THE MEMBRANE OF THE ALLWÖRDEN SACS AS A STRUCTURAL ELEMENT OF WOOL

by

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INTRODUCTION

In 1917 VON ALLWÖRDEN[1] treated wool with chlorine water and observed the formation of small swellings on the wool surface. These optically empty sacs are sharply outlined against the surrounding medium by their extremely thin border line.

According to STIRM AND COLLÉ[13] this surface is merely the boundary layer between two liquids: the viscous contents of the sac and the liquid surrounding the wool.

Most workers on this subject, such as KRAIS AND WAENTIG[4], LEHMANN[6], KRONACHER AND SAXINGER[5], MÜLLER[11, 12], HOCK, RAMSAY AND HARRIS[3] and GORTER (unpublished), could not agree with this concept. Because of the osmotic behaviour of the sacs and on the strength of experiments with a micromanipulator needle, they all came to the conclusion that a very thin, elastic, semipermeable membrane surrounds the sacs.

The possibility of verifying the latter concept is provided by the results of LINDBERG, PHILIP AND GRALÉN[10], who proved with the aid of the electron microscope that the wool fibre is surrounded by an extremely thin membrane. These authors[8, 9] suggest that this membrane is identical with the one surrounding the ALLWÖRDEN sacs.

It is important to follow up this idea in order to understand the ALLWÖRDEN reaction.

NOMENCLATURE

In connection with the discovery of this new structural element, we consider a critical survey of the histological nomenclature very necessary.

Histologically, the hair is composed of cellular units which usually are arranged in three layers—an outer layer of thin, scale-like elements, a middle region of spindle-shaped cells usually called the cortex, and a central tissue of more or less isodiametric cells, often filled with air, usually called medulla. This nomenclature for the two innermost layers is justified since, in histology, these names are generally used. We are therefore inclined to keep to the names *medulla* and *cortex*.

The nomenclature of the outer scale layer, often called the cuticle, is quite a different matter. The ontogeny of the hair clearly proves that this scale layer develops as a one-layer tissue, and for this reason it should be called the epidermis. In histological literature an epidermis is a tissue consisting of one or more layers of cells bordering an organism on the outside, whereas a cuticle is a usually very resistent membrane which the epi-

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dermis cells form on their outer side. For similar reasons several authors (*e.g.* HELM[2]) have suggested dropping the word cuticle as the name of the scale layer.

The situation becomes acute now that LINDBERG, PHILIP AND GRALÉN have discovered a structural element which does correspond to the definition of a cuticle. It is a resistent membrane which all scale cells share in common on their outside.

LINDBERG, PHILIP AND GRALÉN introduced the name epicuticle for the layer, a quite logical solution considering that they call the scale layer cuticle. However, it seems advisable to follow the general nomenclature used in histology. This prevents confusion and complication in terminology.

We therefore suggest the term *epidermis* to designate the scale layer of the hair (including subcutis or epidermis membrane), and *cuticle* to designate the membrane discovered by LINDBERG, PHILIP AND GRALÉN.

This nomenclature will be used in what follows.

INVESTIGATION OF THE CUTICLE

We shall now verify the existence of the membrane and try to demonstrate the part it plays in the formation of the ALLWÖRDEN sacs. In this investigation we mainly followed the methods applied by LINDBERG, PHILIP AND GRALÉN*.

According to the sodium sulphide method a sample of wool is treated for one month at room temperature with 0.3 *M* sodium sulphide (30 ml per gram wool). After this period the wool had dissolved completely, forming an opalescent solution which was then centrifuged. The sediment was washed with distilled water and centrifuged again, after which it was suspended in a little distilled water. Samples made from this suspension were shadowed with gold-manganin at an inclination of 1:2. Fig. 1 illustrates a membrane obtained in this way.

According to the chlorination method a sample of wool is treated for 2 minutes with sodium hypochlorite (2% active chlorine calculated on wool) acidified to p_H 1.5. After washing the wool was vibrated ultrasonically for 3 minutes at a frequency of 220 kc and an energy of about 5 Watt per cm² per second. The membranes obtained in this way are extremely thin (about 60 Å) and often show a granular surface (Fig. 2).

