KERATIN FIBRES

IV.* STRUCTURE OF CUTICLE

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Summary

The cuticle structure of keratin fibres of placental mammals, marsupials, and monotremes has been examined by the techniques of light microscopy and scanning electron microscopy. These techniques have been used to study both intact fibres and whole cuticle cells separated by formic acid treatment.

Observations have been made on: (1) the size and shape of individual cuticle cells and the manner in which they are arranged in the surface of the fibre; (2) the large number of false scale edges (i.e. scale markings which do not correspond with the distal edge of a cuticle cell) for kangaroo and platypus fur; (3) variations in scale pattern along the length of seal and platypus fur and guard hair. The mechanism of scale formation in the follicle is discussed.

I. Introduction

The gross features of the scale patterns on keratin fibres have been revealed by light microscopy, and the detailed structure of the cuticle cell on intact fibres by electron microscopy of longitudinal and cross sections (reviewed by Ryder 1963; Lundgren and Ward 1963). However, there is a gap in our knowledge which results from the limitations of the microscopic techniques.

Extension of a technique used by Hock, Ramsay, and Harris (1941) has resulted in the separation of intact cuticle cells for study by light microscopy (Leeder and Bradbury 1968). The integrity of these cells can be checked by the fact that they develop Allwörden sacs on treatment with chlorine water, whereas cuticle cell fragments produced by mechanical agitation (Bradbury and Chapman 1964; Bradbury, Chapman, and King 1965) do not. Furthermore, the use of scanning electron microscopy has enabled surface structures to be examined in considerable detail.

In this paper we report on a microscopic study of the cuticle structure of a wide range of keratin fibres; particular attention was devoted to the shape and size of cuticle cells and the manner in which they are arranged in the surface of the fibre.

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II. Experimental

Merino 64's fleece wool and Lincoln 36's wool processed to "top" form was cleaned as described previously (Bradbury *et al.* 1966). The red human hair was from an 8-year-old girl, the source of the kangaroo fur, platypus fur and guard hair was given previously (Bradbury and O'Shea 1969), the Alaskan seal fur was provided by Dr. J. Menkart, Harris Research Laboratories, Washington, D.C., and seal guard hair by the Returned Soldiers and Sailors Woollen Mills, Geelong, Vic. The hair and fur samples were washed with 1% aqueous detergent (Gardinol BW) (Bradbury and Chapman 1964), water, and dried in air. They were then washed in heptane, ethanol, and dried in air.

(*a*) *Production of Separated Cuticle Cells*

Earlier methods used to prepare histologically pure samples of cuticle cells have involved ultrasonic disintegration (Bradbury and Chapman 1964) or mechanical agitation (Bradbury, Chapman, and King 1965) in 98–100% formic acid. Fragmented cuticle cells are produced by these methods, since very few are large enough to represent complete cells and it is not possible to develop Allwörden sacs on them (King 1967; Leeder and Bradbury 1968). Single, intact, cuticle cells were obtained in small amounts by heating fibres in formic acid for 1 hr at 100°C without mechanical agitation. The cells were separated from the bulk of fibres, concentrated by centrifugation, and washed with water. The aqueous suspension was examined microscopically.

It was found that sheets of cuticle cells could be produced by treatment with 0·01M HCl at 100°C for 20 hr followed by 1 hr in formic acid at 100°C. The cuticle cell sheets were washed with water and examined microscopically.

(*b*) *Allwörden Reaction*

Saturated chlorine water was freshly prepared by bubbling chlorine gas through distilled water at 0°C and then allowing the solid mass to heat up to room temperature. Fibres, or in some cases separated cuticle cells, were treated with chlorine water on the microscope slide and the development of the Allwörden sac (Allwörden 1916) observed microscopically.

