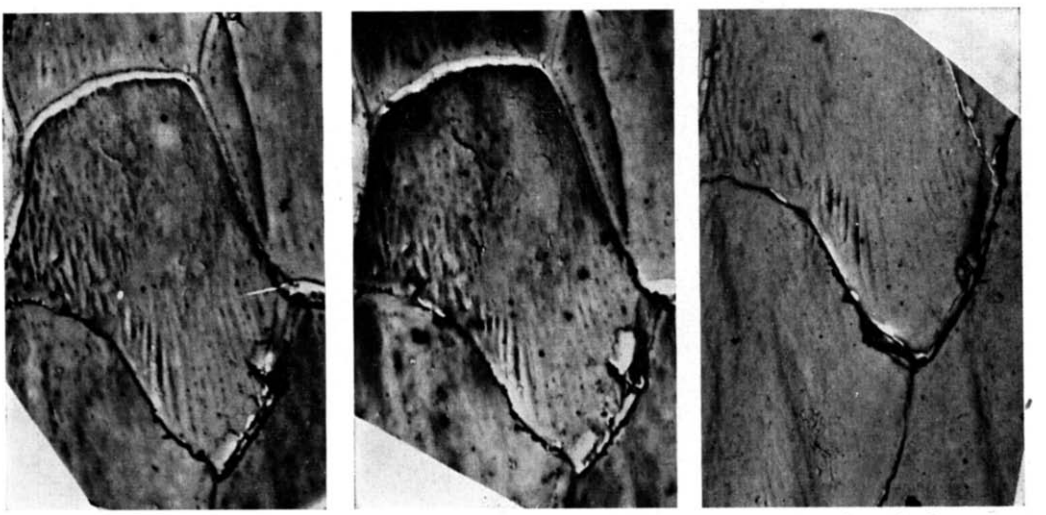

Fig. 2. Lincoln wool fibre. Two stage (Ag/SiO) surface replicas. a. Untreated surface; b. Repeat replica from the same area as in (a); c. Same area as (a) and (b) after mild treatment with sulphuryl chloride. Photographic prints from original micrographs. × 3,600.

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induced by mild surface treatments could be detected unambiguously. The same fibre used for the replicas of Fig. 2 (a) and (b) was therefore exposed for a short time to the action of sulphuryl chloride, and a final replica of the surface thus treated gave the micrograph of Fig. 2 (c). It will be seen that there is a general "cleaning up" of the surface and some modification of the scale edges. It is emphasized here that the treatment was purposely kept as mild as possible in order to have only slight changes in the surface texture. It is, of course, realized that this technique will be of most value when applied to fibres which have been subjected to comparatively mild treatments; furthermore, the production of a series of replicas such as those in Fig. 2 must be regarded as something of a *tour de force*, and is not to be lightly undertaken as a routine procedure. We have, therefore, in our examination of the effects of a number of more drastic treatments, confined our attention to replicas of the treated surfaces, since we wished, at this stage, to make a survey of the field rather than a detailed study of any particular effect.

(d) *Stereoscopic micrographs*

Interpretation of the micrographs of SiO replicas is rather more difficult than in the case of plastic replicas because the film of SiO is approximately constant in thickness[13] and contrast arises from variations in transmitted electron density caused by local variations in the slope of the replica, whereas in plastic replicas one face is approximately flat and differences in thickness (and therefore in electron transmission) are directly related to the surface contours. The situation is, however, even more complicated by the possibility of "self-shadowing"[14] by the SiO—an effect impossible to get rid of on account of the curvature of the fibre surface and consequently of the intermediate silver replica. It is true that by use of metal shadowing assessment of the true nature of the contours might be facilitated, but to adopt this device would be undesirable in view of the already adequate contrast appearing in the unshadowed replicas. It appeared, therefore, that the examination of the surface replicas might be a fruitful field for stereoscopic methods, by means of which the true nature of the contour should be detectable. In the event, it was found that the application of this technique produced results which might be said, with some justification, to promise a major revolution in the field of study of wool fibre surfaces.

To obtain the stereoscopic pairs the simple technique of tilting the specimen through about 5° on either side of the plane perpendicular to the microscope axis was adopted. The axis of tilt was, of course, fixed in the instrument, and in the micrographs the direction of the stereo base line depended on the magnification, owing to rotation of the image[13]. The rotation was therefore calibrated by taking pictures at various magnifications of a replica of a line grating with the lines set perpendicular to the axis of tilt. In this way the base line could be located with sufficient accuracy for qualitative work. One difficulty in interpretation was, however, still present even in the stereoscopic pairs. This was concerned with whether the replica was mounted in the microscope with its "obverse" or "reverse" face upwards; since the replica was picked up from water after washing, it was impossible to control this orientation. Thus, when viewing the stereoscopic pair it is still uncertain whether one is adopting the external or internal viewpoint. The simplest solution of this problem in the present experiments was to observe the position of the formvar backing film, which was found in places to be slightly separated from the SiO replica.