Our own investigations therefore confirm the results of the Swedish authors.

THE MEMBRANE OF THE ALLWÖRDEN SACS

The next question is whether or not the ALLWÖRDEN sacs are surrounded by a membrane and whether it is identical with the membrane under discussion.

It seems reasonable to expect that a membrane of this kind can be freed by ultrasonic vibration. Accordingly, a sample of wool was treated with saturated bromine water for 10 minutes. Once the presence of ALLWÖRDEN sacs had been proved, the wool was repeatedly washed very carefully with redistilled water until the wool had quite lost its colour and no more bromine could be found in the liquid. After that the wool was submerged in a minimum of redistilled water. Ultrasonic vibration (3 minutes at

* The investigation was carried out with the electron microscope and the crystal oscillator of the "Technisch Physische Dienst T.N.O. and T.H.". Thanks are due to Mr A. J. NIEUWENHUIS who made the photographs and to Messrs J. VAN DER HARST AND H. M. A. VAN HEUVEN for their help in the treatment with ultrasonic waves.

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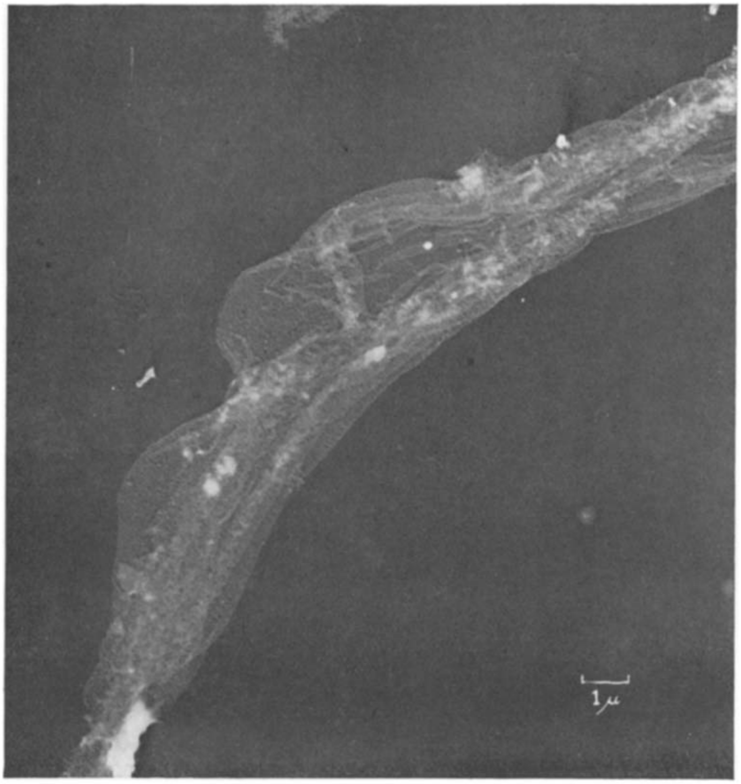

Fig. 1. Membrane obtained with the sodium sulphide method. Thickness about 70 Å. Gold-manganin shadowcast 1:5. El. Opt. 6000 ×