(*c*) *Microscopy*

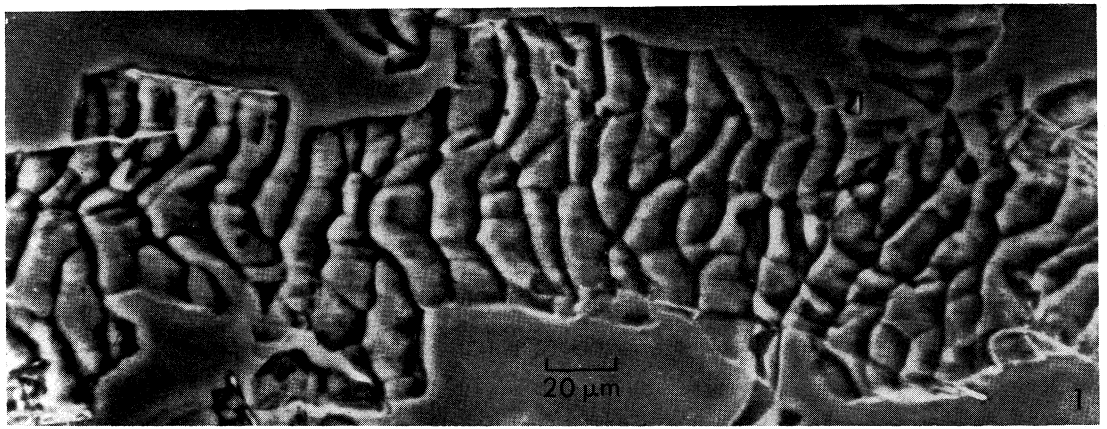
A Leitz Dialux microscope was used with phase-contrast illumination for examination of separated cuticle cells and Allwörden sacs. Fibre cross-sections were prepared using a Hardy microtome. Scanning electron micrographs were obtained with the Cambridge stereoscan electron microscopes located at the Cambridge Instrument Company, England, or at the Defence Standards Laboratory, Melbourne. All samples for scanning electron microscopy were coated with a 30 nm thick layer of gold–palladium prior to examination.

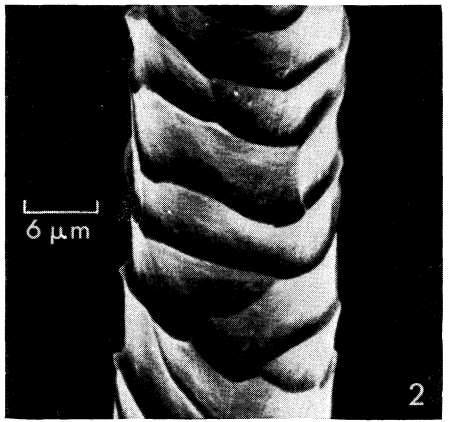
III. Results and Discussion

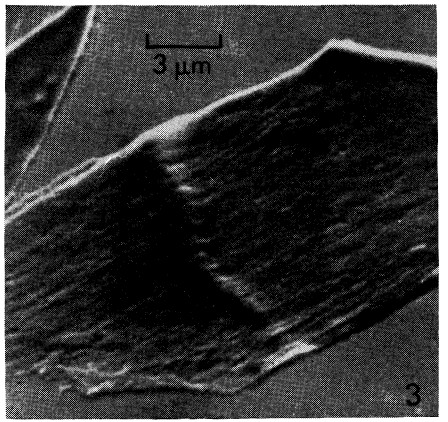
(*a*) *Merino Fibres*

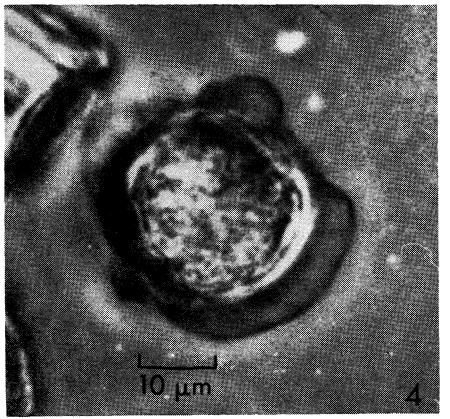
The average distance between successive scale edges was measured on optical micrographs of intact fibres and on cuticle sheets (Fig. 1) and found to be 12 μm. On the other hand the length of fibre surface spanned by Allwörden sacs (Fig. 1 of Leeder and Bradbury 1968) was rather more variable with an average value of about 18 μm. It is assumed that each Allwörden sac covers a single cuticle cell (Muller 1939; Leeder and Bradbury 1968) and on this basis about one-half of the cuticle cells would have a second or false scale edge as shown in Figure 3 and found by Bradbury and Rogers (1963) and Bradbury and Chapman (1964).

The length of those cuticle cells which are able to form Allwörden sacs (Fig. 3 of Leeder and Bradbury 1968) and hence are considered to be intact is very variable with an average value of about 30 μm and a maximum of 60 μm. This distance