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Now, it is the face of the replica which was originally in contact with the silver intermediate which is the actual reproduction of the outside surface of the fibre, and the formvar film covers the other face. Hence, when the viewpoint is external the backing film will appear to be behind the replica. Another criterion which was also found useful was the fact that, in Lincoln wool, the scales were in general smoother near their tips than near their roots, where broad striations usually appear. This feature was confirmed by checking the electron micrograph against a view of the fibre in the optical microscope, where tip and root directions of the fibre can be determined without any difficulty.

Fig. 3. Lincoln wool fibre, untreated. Two stage (Ag/SiO) surface replica. Photographic print from original micrograph. × 4,600.

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RESULTS AND DISCUSSION

(a) *Untreated Lincoln wool fibres*

Perhaps the most obvious feature of the replicas of Lincoln wool fibres is the extraordinary variety of surface detail which they show; this makes it difficult to pick out characteristic features. Most of the important points which will be mentioned in the following summary of our observations are illustrated in Figs. 3, 5 and 6, in Fig. 4 a diagrammatic key to Fig. 3 is given, and the following description will refer to features shown there. In this micrograph there appear three almost complete scales which we call X, Y, and Z, using suffixes to refer to subdivisions of the scale surfaces.

The feature represented by the line ABC running across the scales Y and Z is of widespread occurrence in this wool; it is due to a fold or weld in the epicuticle and is continuous across the scale boundary at B. A somewhat similar line is often seen to run parallel to the scale edge, for example, DE in the figure. The continuation EF of this is almost certainly the edge of an epicuticle sheet covering X_1; near E the line has an appearance suggestive of torn epicuticle, but on passing towards D its character changes to something more suggestive of a weld or fold. The occurrence of such epicuticular features confirms the conclusions of GRALÉN[15] who observed what he called welds on isolated pieces of epicuticle. A quite remarkable characteristic of the line ABC occurs at B, at the tip of the scale Z. Here the fibre surface falls rather abruptly as we pass from Z to the underlying scale Y, and the epicuticle forms a thin solid ridge running from the top of the escarpment across the broad valley. A similar knife-edge ridge also appears in Fig. 5 at X; we have observed in some repeated replicas that this feature is reproducible, and this is a striking tribute to the ease of stripping of the silver intermediate replica.

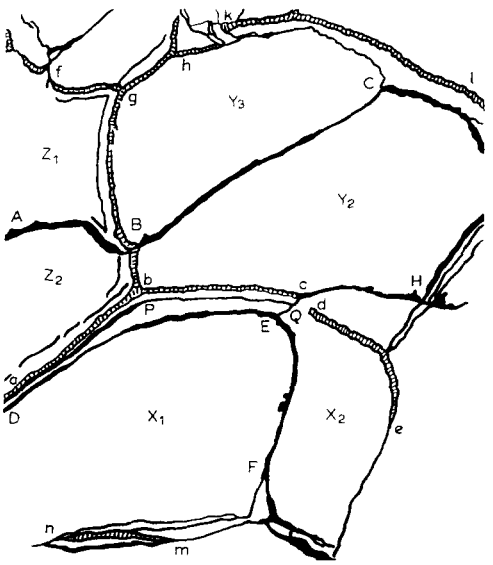

Fig. 4. Diagrammatic key to Fig. 3.

It will be seen in Fig. 3 that the scale surface Y appears to be modified in two ways which are associated with the line BC. In the first place, the clarity of the surface details is much better in Y_2 than in Y_3; all the indications suggest that Y_2 is covered by epicuticle (its edge may be clearly seen at cH); it is conceivable, but in our view unlikely, that Y_3 may be overlaid by a double epicuticle, which would obscure the underlying details of structure. A more reasonable supposition would be that the epicuticle covering Y_3 is more tightly stretched and thus not so closely moulded to the underlying exocuticle. Secondly, BC appears to lie in a shallow valley in the scale surface, and the scale edge at B shows a slight change in direction. A possible explanation of such details is that they represent traces left on the cuticle of features of the inner root sheath against which the scales were moulded during