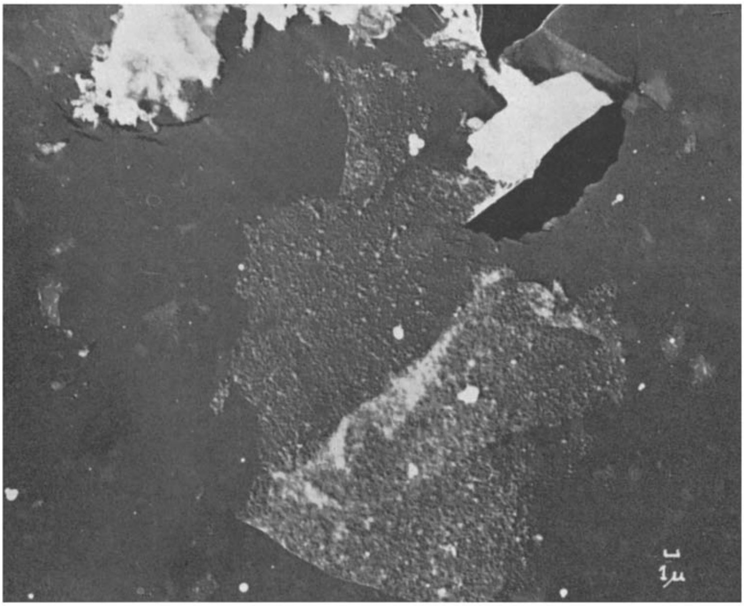

Fig. 2. Membrane obtained by the chlorination method. Thickness about 200 Å. Chromium shadowcast 1:5. El. Opt. 2000 ×

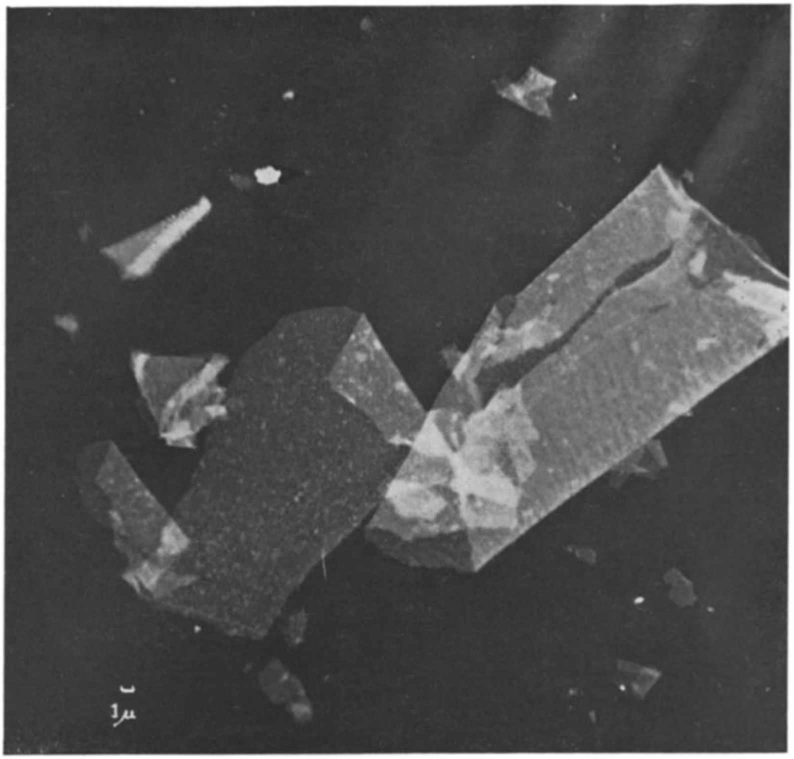

Fig. 3. Membranes of Allwörden sacs. Thickness about 100 Å. Non-striated membranes and one membrane with clear striation. To the left a membrane with faint striation. Chromium shadowcast 1:5. El. Opt. 2000 ×

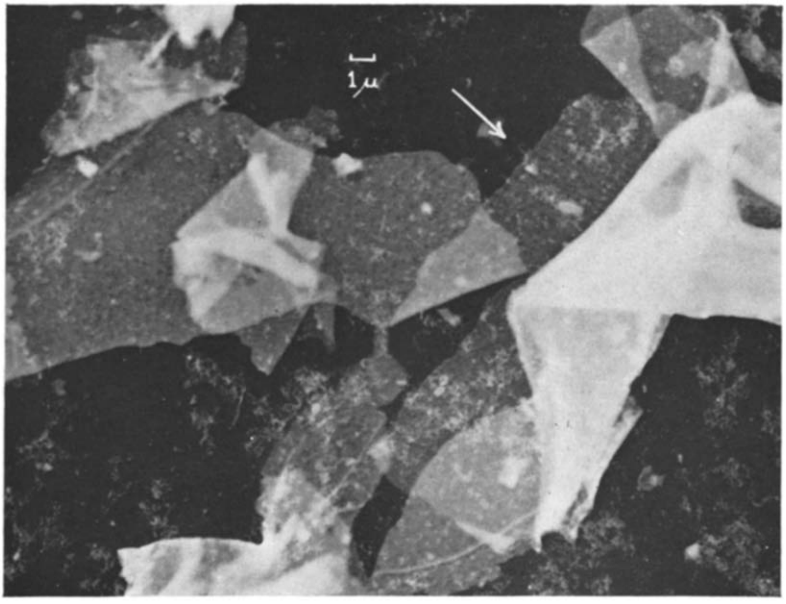

Fig. 4. Membrane of Allwörden sacs of which a small part (→) is striated. Thickness about 125 Å. Chromium shadowcast 1:5. El. Opt. 3120 ×

