ELECTRON MICROSCOPY OF THE SURFACE STRUCTURE OF FEATHER

by

BO PHILIP, GÖSTA LAGERMALM, AND NILS GRALÉN
Swedish Institute for Textile Research, Gothenburg (Sweden)

INTRODUCTION

Both feathers and animal hairs are built up from keratins, and general analogies might be expected both in the inner structure and in the surface layers. As regards the surface, this is indicated by, for instance, the low friction of both feather and wool, and also by the water-repellency, even more pronounced for feather than for wool but nevertheless a common characteristic of both. It has been shown by LINDBERG, PHILIP AND GRALÉN that the surface of wool and other animal hairs is covered with a continous thin membrane, the epicuticle[1]. The following investigation was carried out in order to see whether any substance analogous to or identical with the wool epicuticle could be isolated from feather. This being the main purpose of the work, we have not discussed in detail all the purely morphological questions which may arise.

The epicuticle of wool and hair can be loosened from the main body of the fibre by a slight chlorination, or it can be isolated as the chemically most resistant component when the fibres are dissolved in sodium sulphide[1]. As revealed by electron microscopic investigation, the scale region of an animal hair can be divided into at least three different layers: the epicuticle, the exocuticle and the endocuticle. The thickness of the epicuticle, which most probably is non-protein, varies from 50 to 250 Å. It is chemically rather resistant. The exocuticle serves as a cementing material which fastens the epicuticle to the endocuticle, the main body of the scales. It is less resistant, but can be hardened by chemical treatment.

Optical microscopy

Through the work of several investigators the morphology of feather, to the limit of the resolution of the optical microscope, is very well known. We here briefly review the main structural features of an ordinary contour feather, referring to a paper by CHANDLER and using the nomenclature suggested by him[2].

A typical contour feather consists of a shaft or quill, the lower part of which is covered with down and the upper carrying the vane. The down is less specialized than the barbs of the vane and approaches animal hair in appearence. Thus the nodes of the downy barbules might be said to be analogous to the scales of hairs. The vane consists of barbs carrying two rows of barbules which are furnished with hooklets and cilia.

References p. 507.