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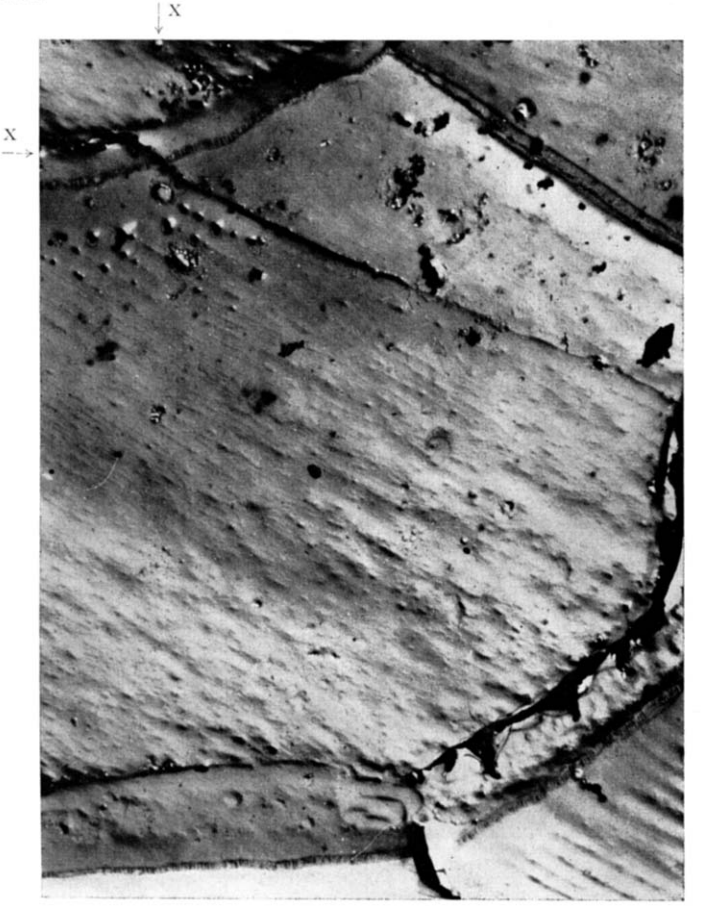

Fig. 5. Lincoln wool fibre, untreated. Two stage (Ag/SiO) surface replica. Photographic print from original micrograph. × 7,300.

growth[16]. It is even possible that the epicuticle may, in fact, have originated as an inner layer of the root sheath, to be transferred to the fibre at some level in the follicle.

In general, Lincoln wool scales are, as we have mentioned above, broadly striated or corrugated near their root ends and smoother towards their tips. Even there, however, we do not observe the "lacquered" appearance described by SWERDLOW AND SEEMAN[2], for the surface has numerous pits and longitudinal grooves which are shallower than the corrugations. How far these are features of the exocuticle itself or are due to the epicuticle is a matter of some importance. The usual interpretation of the corrugations, which SWERDLOW AND SEEMAN also observed, is that they are due, in the end, to the endocuticle, to whose contours the exocuticle conforms; and we are inclined to agree with this interpretation. The comparative smoothness of the scale tip regions in the replicas may imply that the endocuticular corrugations are absent there, or that they are obscured by the exocuticle or by the epicuticle. The last alternative is one which would warrant further study, for the idea that the epicuticle may be tightly stretched across the crests of the corrugations

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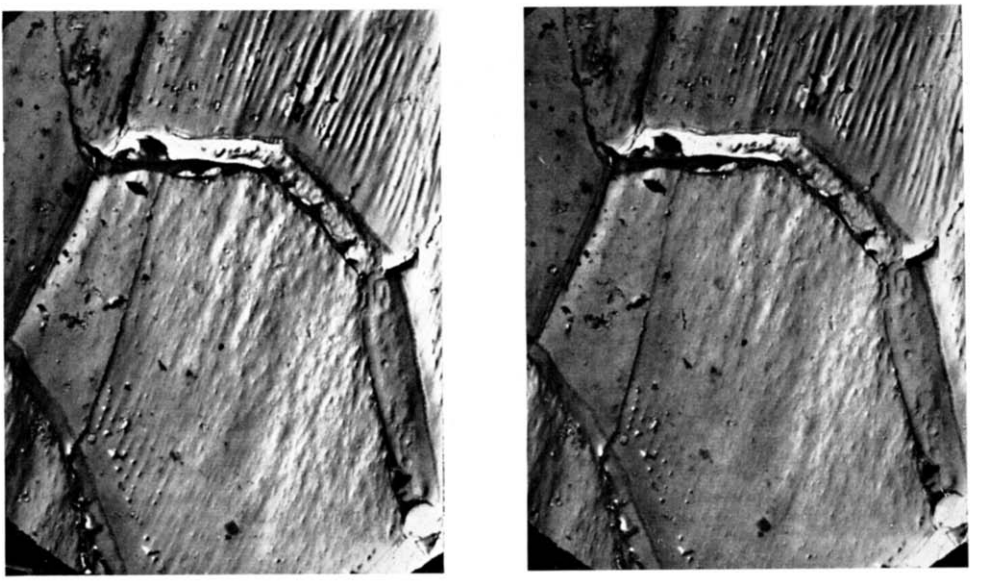

Fig. 6. Lincoln wool fibre, untreated. Two stage (Ag/SiO) surface replica. Stereoscopic pair showing a larger area of the surface illustrated in Fig. 5, external view point. Photographic prints from original micrographs. × 3,500.

is one having some attractive implications. For example, it would leave open channels for the penetration of reagents from the scale tips towards the roots and thus give a simple explanation of the striated staining which occurs in lightly damaged fibres[17]; the epicuticle would also be more susceptible to mechanical damage in the regions where it is not so closely moulded to the underlying material, and the occurrence of preferential tip-end damage, which is well established, could be thus explained. It must be admitted, however, that no clear evidence is as yet available by which such ideas can be tested; we intend, however, to extend our experiments to examine fibres which have been subjected to mechanical action, since this is known to damage the epicuticle.