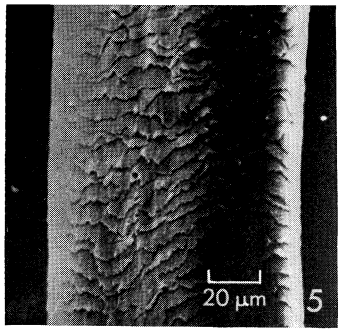


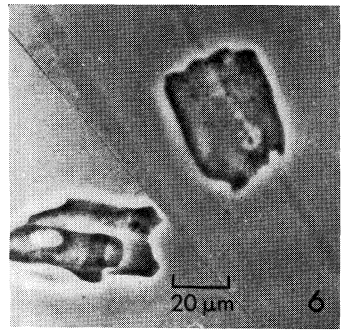


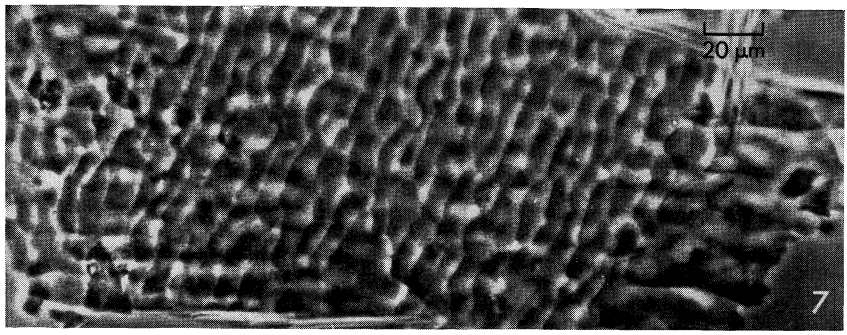


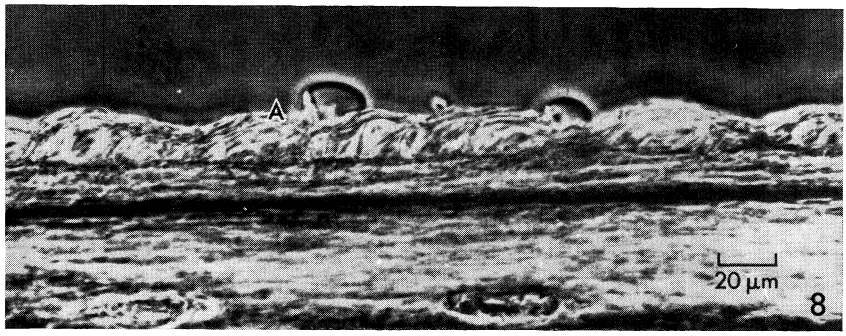
Figs. 1 and 4.—Light micrographs of : *1*, sheets of Merino cuticle; *4*, cross-section of Merino fibre treated with chlorine water, showing Allwörden sac which surrounds about one-half of the circumference of the fibre.

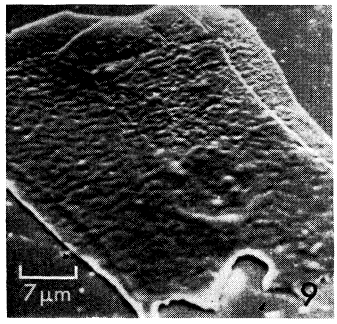
Figs. 2 and 3.—Scanning electron micrographs of: *2*, Merino fibre; *3*, a fragment of Merino cuticle.











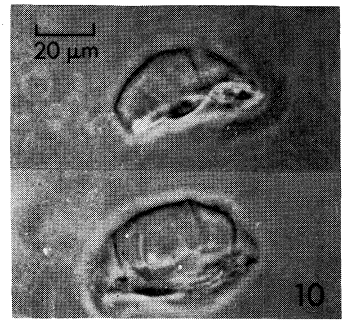


Fig. 5.—Scanning electron micrograph of human hair.

Fig. 6.—Cuticle cells from human hair as shown by light microscopy.

Fig. 7.—Light micrograph of sheet of human hair cuticle.

Fig. 8.—Light micrograph of human hair after treatment with chlorine water. Note the scale flap at A.

represents the transverse dimension of the scale on the intact fibre of diameter about 20 μm, as shown in the cross-section in Figure 4. On the average there appear to be two scale cells around the circumference of the fibre, in agreement with Wildman (1955). However, the variability of the structure is further emphasized by examination of the sheets of Merino cuticle shown in Figure 1.

Kassenbeck (1958) postulated the occurrence of orthocuticle and paracuticle cells which were apparently associated with the orthocortex and paracortex of wool fibres (Ryder 1963). He proposed that false scale edges occur only on the orthocuticle. If this were the case one might expect the Allwörden sacs on one side of the fibre to be longer than on the other side. We have not observed this. Secondly, because of the extreme variability of the distribution of scale junctions in the transverse direction of the fibre (see Figs. 1 and 2) it seems very unlikely that there are differences of this type in the cuticle cells capable of producing the ortho-para effect in Merino fibres (Chapman and Bradbury 1968).