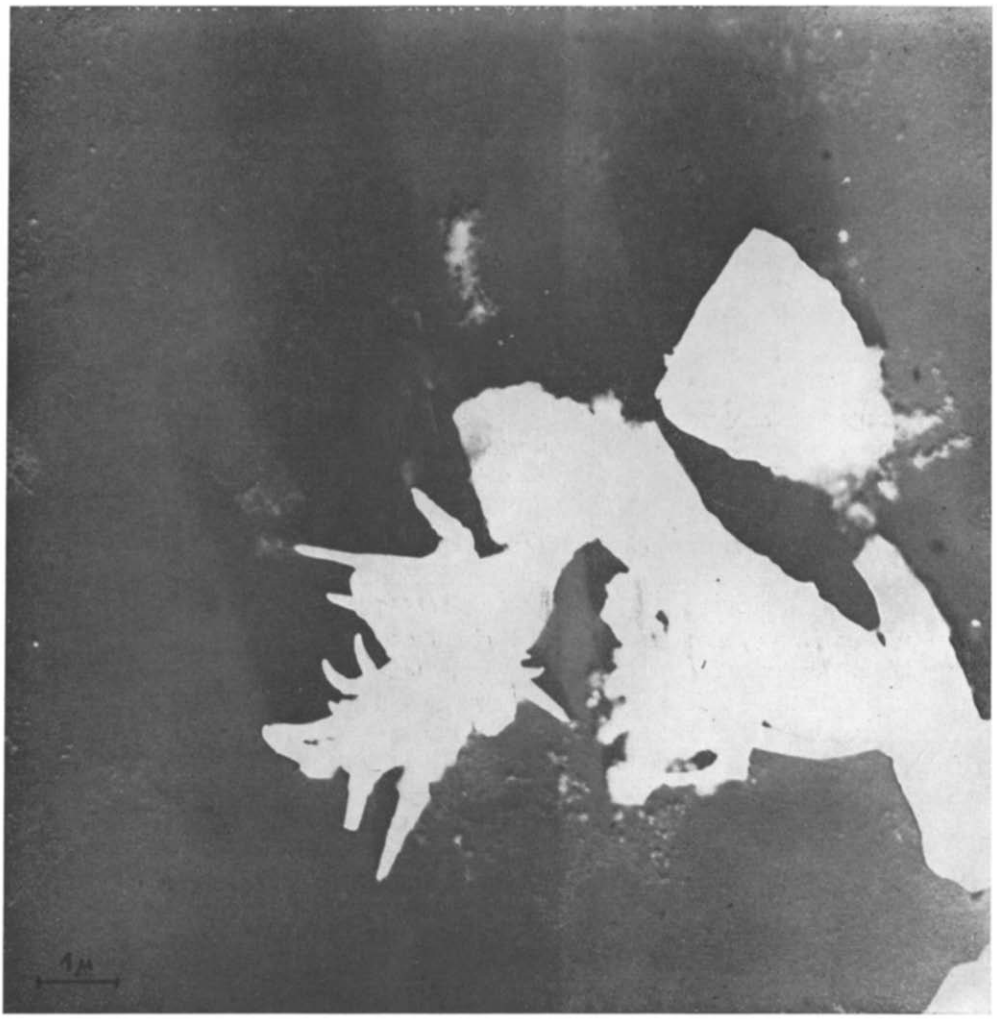

Fig. 1. Fragment from feather barbs after treatment with ultrasonics. Pd shadowing, 10 Å, 4:1. Note the small rods on the left of the specimen

One side of the barbule is flattened out to a thin membrane in which it is possible to distinguish border lines between the different cells.

Material

The material used for the preparations was vane and down from contour feathers of white domestic fowl. The feathers were first extracted repeatedly in toluene and ether-alcohol to remove grease. The vane and down were separated from the shaft and treated individually.

Direct observation in the Electron Microscope

Feather barbs and even barbules are much too thick to be examined in the EM. Some attempts were made to do this by simply placing a barbule on the specimen grid,

References p. 507.

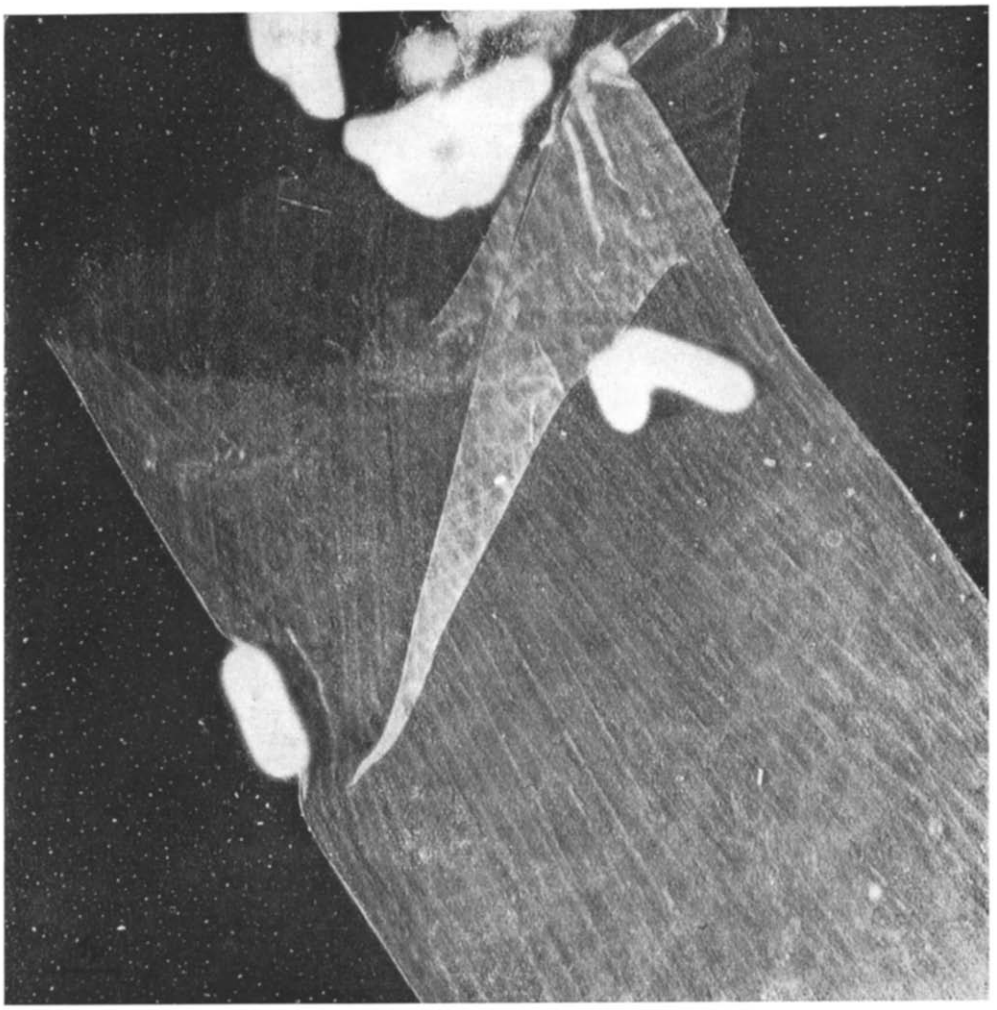

Fig. 2. Striated tubular membrane from tryptic digest of phenol-treated feather down. Pd, 10 Å, 4:1

but no satisfactory results were obtained. However, after mechanical disintegration of the feather barbs by treatment with ultrasonics (22,000 c/s) in water suspension, we were able to obtain pictures of some smaller fragments, as shown in Fig. 1.