If, however, it is supposed that the absence of corrugations near the scale tips is a true feature of the scale itself, the simple picture of the cuticle layers put forward by LINDBERG, MERCER, PHILIP, AND GRALÉN[18] may need revision. At this stage it would be fruitless to do more than draw attention to the region X_2 in Fig. 3, where the scale tip region is free from overlying epicuticle and the corrugations are clearly shown. In our experience, however, the effect seen here is quite abnormal, and it would be unwise to stress unduly its importance.

We turn now to an examination of the scale edges themselves. The first difficulty is to decide what, in fact, corresponds in the replicas to the scale edge, and, as has been said, it is only by using stereoscopic methods that a true conclusion has been reached. The fact that optical illusions can occur in viewing shadowed electron micrographs is, of course, well known. (An example may, in fact, be seen in Fig. 3 where the surface of the scale Z_1 appears to be lower than that of the scale on the other side of the line fg, but if the figure is turned through 180° the relative heights are

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reversed.) The observations set out below are based, in the main, on stereoscopic examination; we re-emphasize here that they refer to Lincoln wool.

A typical contour over a scale edge (as found in the replicas) would be something like that shown in Fig. 7. Here AB is the surface of the overlying scale, DEF that of the underlying one, BC is what is usually referred to as the scale edge, and the allocation of CD to one scale or the other is a matter requiring some thought (see below). At B an epicuticle edge or fold, perhaps with torn pieces protruding, can often be seen. A striking example occurs in the stereoscopic pair of Fig. 6. We shall give reasons later for our belief that what are being observed here are actual fragments of the epicuticle which have been torn from the fibre by the silver and thence transferred to the SiO replica. The regions CD and DE are relatively smooth, without the pits and grooves which are such a common feature of the rest of the scale surface; at D, separating these smooth regions, there appears a remarkable band running parallel to the scale edge and transversely striated (see abcde and fghkl in Fig. 4). The width of this band may vary between about 1000 and 3000 A, and the striations have a spacing of the order of 500 A. The region BC has rather different characteristics at the tips and sides of the scales. At the tips the slope is steep and the texture is rough with an ill-defined structure; at the sides the slope may be more gradual and the texture much smoother. The length of the region CD is variable; the banded structure at D is common but not always observable in the replicas.

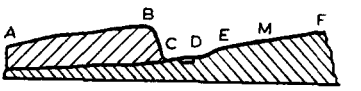

Fig. 7. Lincoln wool fibre, typical edge contour (*cf.* Fig. 8).

We shall discuss first the scale edge BC with particular reference to the tip of the scale. Is the picture of this given by the replica a true one? If it is, the widely-held concept of re-entrant scale edge contours must be rejected for Lincoln wool, at least. It is not easy to predict with certainty what the effect of a re-entrant contour would be on the replica, but if the silver used to form the intermediate replica does not fill the spaces underneath the protruding scale edges that replica should have a steep step at the scale edge and a discontinuity if the silver layer is thinner than the scale depth at the tip, and these features should reproduce themselves in the SiO replica. This does not correspond to what is usually observed, though sometimes there does appear to be a break in the replica at the scale tip. If the silver fills the re-entrant angle, stripping will be difficult without tearing or severely distorting either the silver or the keratin, and in the absence of such effects the SiO replica should have a discontinuity, which, as we have said, is not usual. There is left, then, the possibility that the region BC represents either the fractured surface of the torn silver replica or the fractured surface of the scale tip torn away with the silver. Neither alternative is very attractive, in view of the general success of the stripping process; if such tearing does take place it might be expected that more definite signs of it would appear in regions more remote from the scale edges. In this connection, it might be argued that some such effect might be responsible for the abnormal "stripped" region X_2 in Fig. 3.

It must also be pointed out that the appearance of the scale edge contour of Lincoln wool fibres, as seen in the optical microscope, is not particularly suggestive of re-entrant angles. It might be thought that an examination of the apparent edge contour in micrographs such as that in Fig. 1 (or in corresponding optical micrographs

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such as those shown by WILDMAN[1]) should provide information about the shape of the "overlap" region. In fact, however, this edge contour usually represents a section of the surface by a non-diametrical plane, and the apparent overlap is therefore exaggerated. The conclusion that the representation of the scale edge in our replicas is generally a faithful one is strengthened by the results of the repeated replication experiments although, so far, the technique has not been used by design to investigate this particular problem in detail.