(*b*) *Lincoln Fibres*

Cuticle cells from Lincoln 36's fibres (diam. *c.* 60 μm) have approximately the same dimensions as those of Merino fibres. Furthermore, the dimensions of the Allwörden sacs on the fibre and of the isolated cuticle cells are the same as those of Merino fibres. This indicates a similar frequency of occurrence of false scale edges and a similar degree of overlap of cuticle cells in the longitudinal direction (about one-sixth the length of a cell—Appleyard and Greville 1950) with both Merino and Lincoln fibres. A consequence of the threefold increase in diameter of the Lincoln fibre over the Merino fibre is that there should be on the average about three times as many cuticle cells around the circumference of the former, viz. about six.

(*c*) *Human Hair*

Whereas the cuticle of wool is normally one cell thick except for the region where adjacent scales overlap, the cuticle of human hair (diam. *c.* 125 μm) and of most coarse keratin fibres is many cells thick (Rudall 1941; Appleyard and Greville 1950). This is confirmed by the present work where the average distance between successive scale edges in Figures 5 and 7 is about 6 μm, whereas the average dimensions of isolated cuticle cells is about 30 by 40 μm (Figs. 6, 9, 10). Thus only about one-fifth to one-sixth of each cuticle cell in the longitudinal direction is exposed on the surface of the fibre, the remainder is covered by overlapping neighbouring cells. This agrees well with the result obtained by Appleyard and Greville (1950) using a different method.

Because of the constraints imposed by these overlapping cells, the Allwörden sacs are unable to develop as on wool fibres, but rather the whole surface is raised (Fig. 8). Also, cuticle cells which have been dislodged from the surface are able to form Allwörden sacs in the normal manner. A similar effect is observed at the end of a cut fibre, where the last cuticle cell is not constrained by adjacent cells and hence forms a normal sac. Cuticle cells isolated from the fibre also show the same type of Allwörden sac as those obtained from wool (Fig. 10).

Fig. 9.—Scanning electron micrograph of a cuticle cell from human hair.

Fig. 10.—Allwörden sacs developed by treatment of cuticle cells from human hair with chlorine water.

A general feature of the cuticle cells from human hair is the occurrence of the small, circular, thin regions shown by light microscopy (Fig. 6) and reflection electron microscopy (Fig. 9). The origin of this feature is not known, but perhaps it may arise from the nucleus of the cuticle cell. Close examination of the cuticle cells showed no evidence for the occurrence of false scale edges.

(*d*) *Kangaroo Fur*

Although kangaroo fur is superficially similar in its cuticle structure and diameter to a Merino fibre (Fig. 11) it is very different histologically because it contains a significant amount of medulla (Bradbury and O'Shea 1969). Furthermore, isolated cuticle cells have the structure shown in Figure 13, the approximate dimensions being 30–50 μm long and 20 μm wide. These cells represent whole single cells since a single sac is produced on each cell (Fig. 14). The longitudinal distance between scale edges in Figure 11 is about 10–15 μm and the longitudinal dimension of Allwörden sacs in intact fibres (Fig. 12) is 20–45 μm. This shows that (1) the cuticle cells are oriented with their larger dimension (30–50 μm) in the direction of the fibre; (2) there is only a small degree of overlap of cuticle cells on the longitudinal direction; and (3) there are about three false scale edges per cuticle cell.

Since the shorter dimension (20 μm) is placed across the fibre there are about four cuticle cells around the circumference of the fibre as compared with only about two for Merino wool. However, the more striking difference is the occurrence of about two or three false scale edges per cuticle cell (Fig. 13) as compared with only one false scale edge on every second cell for Merino wool. This greatly reduced overlap of cuticle cells along the fibre, combined with the smaller amount of wrapping around the fibre, probably accounts for our observations of a much more rapid breakdown of kangaroo fur than Merino wool when agitated gently in formic acid.

(*e*) *Seal Fur and Seal Guard Hair*

The seal fur fibre (diam. 15 μm) shown in Figure 15 has a surface which appears to be devoid of surface cracks or crevices and in which the scale cells do not project from the surface as much as with the other fibres (Figs. 2, 5, 11). This appears to be the reason for the complete lack of breakdown of the fibres when treated according to the methods used in this paper or on prolonged subjection to ultrasonic disintegration in formic acid (Bradbury and Chapman 1964). In addition, Allwörden sacs require 30–60 min for full development as compared with 15 sec for Merino fibres (Leeder 1969). There appear to be several sacs covering each scale cell (Fig. 16), but it was not possible to study this further because of inability to disrupt the fibre.

The guard hair of the seal is very variable in diameter (40–200 μm) and the scale pattern changes greatly along the length of a single fibre. This has been noted previously in some keratin fibres by Hardy and Plitt (1940), Lyne and McMahon (1951), and Wildman (1955). Figures 17–19 show the change of scale pattern from the base of the fibre [which resembles coarse wool fibres (Wildman 1955)], to mid-shaft (which shows a "diamond"-type pattern similar to that of seal fur) to the distal end

Fig. 15.—Scanning electron micrograph of seal fur fibres.