Chemical and enzymic treatments

The chemical treatment of feather for preparing specimens suitable for the EM was essentially the same as used in this laboratory for studying wool fibres[3].

Phenolic treatment

Following a method of ELÖD AND ZAHN[4, 5], the barbs of the vane and the down were treated for 2 hours at 100°C in 50% phenol in water, washed with warm water and digested with trypsin at p_H about 9. The breakdown of the keratin was quite rapid

References p. 507.

Fig. 3. Double, folded membrane from tryptic digest of phenol-treated feather vane. Pd, 10 Å, 4:1

and took place in less than 24 hours. A white residue remained which still retained the shape of the original material. By shaking in water it was easily disintegrated into small fragments.

The specimen was prepared by placing a drop of the suspension on the collodion film of the specimen grid. After drying it was shadowed with 10 Å Pd at an angle of 4 to 1. In the EM it was found that most of the residue from the enzymic digestion had the character of membranous, often folded, sheets. Three kinds of structures were specially observed.

The longitudinally-striated flattened tube shown in Fig. 2 is from a preparation containing only downy barbules, but also in specimens from the vane similar tubes were found rather frequently, sometimes stretching over the whole opening of the specimen grid. This corresponds to a length of at least 50 microns, and the width is several microns.

References p. 507.

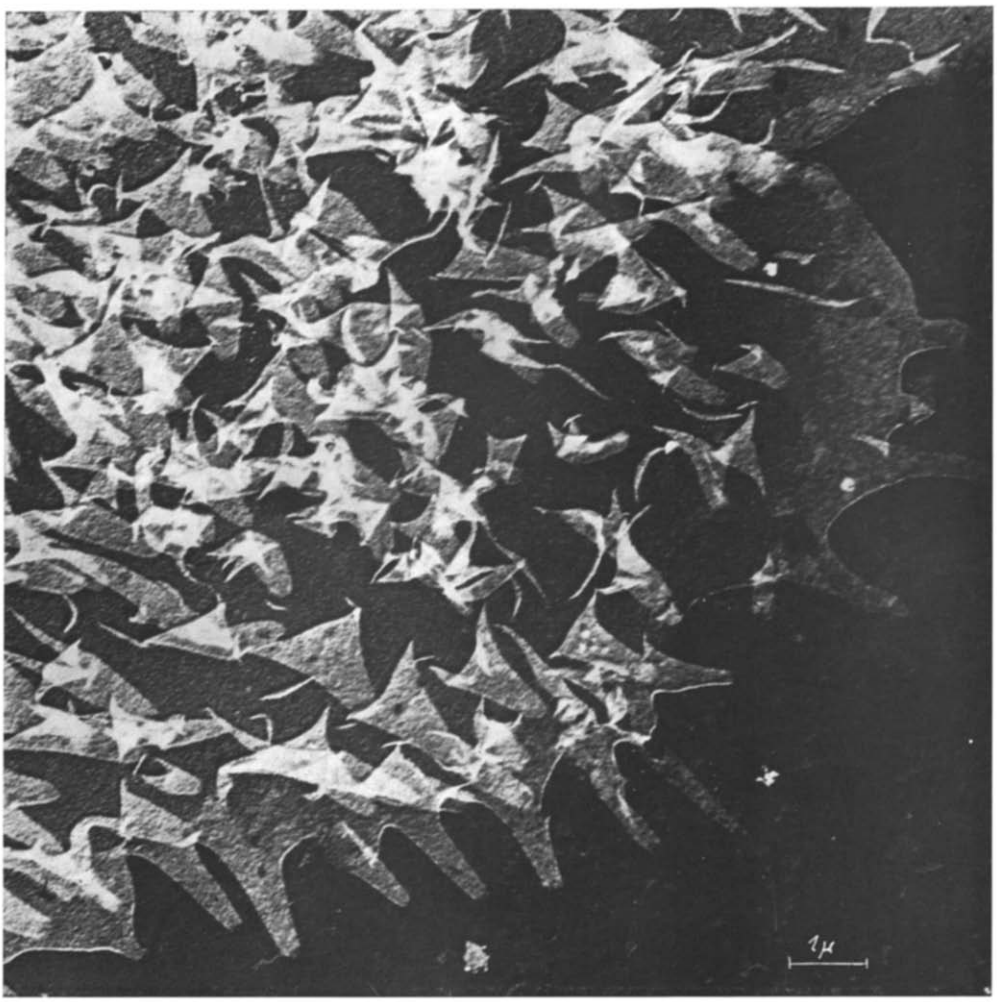

Fig. 4. Membrane covered with cornet-like sacs from tryptic digest of phenol-treated feather vane Pd, 10 Å, 4:1

The thickness is about 200 Å. They are the remnants from downy barbules or such parts on the vane as the cilia and hooklets. Their tips are usually broader with a thickened edge, corresponding to nodes or junctions with other parts. The striations give the impression of a replica of bundles of microfibrils.

Another kind of membrane of the same thickness also found quite frequently in preparations from the vane is illustrated by the large sheets shown in Fig. 3. They are often folded and in most cases double. The striations are found even here, though less marked. These sheets probably originate from the lamellar base of the barbules.

The most interesting kind of membrane, however, is the one shown in Figs. 4 and 5. The main part of it is very thin, uniform and even, but the surface is covered by numerous flattened cornet-shaped sacs, most of them having the same general shape and order of magnitude, though in some places only rudimentary. The sacs all show the same

References p. 507.

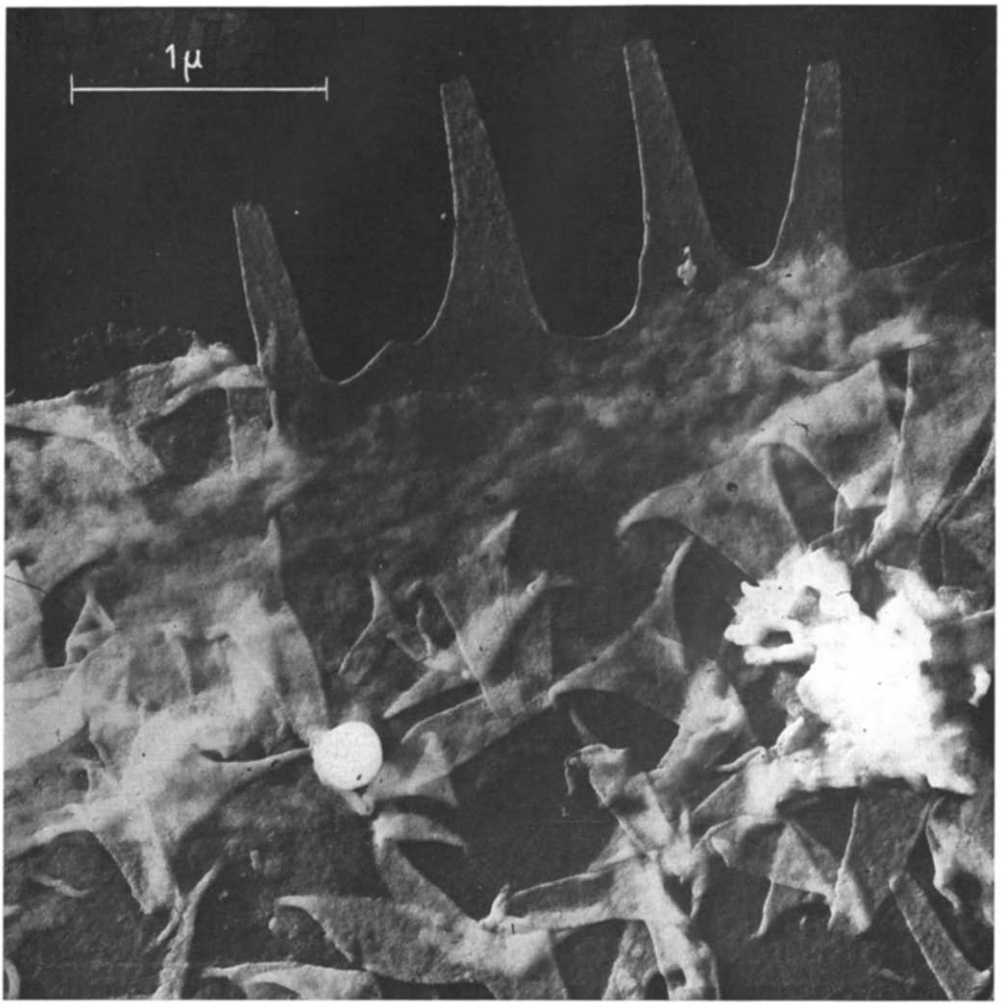

Fig. 5. Detail of cornet-like sacs from same specimen as Fig. 4. Pd, 10 Å, 4:1

slightly curved base and a contour reminiscent of a steep normal frequency curve. The basal membrane is a single layer, but near the edges it is folded along the base-line of some of the sacs, which therefore point outwards from the membrane itself.