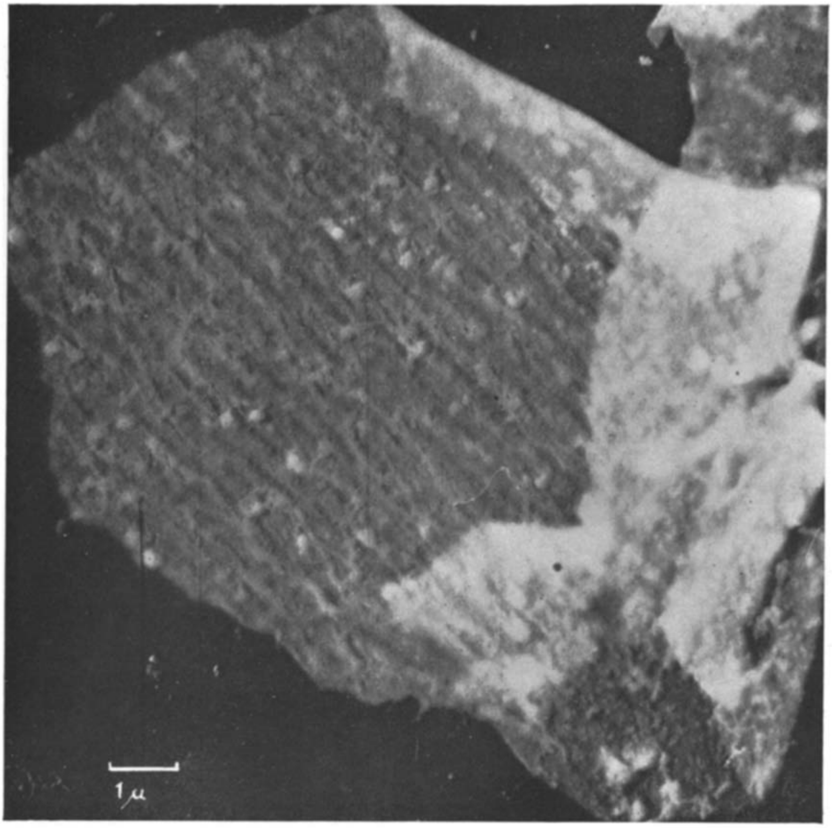

Fig. 5. Striated membrane of Allwörden sacs. Thickness about 170 Å. Chromium shadowcast 1:5. El. Opt. 9360 ×

220 kc) produced an opalescent suspension containing numerous membranes. The thickness of these membranes proved to be about 100 Å; most of them showed no structure whatever, except for a small percentage which appeared striated. Fig. 3 shows a number of non-striated membranes and one in which the striation can be seen clearly. Special attention is directed to Fig. 4, because this shows a membrane only a small part of which (→) is striated. This proves that there are not two kinds of membranes.

In Figs. 3 and 4 the stripes run beautifully parallel. This regularity is less pronounced in Fig. 5 which shows another striated membrane. The striation is caused by a difference in the thickness of the membrane, as is evident from the crenation (Fig. 3) visible here and there at the folds.

Probably the striation has something to do with the structure of the underlying scales. Accordingly, it is important to note the striking resemblance of pattern and measurements between the striation of the membrane in Fig. 5 and the fibrillar structure of the trypsin-treated scale in Fig. 6.

The striation is obviously caused by interfibrillar scale material adhering to the membrane as it is loosened. Bromine easily affects the interfibrillar keratin and we might therefore expect the striation to disappear after prolonged bromine treatment. The following investigation confirms this supposition.