Fig. 16.—Light micrograph of Allwörden sacs produced after 15 min treatment of seal fur with chlorine water.

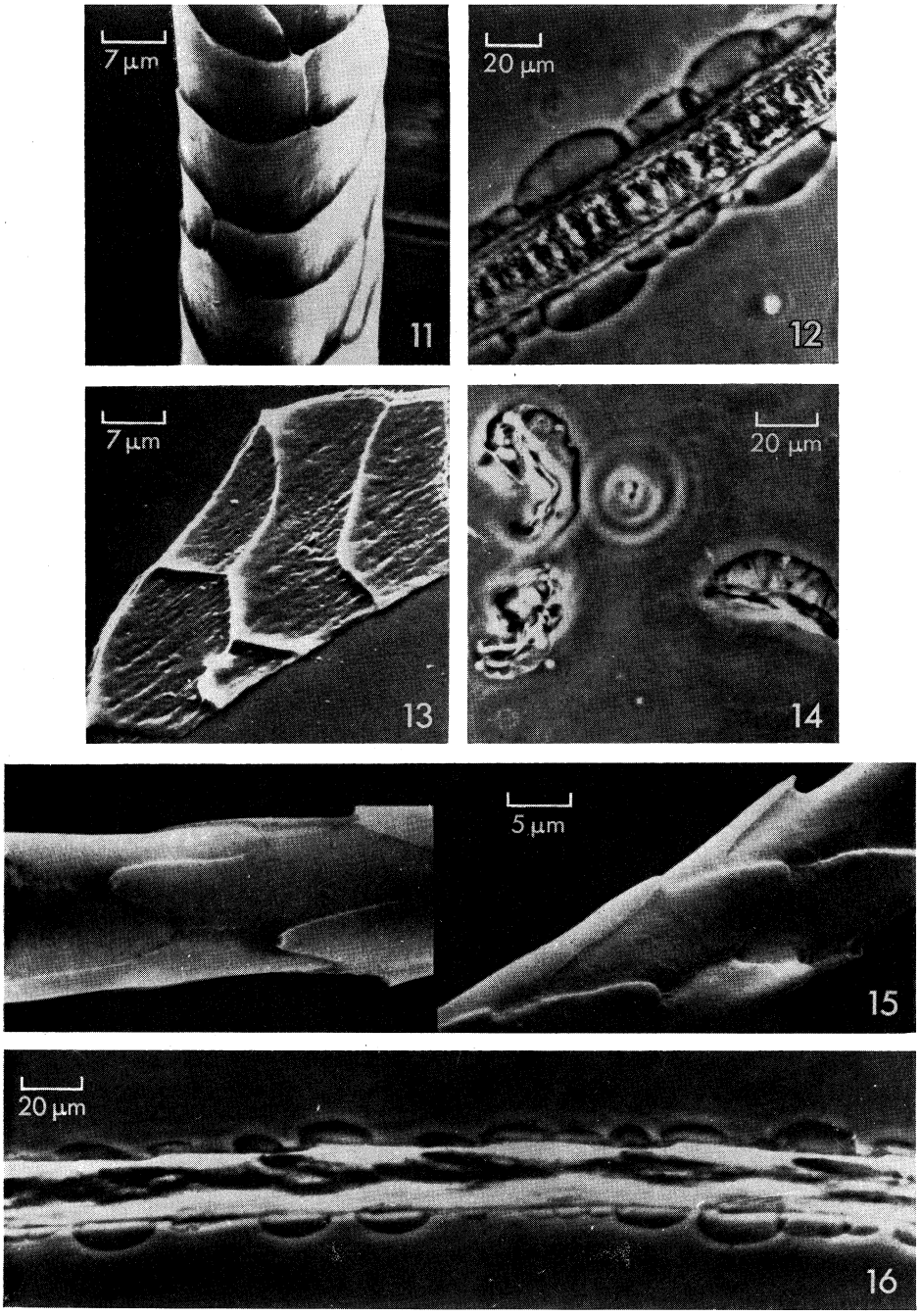


Fig. 11.—Scanning electron micrograph of kangaroo fur.

Fig. 12.—Light micrograph of Allwörden sacs on a kangaroo fibre after treatment with chlorine water.

Fig. 13.—Cuticle cell isolated from kangaroo fur; scanning electron micrograph.

Fig. 14.—Light micrograph of Allwörden sacs on cuticle cells isolated from kangaroo fur.