The membranes of this kind are sometimes very large, stretching over several openings in the specimen grid. This corresponds to a length of more than 0.1–0.2 mm. The length of the sacs is slightly more than 1 micron. They are just at the limit of resolution of the optical microscope and are probably identical with small dots which can be seen on the horny keel of the barb using oil immersion. Whether the barbules, too, are equipped with formations of this kind cannot be determined from optical microscopy. The thickness of the membrane, calculated from the length of the shadows in the electron micrographs, is approximately 60 Å, which is almost the same as for the epicuticle of wool when isolated in a similar manner by the action of phenol and

References p. 507.

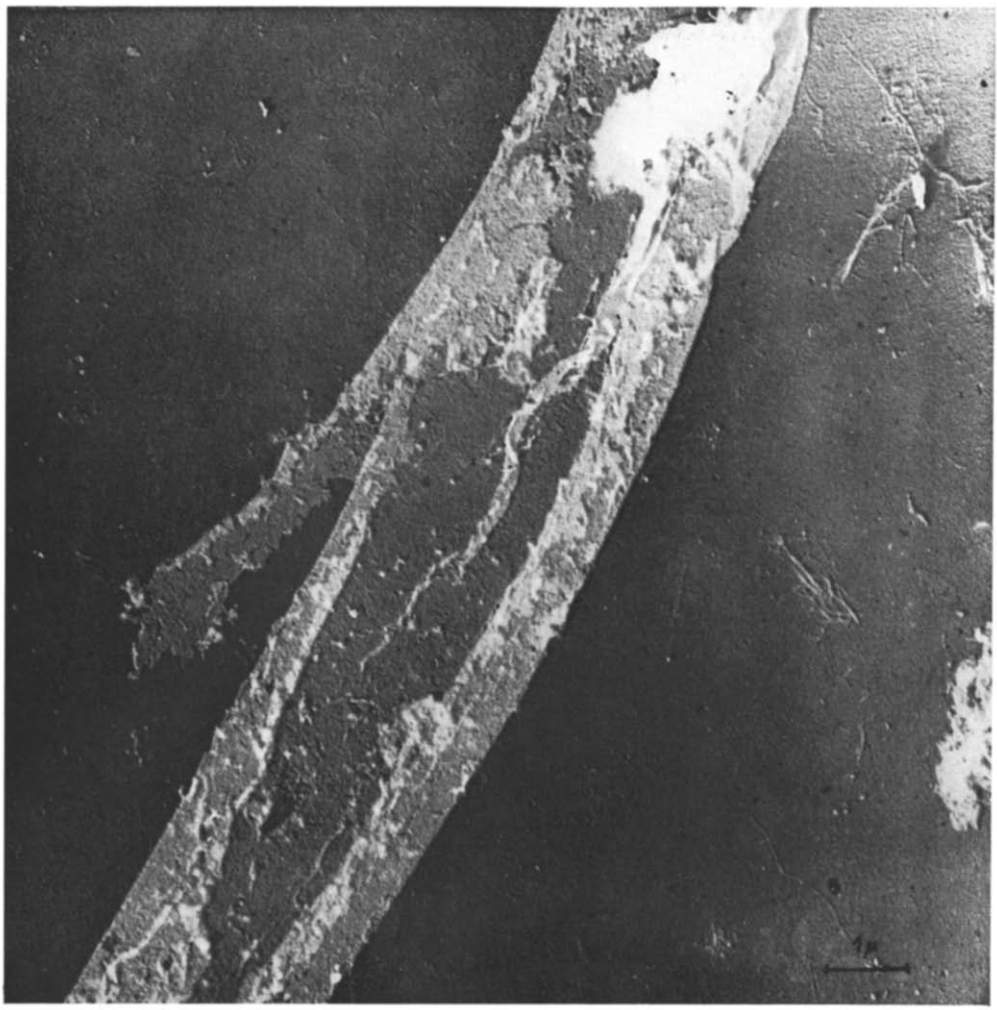

Fig. 6. Folded membrane from brominated feather barbs. Pd, 10 Å, 4:1

trypsin. The sacs are certainly the residues from the "rods" or "microhorns" in Fig. 1, but here the keratinous body has been dissolved away and only the empty sacs are left. Even when the sacs have always a curved base, it can be noticed that both the curvature of the base-line and also the height of the sacs vary. The curvature of the base-line of the empty sacs after falling down must depend on the cross-section and the stiffness of the bell-like formations and also on the shape of the line of junction between the bells and the basal membrane. This border line is probably not very distinct, if at all noticeable. The micro-horns will give the surface a velvet-like appearence and may be considered as some kind of villi.