The cross-striated band at D (Fig. 7) is a feature of exceptional interest because nothing of the sort has ever been observed before, and its interpretation raises many problems. It will be seen in Figs. 3, 5 and 6 that the band is clearly evident in the neighbourhood of the epicuticular bridges across the scale-edge valleys. Furthermore, it seems clear that the epicuticle usually covers the scale edge regions BC (Fig. 7), although sometimes it may be torn away there, especially at the scale tips. Consequently, the continuity of the epicuticle at the bridges forces us to the conclusion that it does, in general, extend across the band at D. Now nothing like this striated band has, as far as we know, ever been observed in isolated epicuticle, and this suggests that the epicuticle is moulded round the striae which are actually a feature of the scale itself. The evidence is, in fact, strongly in favour of this idea, for the following observations admit of no other reasonable interpretation. On examination, three variations in the appearance of the band were noted. In one, the region CDE (Fig. 7) appears rather flat, and the striations show as a feature of an otherwise smooth surface. This, it is inferred, happens when the epicuticle is undamaged and closely applied to the underlying exocuticle. In the second variation, the striations are fainter or are obscured more or less completely by what can be seen to be a film covering them — presumably the epicuticle partly detached from the surface and with its corrugations smoothed out. When this is observed in a first replica the striations may appear much more clearly in a second because the stripping of the silver completes the disruption of the epicuticle and may remove the torn fragments altogether (see below). In the third variation (of which the last noted effect is an example) the striations are definitely at a lower level than the edges of the band, and often an epicuticular edge can be seen projecting slightly over the striated crevice. It may be concluded that here the epicuticle has been torn away along the band, leaving the striated exocuticle exposed. If this interpretation is correct the position of the band must be regarded as a line of weakness in the epicuticle; we can thus explain why some observers[19] have obtained isolated fragments of epicuticle approximately equal to the scales in area, whereas others[20] have been able to isolate much larger sheets.

The principal objection to the idea that the striated band is a feature of the scale itself and not only of the epicuticle is that nothing of the sort has been observed in isolated cuticle scales. However, since methods of isolation may usually be expected to entail some attack on the cuticle, it may reasonably be argued that the detail is thus lost. The origin and possible function of the band are, at this stage, matters of speculation. It may be noted, however, that its location is often well removed from the foot of the steep scale tip slope, and two possible interpretations of this are that the band is in the exocuticle of the underlying scale or that it marks the actual boundary of the two scales. At first sight one is inclined to take the point C in Fig. 7 as being at the actual tip of the underlying scale, and then the first alter-

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native must be chosen. The band usually lies in a shallow valley where the exocuticle may be rather thin, and this suggests as a possible cause shrinkage strains in the later stages of fibre formation leading to incipient or partial rupture of the exocuticle. The alternative, that the band marks the actual tip of the overlying scale, implies that when the scale is being moulded against the inner root sheath a short flange may be formed round the edge which fits into a corresponding recess in the lower scale (Fig. 8), and the band may be formed by the shrinkage of the flange back towards the main body of the scale.

Fig. 8. Lincoln wool fibre, alternative scheme of scale edge contour (*cf.* Fig. 7).

(b) *Fibres with surface modification*

In the replicas of untreated fibres a feature of rather frequent occurrence is the appearance at the scale edges, and particularly at the scale tips, of fragments of a thin membrane, which we have identified with the epicuticle, projecting from the surface. The most likely interpretation of such effects is that in the untreated fibre the epicuticle had become loosened in these regions, and scraps of it were torn off to adhere to the intermediate silver replica. These were then covered by the SiO, to which they remained attached when the silver was dissolved. A test of the validity of this idea would be to examine fibres which had been treated in trypsin, which, according to MERCER AND REES[21] attacks the exocuticle and as a consequence should lead to a more widespread transfer of epicuticle to the replica. In fact, this expectation was completely justified, as may be seen from an examination of Fig. 9. In the nearly complete scale shown there, the epicuticle can be seen covering the region A, whereas it is absent from region B; the edge along which it was torn is clearly seen separating the two regions. Projecting upwards from the replica, the loosened epicuticle from B