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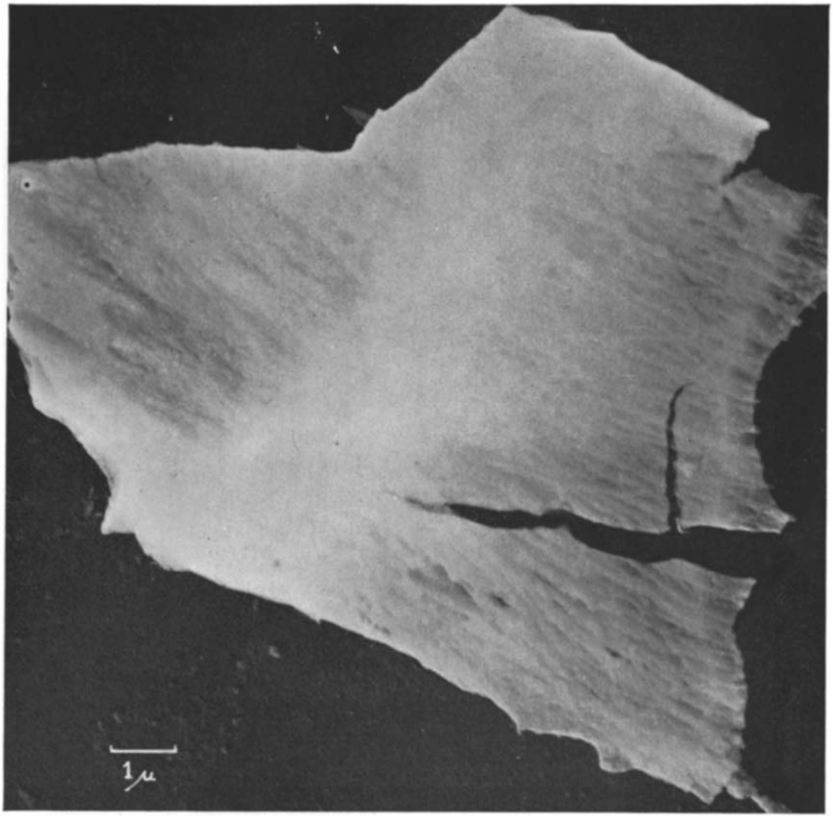

Fig. 6. Part of a scale treated with trypsin. The greater part of the fibrils run lengthwise of the fibre. Thickness about 1000 Å. Chromium shadowcast 1:5. El. Opt. 9360 ×

Four lots of degreased, washed wool were treated under exactly similar circumstances for 1, 2, 4, and 10 minutes with saturated bromine water, and then immediately washed with distilled water until no more free bromine was present. After that the samples were treated ultrasonically in a little distilled water. Of each of these suspensions 3 preparations were made for electron microscopy. Several electron micrographs of each were made, with the following results:

Duration of the bromine treatment in minutes	Percentage striated membranes
1	10 to 20
2	20 to 30
4	about 5
10	0

This table shows that the percentage of striated membranes diminishes with longer treatment. The striation, besides, was less pronounced after the 4 minutes' treatment than after the shorter treatments.

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These investigations clearly prove the presence of a membrane surrounding the ALLWÖRDEN sacs. Furthermore, the conformity in thickness between this membrane and those obtained with sodium sulphide and chlorination justifies the conclusion that the skin of the sac and the cuticle are identical.

The following experiment also supports this conclusion.

Two 50 mg samples of wool were treated for 10 minutes with 4 ml of a 3% solution of bromine in carbon tetrachloride. The bromine was removed by repeated washing with carbon tetrachloride. After this treatment no microscopically visible changes had taken place and the ALLWÖRDEN sacs had not developed.

Sample 1 was then treated with redistilled water and immediately a general formation of ALLWÖRDEN sacs took place. This proves that the bromine in the carbon tetrachloride did affect the wool but no swelling occurred for lack of water. The sample was now vibrated ultrasonically with a frequency of 220 kc for 3 minutes. Microscopical examination showed that the vibration had broken all the sacs and with the electron microscope we were able to observe a great number of membranes as described above.