Bromination of feather barbs

Most animal hairs when subjected to a slight action of chlorine or bromine show

References p. 507.

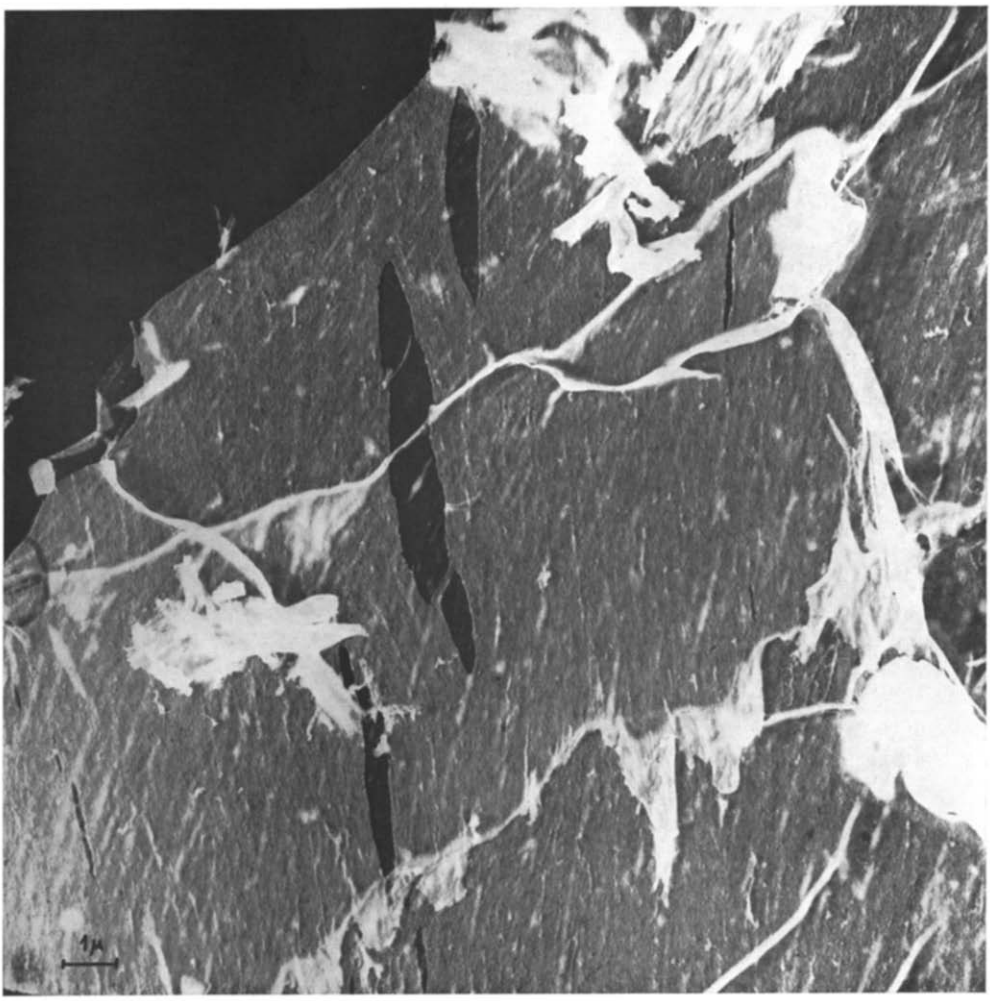

Fig. 7. Double, striated membrane from feather vane treated with alcohol-bisulphite. The white ridges may belong to cell walls. Pd, 10 Å, 4:1

the ALLWÖRDEN reaction[6]. The epicuticle acts as a semipermeable membrane and, because of the osmotic pressure caused by the degradation products from the underlying layer, forms small blisters covering the surface of the fibre[7].