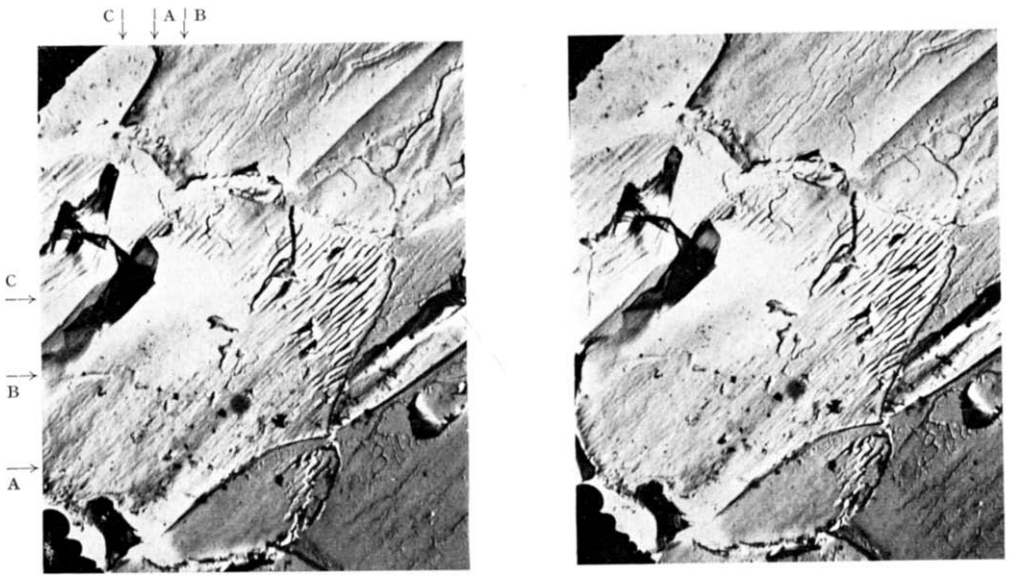

Fig. 9. Lincoln wool fibre after treatment in trypsin for 24 h. Two stage (Ag/SiO) surface replica. Stereoscopic pair, external view point. Photographic prints from original micrographs. × 1,800.

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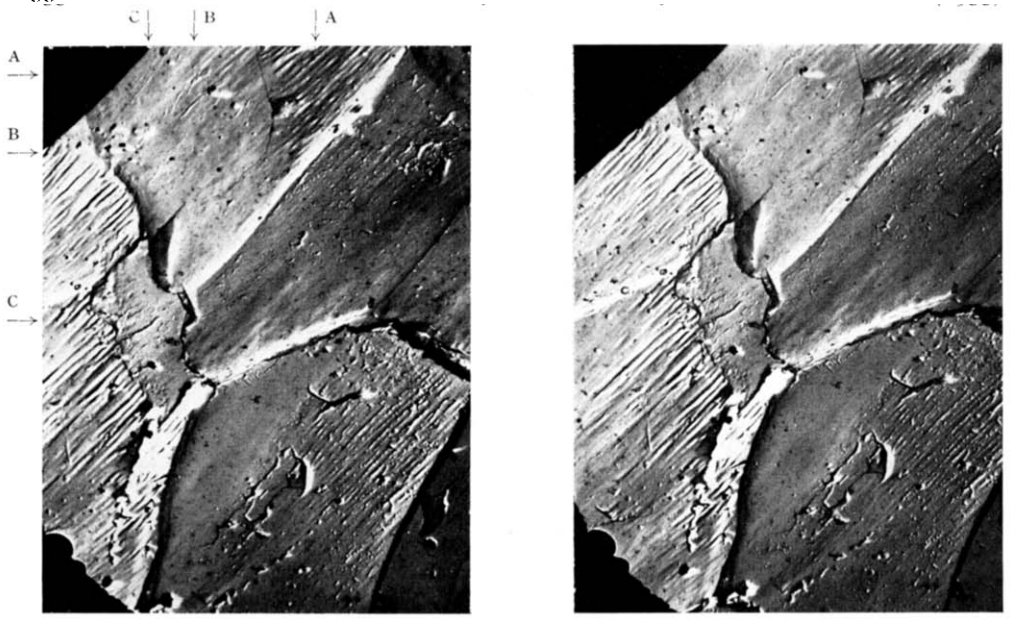

Fig. 10. (Same legend as for Fig. 9).

may be seen at C. Careful examination shows that the epicuticle C, which was stripped from the fibre by the silver and thence transferred to the final replica, forms a continuation, without any break, of the epicuticle covering A, from which it must be concluded that this latter is also transferred epicuticle, and not a replica. The region A is, in fact, a pseudo-replica[22] showing the actual under-surface of the epicuticle; in contrast, the region B shows a replica of the outer surface of the epicuticle. This interpretation emphasizes two points; one is that replicas of treated wool fibre surfaces may need very careful examination to decide whether pseudo-replication has occurred; repeated replication of such surfaces seems to be almost essential if the complexities which are apparent in such micrographs as Fig. 9 are to be resolved. Fortunately, this particular example was one in which the character of the epicuticle could be discerned, but this is a contingency which cannot always be expected (*cf.* Fig. 10, below). The other point is that in the one micrograph can be seen pictures of both sides of the epicuticle; the greater detail seen in A than in B is probably due to the transfer of some exocuticular material which is adhering to the epicuticle. In the other scales seen in Fig. 9 the replica is that of the external surface of the epicuticle, but whether the latter has been stripped by the silver and subsequently lost, or remains on the fibre, cannot be decided.