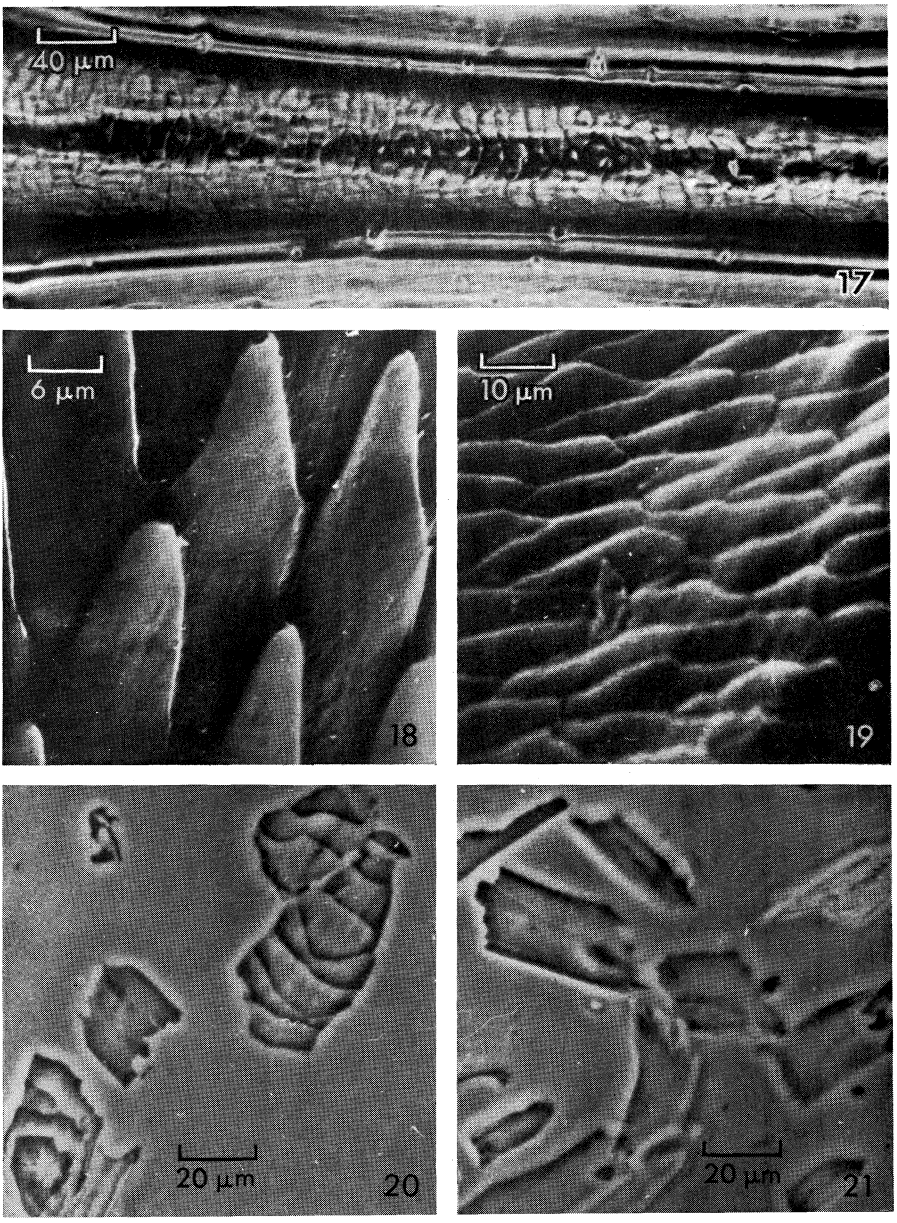


Fig. 17.—Light micrograph of seal hair near the basal (root) end, mounted in Sellotape. Note the dark core of medulla.

Figs. 18 and 19.—Scanning electron micrograph of seal hair at mid-shaft (*18*) and at distal end (tip of fibre) (*19*).

Figs. 20 and 21.—Light micrograph of seal hair cuticle cells from basal (*20*) and distal (*21*) end of fibre.