When feather was treated in a similar way no such reaction was observed. On shaking the feather vanes with water after bromination in saturated bromine-water, the water became slightly opalescent. This indicates that some surface material has been loosened by the halogen, just as with wool fibres. When a droplet was placed on a specimen screen, dried and shadowed, a picture such as Fig. 6 was obtained. The thin (only about 60 Å thick) folded membranes very much resemble the epicuticle obtained in a similar way from wool, but there seem to be some differences. The membranes from feather are more or less ruptured, mostly in one direction. They give the impression of

References p. 507.

being more brittle than the wool epicuticle. This might be the explanation why the ALLWÖRDEN reaction does not occur on feather.

Solution of feather in Na_2S

Since the epicuticle of wool was discovered when treating wool with sodium sulphide solution, the feather barbs were dissolved in 1% Na_2S at 50°C, with the same short-time treatment as was used for wool[8]. The residue when examined in the EM showed membranes very little different from those obtained by tryptic digestion of phenol-treated material. They were perhaps not so completely free from adhering material and the sacs were often filled with keratin.

Other chemical treatment

In a paper by LUNDGREN and co-workers[9] it is reported that feather can be partly dissolved in 50% alcohol with the addition of a reducing agent. Following this method feather barbs were treated with 50% methanol to which had been added 0.1% sodium bisulphite. The mixture was kept near the boiling point for several hours, and the residue was washed with hot 50% methanol.

Under the optical microscope the specimens had exactly the same shape as the original feather components. For EM study they were violently shaken with water in order to obtain a suitable suspension. As after phenol-trypsin treatment big membranous sheets appeared (Fig. 7), now considerably thicker (500–800 Å) than the specimens from phenol-trypsin or Na_2S treatment, and also in most cases double. Here and there compact ridges occured, probably belonging to cell walls. The impression of a replica of more or less parallel fibrils is even more evident, and where double layers occur the striations run in two main directions, suggesting a replica of crossing fibrils. Crossing fibrils in feather have been observed earlier at this institute[10]. The sacs in these preparations also seemed more dense, most of them too dense to show any internal structure.

DISCUSSION

As the cross-linkages between the molecules are less frequent in feather keratin than in wool and other animal hairs, feather is more easily dissolved. Thus it is possible to dissolve 80% of it in alcohol-water mixtures if at the same time disulphide bonds are broken by a reducing agent[9]. In the surface layers of wool the disulphide bonds are more frequent than in the rest of the fibre[11]. This might be the explanation why the cuticle of wool is more resistant to chemical and enzymic treatment than the rest of the fibre. A similar explanation might be valid also for feather.

The phenolic treatment makes the central parts of the keratinous body digestible by trypsin. After the enzymic treatment a tubular residue is left which retains the original shape of the material. This is a feature common to both feathers and hairs. In wool this formation of a tubular residue is ascribed to a hardening action on the exocuticle which makes it stick to the epicuticle[3]. The action on feather seems to be the same as on wool. The keratin of the tubular residue is digestible by prolonged treatment. The only component left will be the thin outer layer which probably is non-protein. As this layer corresponds to the epicuticle of animal hairs we propose that the same name be used with feather also.

The underlying keratinous layer, not so easily attacked as the main part of the

References p. 507.

feather, corresponds to the exocuticle of animal hairs. In feather this layer envelopes the main body built up from fine fibrils, as may be concluded from the impressions found on these membranes when observed in the EM. On account of the general relationship to the exocuticle of animal hairs it may be justifiable to use the same term, exocuticle, also for the second enveloping layer of feather.

As to the rods or micro-horns it seems possible that their function is to complete the hooklets and cilia by increasing friction and thereby strengthening the joints between the constituents of the vane. Thus the air movement through the feather is diminished, which increases both the carrying power in flight and the heat insulation. As the formations illustrated in our pictures originate from a bird which is a very poor flyer, it may be possible that similar formations of a modified type can be found on feathers from other groups of birds.

The epicuticle on feather very probably forms a non-wettable surface and thereby contributes to the water-repellency. The micro-horns may form capillary spaces which are constantly filled with air, thus preventing water from penetrating. On that part of the barb which is a constituent of the surface of the vane, the micro-horns may give the surface properties resembling those of the wool fibre where the friction is very different in different directions. Thus relative positions of the feathers, suitable for the requirements of the bird, may be favoured.

ACKNOWLEDGEMENT

One of us (G.L.) wishes to express his thanks to the International Wool Secretariat for a research fellowship enabling him to carry out studies on the epicuticle.

SUMMARY

Feather vane and down have been subjected to chemical and enzymic treatment and specimens obtained in this way examined in the electron microscope.

It has been found that the main body of the feather parts is enveloped by a cuticular sheath of a somewhat different character. The cuticle can be divided into at least two different layers, the outer being probably of non-protein composition, and the inner a more resistant keratin layer. They are called epi- and exocuticle, in view of the close relationship with corresponding layers in the cuticle of wool and other animal hairs. It seems probable that the epicuticle is responsible for such properties as water repellency, low friction and the chemical inertness of the surface.