Another example of a wool surface attacked by trypsin is given in Fig. 10. Here, again, the epicuticular edge can be seen separating regions A and B, and the interpretation is similar to that given for Fig. 9. It should be noted, however, that had we had to interpret Fig. 10 without having first seen Fig. 9, it would have been quite impossible to decide whether the region A is a true replica or a pseudo-replica. The region C in Fig. 10 shows the scale tip denuded of epicuticle, with severe attack on the underlying exocuticle. The torn edge of the epicuticle can be seen projecting

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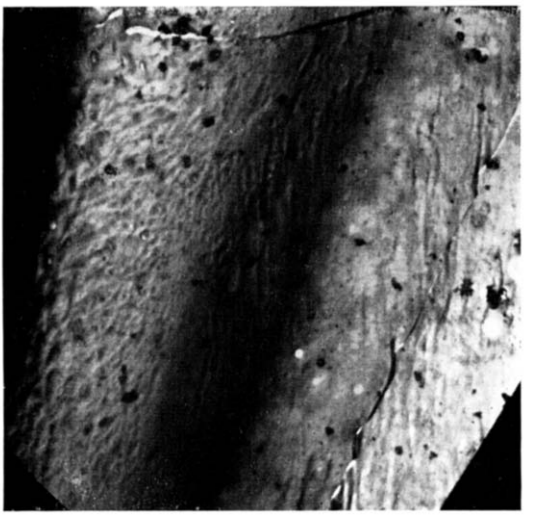

Fig. 11. Cotton fibre, untreated. Direct (negative) surface replica (SiO). Photographic print from original micrograph. × 6,600.

Fig. 12. Jute fibre, untreated. Direct (negative) surface replica (SiO). Photographic print from original micrograph. × 6,600.

upwards. The fibres yielding the replicas of Figs. 9 and 10 were treated comparatively mildly; with more prolonged tryptic attack the epicuticle was transferred to the replicas over very extensive areas, and this gave rise to striking, if not very informative, stereoscopic effects.

(c) *Other fibres*

Although the main object of the work described here was the study of the surfaces of keratin fibres, a few applications of the one-stage technique have been made to other fibre surfaces in order to assess its suitability in such cases. Fig. 11 shows a micrograph of a SiO replica of the surface of a Sea Island cotton fibre which had been cleaned by soxhlet extraction in 50:50 benzene-alcohol for 72 hours. The most obvious feature of this micrograph is the non-uniformity of the surface texture. On one side of the fibre there appears to be a reticulated fibrillar network, similar to that observed by MUHLETHALER[23] in the primary cell wall. The fibrils, however, do not stand out as conspicuously as might be expected from an examination of the electron micrographs of isolated primary cell wall fragments, probably because the cleaning process in our experiment was limited to the extraction of fats and waxes. Over the remainder of the surface little fine detail can be seen, although there are striations suggesting a rather coarse fibrillar system making a small angle with the fibre axis. This is more in line with the observations of BARNES *et al.*[24]. In experiments on the effects of mercerization on the surface details we have observed that this division into regions with different characteristics appears to be a common, if not universal, feature of the cotton fibre surface.

In Fig. 12 we show a SiO replica of jute. Here, too, and even more so than in

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cotton, the obscuring of fine detail by non-cellulosic inclusions is apparent. Longitudinal bands appear to be a feature of the jute surface, also. Fig. 13 shows the surface of an acetate rayon fibre stretched to breaking point by hand. The appearance of zig-zag markings on the surface tallies with the observations previously noted by SIMMENS AND HOWLETT[25], but in Fig. 13 the markings are clearly not continuous trenches or ridges, but consist of lines of pits or protruberances (more precise interpretation is impossible in the absence of stereoscopic micrographs, which were not taken as this work was done before the advantages of stereoscopic methods were fully realized). Fig. 14 shows the surface of a raw silk fibre in which fibrils with a diameter of approximately 500 A are seen to run longitudinally.

These examples are sufficient to illustrate the scope of the single-stage technique; since we have not done any systematic work on these fibres, no further attempts at interpretation will be made here.

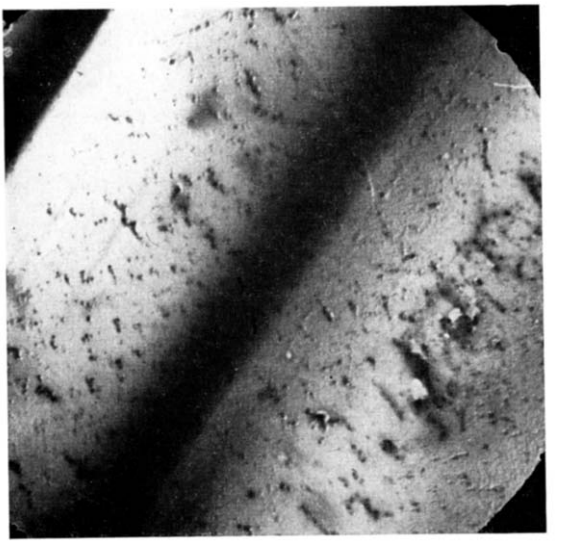

Fig. 13. Acetate rayon fibre, stretched to breaking point. Direct (negative) surface replica (SiO). Photographic print from original micrograph. × 4,000.

Fig. 14. Raw silk fibre. Direct (negative) surface replica (SiO). Photographic print from original micrograph. × 5,600.