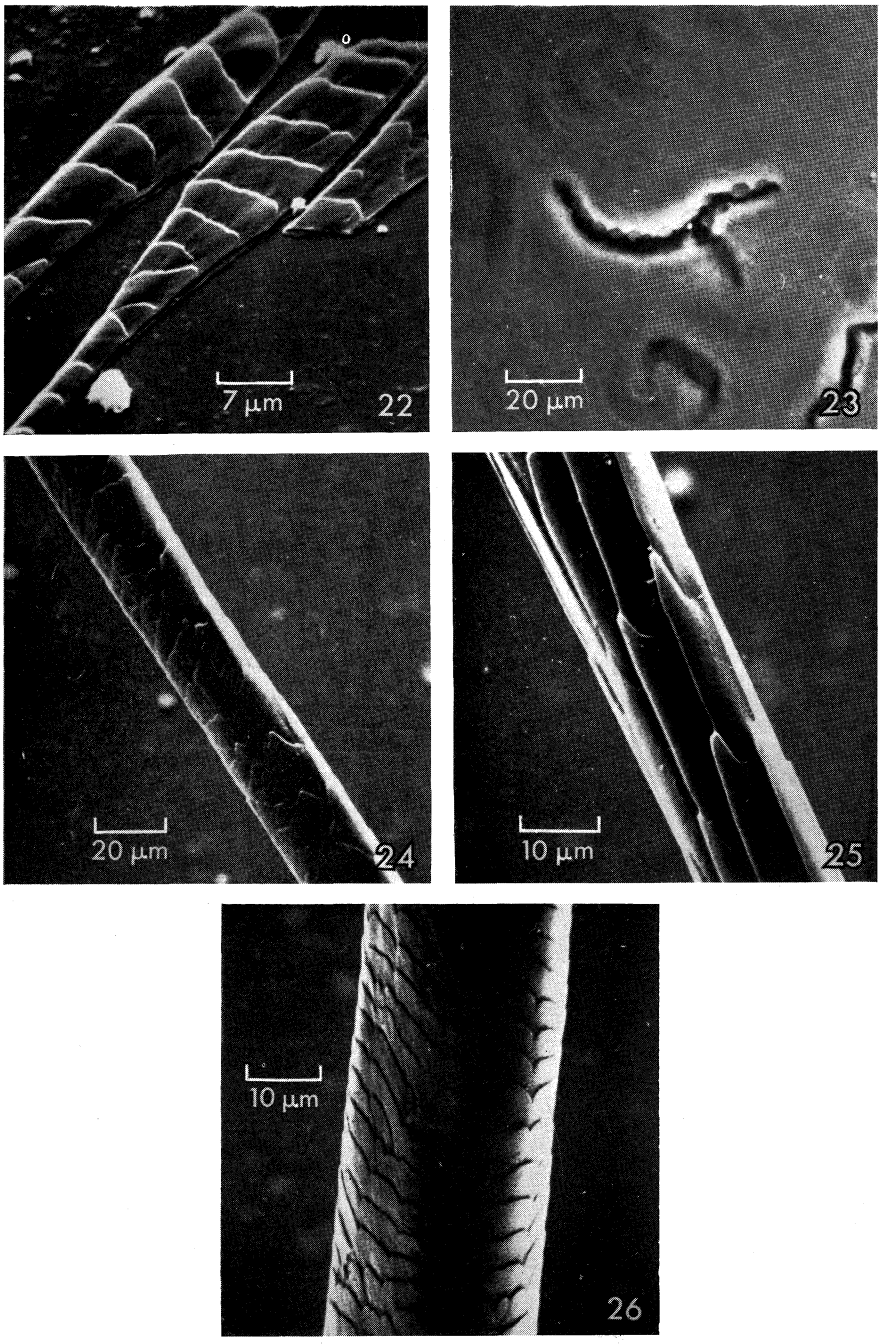


Fig. 22.—Scanning electron micrograph of cuticle isolated from platypus fur by the formic acid treatment.

Fig. 23.—Light micrograph of Allwörden sacs produced by treatment with chlorine water of a cuticle cell of the type shown in Figure 23.

Figs. 24–26.—Scanning electron micrograph of platypus belly guard hair in the basal region (*24*), at mid-shaft (*25*), and at the distal end (*26*).

(which has a cuticle structure similar to that of human hair). Treatment with chlorine water produces the "normal" type of Allwörden sac (shown in Fig. 12 for kangaroo) at the basal end of the fibre and a result similar to that found for human hair (Fig. 8) at the distal end of the fibre. Furthermore, the isolated cuticle cells from the basal end of the fibre shown in Figure 20 resemble closely cuticle cells from kangaroo (Fig. 13) and those from the distal end shown in Figure 21 are similar to those from human hair (Fig. 6). No isolated cuticle cells could be prepared from the diamond-type cuticle observed at mid-shaft, and Allwörden sacs formed very slowly with chlorine water, which in these respects is also similar to the case of seal fur.

The above results give clear evidence for the production of a fibre by the hair follicle which changes from the multiple cuticle (as in human hair) through the diamond-type structure which is so resistant to disruption, to the single, slightly overlapped cuticle with false scale edges shown by kangaroo. Ribbon-like folds are often observed in the transition regions between the different types of scale patterns.