In parts of the vane the surface is covered with small rods (length around 1 micron). These micro-horns give a velvet-like appearence to the surface, which might be helpful in increasing the water-repellency and in reducing the mobility of air, thus increasing the carrying power during flight and the heat insulation. They may also give the feather parts frictional properties which favour suitable relative positions of the feathers.

RÉSUMÉ

Nous avons soumis des barbes de plumes et du duvet à des traitements chimiques et enzymatiques; les spécimens ainsi obtenus ont été examinés ensuite au microscope électronique.

Nous avons trouvé que le corps des parties de plume examinées était enveloppé d'une cuticule en forme de gaine de caractère quelque peu différent du corps principal. L'on peut distinguer dans la cuticule au moins deux couches différentes; la couche extérieure n'est probablement pas de nature protéique, tandis que la couche intérieure est constituée de kératine plus résistante. On appelle ces couches épi- et exocuticule à cause de leur parenté avec les couches correspondantes de la laine et d'autres poils d'origine animale. Il semble probable que l'épicuticule soit responsable de certaines propriétés, telles que la répulsion d'eau, le faible frottement et l'inertie chimique de la surface.

References p. 507.

En certaines régions la surface des barbes est couverte de petites tiges (longueur de 1 micron environ); celles-ci donnent une apparence velouté à la surface qui pourrait rendre cette dernière plus hydrofuge et réduire le mobilité de l'air; ainsi la force portante pendant le vol serait augmentée et l'isolation améliorée. Ces tiges pourraient aussi donner à certaines parties de la plume des propriétés de frottement qui favorisent des positions rélatives convenables des plumes.

ZUSAMMENFASSUNG

Federfahnen und Flaum wurden chemisch und enzymatisch behandelt und die so erhaltenen Präparate im Elektronen-mikroskop untersucht.

Es wurde festgestellt, dass der Grundkörper der untersuchten Federbestandteile von einem hülsenförmigen Häutchen von etwas abweichendem Charakter umgeben ist. In dem Häutchen kann man mindestens zwei verschiedene Schichten unterscheiden, die äussere, die wahrscheinlich nicht Eiweisscharakter hat und die innere, die eine widerstandsfähigere Keratinlage darstellt. Sie werden auf Grund ihrer Verwandschaft mit den entsprechenden Schichten der Wolle und anderer tierischer Haare "Epi-" und "Exokutikula" genannt. Es ist wahrscheinlich, dass erstere für die wasserabhaltenden Eigenschaften, die geringe Reibung und die chemische Trägheit, sowie für sonstige derartige Eigenschaften der Oberfläche verantwortlich ist.

Die Oberfläche der Fahne ist stellenweise von kleinen Stäbchen (Länge ungefähr 1 Mikron) bedeckt. Diese Erhöhungen geben der Oberfläche ein samtartiges Aussehen, welches die wasserabhaltenden Eigenschaften erhöhen und die Bewegung der Luft verringern könnte, wodurch die Tragkraft während des Fluges und die Isolierung verbessert würden. Sie könnten auch den Bestandteilen der Feder Reibungseigenschaften verleihen, die günstige gegenseitige Lagen der Federn bevorzugen würden.

REFERENCES

[1] J. LINDBERG, B. PHILIP, AND N. GRALÉN, *Nature*, 162 (1948) 458.
[2] A. C. CHANDLER, *Univ. of California Publ. in Zoology*, 13 (1916) 243.
[3] E. H. MERCER, J. LINDBERG, AND B. PHILIP, *Textile Research J.*, 19 (1949) 678.
[4] E. ELÖD AND H. ZAHN, *Melliand Textilber.*, 28 (1947) 217.
[5] H. ZAHN, *Thesis*, Heidelberg, 1947.
[6] K. VON ALLWÖRDEN, *Z. angew. Chem.*, 29 (1916) 77.
[7] J. LINDBERG, *Textile Research J.*, 19 (1949) 43.
[8] G. LAGERMALM AND B. PHILIP, *Textile Research J.*, 20 (1950) 668.
[9] H. P. LUNDGREN, A. M. STEIN, V. M. KOORN, AND R. A. O'CONNELL, *J. Phys. Colloid Chem.*, 52 (1948) 180.
[10] B. PHILIP, E. H. MERCER, Unpublished results.
[11] W. B. GEIGER, *J. Research Natl. Bur. Standards*, 32 (1944) 127.

Received October 8th, 1950