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SUMMARY

Two methods of obtaining SiO replicas of fibre surfaces are described; both make use of a technique in which the fibre is partly embedded in a suitable plastic, leaving only a fraction of the surface exposed. In one, a direct replica is obtained by evaporating SiO, which is freed from the fibre by dissolving the embedding material; in the other, an intermediate silver replica (stripped from the fibre without disturbing it) is first taken, and a SiO positive made from it.

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Both techniques yield replicas showing much more detail than has previously been reported to be present in wool fibre surfaces. The two-stage technique lends itself to an experimental investigation of the reproducibility of the replicas, and to the possibility of comparing the same surface area before and after some surface treatment. Interpretation of the micrographs is facilitated by using stereoscopic methods, and in this way several new features of the wool fibre cuticle have been observed. In particular, the epicuticle is often torn at the scale tips; epicuticular welds or folds are found to run across the scale edges; and the individual scales are often bordered by a remarkable striated band. In general the scale tends to be broadly corrugated proximally and much smoother distally, while the whole surface is traversed by a system of fine longitudinal striations. These results have been obtained with Lincoln wool fibres.

A few applications of the single-stage technique have been made to fibres other than wool, and micrographs of the surfaces of cotton, jute, acetate rayon, and raw silk are given.

RÉSUMÉ

Deux méthodes permettant d'obtenir des empreintes en SiO de la surface d'une fibre sont décrites; elles utilisent toutes les deux une technique qui consiste à envelopper partiellement la fibre dans un plastique convenable, en laissant une partie seulement de la surface exposée. Dans l'une, une empreinte directe est obtenue en évaporant SiO, qui est ensuite détaché de la fibre en dissolvant le produit enveloppant; dans l'autre une empreinte intermédiaire en argent (ôtée de la fibre sans la détruire) est d'abord prise, et une empreinte positive en SiO en est faite. Les deux techniques fournissent des empreintes qui montrent, à la surface des fibres de laine, beaucoup plus de détails qu'il n'en avait été décrit antérieurement. La technique en deux étapes se prête à une étude expérimentale de la reproductibilité des empreintes et permet de comparer la même portion de surface avant et après un traitement quelconque de cette surface. L'interprétation des micrographies est facilitée par l'usage de méthodes stéréoscopiques, et de cette façon, plusieurs nouvelles propriétés de la cuticule de la fibre de laine ont été observées. En particulier, l'épicuticule est souvent déchirée aux extrémités de l'écaille; on observe des raccords ou des plis épicuticulaires qui traversent les bords de l'écaille; enfin les écailles individuelles sont souvent bordées d'une bande striée remarquable. En général, l'écaille a tendance à être plissée fortement dans sa partie proximale et plus légèrement dans sa partie distale, tandis que la surface entière est traversée par un système de belles stries longitudinales. Ces résultats ont été obtenus avec des fibres de laine Lincoln.

Quelques applications de la technique à une seule étape ont été faites à des fibres autres que la laine, et des micrographies des surfaces des fibres de coton, de jute, de rayonne à l'acétate et de soie brute sont données.

ZUSAMMENFASSUNG

Zwei Methoden zum Erhalten von Faseroberflächenmodellen aus SiO werden beschrieben; beide bedienen sich einer Technik, derzufolge die Faser teilweise in ein entsprechendes plastisches Material gebettet wird und nur ein Bruchteil der Oberfläche unbedeckt bleibt. Die eine Technik ergibt ein direktes Modell, durch Eindampfung des SiO, welches von der Faser befreit wird, indem es das einbettende Material auflöst. Die andere Technik bedient sich eines Silber-Zwischenmodells, welches von der Faser abgezogen wird, ohne die Struktur derselben zu verändern, wonach man ein SiO-Positiv anfertigt. Beide Techniken führen zu Modellen, welche weit mehr Einzelheiten aufweisen, als laut früherer Berichte auf Wollfaseroberflächen gefunden worden waren. Die indirekte Technik ermöglicht das experimentelle Studium der Reproduktibilität der Modelle, sowie den Vergleich desselben Oberflächengebietes vor und nach einer Oberflächenbehandlung. Die Auswertung der Mikrographien wird durch stereoskopische Methoden erleichtert; auf diese Weise konnten mehrere unbekannte Eigenschaften der Wollfasermembran beobachtet werden. So sind insbesondere die Epikutikeln oft an den Schuppenenden zerrissen; epikutikulare Verschmelzungen oder Falten werden an den Schuppenendungen beobachtet; die einzelnen Schuppen sind oft von einem bemerkenswerten gestreiften Band umgeben. Im Allgemeinen neigt die Schuppe zu weiten proximalen Falten und verhältnismässiger distaler Glätte, während die ganze Oberfläche durch ein System von feinen Längsstreifen durchzogen wird. Diese Ergebnisse wurden mit Lincoln Wollfasern erhalten.

Die direkte Technik wurde einigemale auf Nicht-Wollfasern angewandt und Mikrographien der Oberflächen von Baumwolle, Jute, Azetat-Kunstseide und Rohseide werden gebracht.

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