(*f*) *Platypus Fur*

Fibres of platypus fur (diam. 10–15 μm) show a scale structure at the basal end which resembles seal fur and is likewise resistant to disruption. The distal end of the fibre resembles Merino wool or kangaroo fur and breaks down very readily to give cuticle cell fragments of the type shown in Figure 22. Treatment with chlorine water gives a large number of very small Allwörden sacs as shown in Figure 23 and the same type of sac formation was noted on treatment of the distal end of whole fibres. However, after several hours in chlorine water some of the small sacs coalesced to form long flat sacs similar to those which form on the basal regions of the fibre. The material shown in Figure 22 consists of pieces of about the same size and shape and even prolonged digestion in formic acid or hydrochloric acid followed by formic acid failed to effect subdivision into smaller units. It is therefore likely that each piece represents a single cuticle cell containing a large number (10–16) of false scale edges.

(*g*) *Platypus Guard Hair*

The guard hair from the belly of the platypus (Figs. 24–26) occurs in admixture with finer fur fibres and shows the same type of change in cuticle structure from base to tip as that observed with seal guard hair. However, the guard hair from the tail of the platypus showed no variation in scale pattern along its length and is identical in structure with that shown in Figure 26.

(*h*) *Summary of Cuticle Histology*

The data which have been given for the various fibres are summarized in Table 1. The first impression is one of great variability in properties over the various keratin fibres of placental mammals, marsupials, and monotremes. It is interesting to consider the present results in conjunction with the mechanism of formation of the scale pattern in the follicle (Birbeck and Mercer 1957; Rogers 1964). The inner root sheath which surrounds the growing fibre keratinizes (hardens) at a lower level in the follicle than the fibre and hence provides the template or mould for the scale structure of the fibre. Subsequently, the fibre keratinizes and the scale structure is

thus stabilized. As the fibre and inner root sheath move up the follicle, the latter is removed by a mechanism which does not concern us here and the fully formed fibre finally emerges from the follicle.

In the case of fibres with multiple cuticle layers such as human hair the scale edge on the fibre always corresponds with the distal edge of a cuticle cell. This would result from a situation in the follicle in which each cuticle cell was centred in the imbrication made by the inner root sheath. For wool fibres in which about one-half of the cuticle cells have one false scale edge, the cuticle cell is apparently able to distribute itself over two longitudinal imbrications of the inner root sheath. The results for kangaroo fur and the distal end of platypus fur indicate that the cuticle cells are spread over about 4 and 10–16 imbrications, respectively, in the longitudinal direction.

TABLE 1

SUMMARY OF CUTICLE PROPERTIES OF FIBRES

U, unknown

Fibre	Properties on the Fibre				Cuticle Cells	
	Multiplicity of Cuticle	No. of Cuticle Cells around Circumference	Variable Pattern along Fibre	Rate of Disruption in Formic Acid*	Size (μm)†	No. of False Scale Edges
Merino wool	1	2	Nil	Medium	20 by 30	0·5
Lincoln wool	1	6	Nil	Medium	20 by 30	0·5
Kangaroo fur	1	4	Nil	Fast	40 by 20	3
Human hair	*c.* 6	10–12	Nil	Medium	40 by 30‡	0
Seal fur	U	U	Nil	Negligible	U	U
Platypus fur			Yes			
Basal	U	U		Negligible	U	U
Distal	1	*c.* 3		Fast	80 by 10	10–16
Seal guard hair			Yes			
Basal	1	U		Medium	40 by 25	*c.* 4
Mid-shaft	U	U		Negligible	U	U
Distal	Multiple	U		Medium	40 by 20‡	0
Platypus belly guard hair §						
Platypus tail guard hair ¶						

* Using mechanical agitation (Bradbury, Chapman, and King 1965) or ultrasonic disintegration (Bradbury and Chapman 1964).

† The first dimension represents that in the longitudinal direction, the second that in the transverse direction of the fibre.

‡ Orientation on the fibre is uncertain. § Resembles seal guard hair. ¶ Resembles human hair.

The variability of the cuticle pattern along the length of single fibres of seal guard hair, platypus fur, and platypus belly guard hair obviously results from variation in the properties of the inner root sheath over the period of growth of the fibre. Perhaps this is the only factor involved since the actual size of the cuticle cells (assuming uniform thickness of cells) is about the same at both ends of the fibre of seal guard hair (Table 1).

The factors which control the ease of disruption of the various fibres in formic acid are: (1) the large degree of swelling of fibres in formic acid (Bradbury and Chapman 1963, 1964); (2) the dissolution of lipid and protein material from the cell membranes between and underlying the cuticle cells (Bradbury *et al.* 1965, 1966; Leeder 1969); and (3) various geometrical and other factors of the cuticle which are largely unknown. At this stage we are unable to explain the great stability of fibres containing the diamond pattern cuticle structure.

IV. Acknowledgments

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