Immediately after washing with carbon tetrachloride sample 2 was vibrated ultrasonically in the same way as sample 1. Although no suspended membranes could be found in the carbon tetrachloride, they must have been damaged by the vibration because after treatment with redistilled water the number of ALLWÖRDEN sacs was definitely less than normal.

DISCUSSION OF THE RESULTS

Our experiments have confirmed the existence of the structureless membrane described by LINDBERG, PHILIP AND GRALÉN. These authors suggested that the skin of the ALLWÖRDEN sacs is identical with the cuticle they had discovered. The suggestion does not agree with that of LEHMANN[6], who supposed that the skin of the ALLWÖRDEN sac is formed by the "epidermis membrane" which lies between the epidermis and the cortex. According to this investigator the swelling pressure of the reaction products formed during bromination pushes the "epidermis membrane" outwards, where it forms the skin of the sac.

From our experiments we are inclined to reject this concept and to support the one proposed by LINDBERG, PHILIP AND GRALÉN for the following reasons:

In the first place, because the thickness of the skin and the cuticle are both of a size, namely about 100 Å, whereas the thickness of the epidermis membrane differs greatly from that of the skin. From data given by LEHMANN[7] the thickness of the epidermis membrane can be estimated at about 3750 Å.

In the second place, because our experiments with carbon tetrachloride prove that the skin of these sacs must originally have formed the surface of the wool fibre. Only a small number of ALLWÖRDEN sacs is formed in water if the fibre is vibrated ultrasonically after the bromination in carbon tetrachloride. This indicates that the membrane has been badly damaged by the ultrasonic vibration. It is not likely that vibration damages the epidermis membrane because it is enclosed by tissue on either side, in contrast to the cuticle which, in the circumstances described, has been loosened from the underlying material by the bromine and is therefore very susceptible to damage.

An explanation must be given of the presence of striation on the membranes of

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the ALLWÖRDEN sacs and its absence from the membranes obtained after treatment with sodium sulphide and sodium hypochlorite.

In accordance with MÜLLER's investigations[11] we believe that, owing to the dissolving effect of the saturated bromine water, the membranes are at first loosened only locally from the underlying scale material. The osmotic pressure of the reaction products tears away the rest of the membrane, which takes along with it part of the interfibrillar keratin. Prolonged bromine treatment will dissolve this keratin too, and the striation of these loosened membrane parts should disappear.

This interpretation finds support in the conformity between the spacing between the stripes and that of the fibrils in the scales acted upon by trypsin (see Figs. 5 and 6). Furthermore, the data of the table on page 330 prove that the number of striated membranes decreases as bromine treatment is prolonged. The table shows that after 10 minutes all striated membranes had disappeared, and in the more extensive material from which Figs. 3 and 4 have been chosen they occur only sporadically. The conclusion is therefore justified that after this period the keratin causing the striation has been dissolved completely. The percentage of striated membranes never exceeded 30, which shows that the bromine quickly loosens a large part of the membrane.

However, in the method of slight chlorination the membrane will be loosened only in those places where the chlorine has already broken the adhesion with the scale material. Since no sacs are formed, it is evident that the osmotic powers necessary for tearing off the parts of the membrane still adhering are lacking. We should not therefore expect any striated membranes in this case, which is in accordance with our results. Neither should striations occur in membranes obtained by the sodium sulphide method, because all the adhering keratin will be dissolved by the sodium sulphide solution.

CONCLUSIONS

1. The observations of LINDBERG, PHILIP AND GRALÉN[10] on a membrane which covers the scale layer have been confirmed.

2. In agreement with the suggestions put forward by LINDBERG[8] and LINDBERG AND GRALÉN[9] we have demonstrated that the membrane is identical with the skin of the ALLWÖRDEN sac.

3. The striated membranes found after bromine treatment are essentially the same as the structureless ones. The striations are due to adhering scale material.

4. It is considered incorrect to suppose that the "epidermis membrane" or "subcutis" forms the skin of the ALLWÖRDEN sac.

ACKNOWLEDGEMENT

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SUMMARY

In order to obtain thin membranes as described by LINDBERG, PHILIP AND GRALÉN, samples of wool were treated by two different methods: one with 0.3 *M* sodium sulphide and one with a slight chlorination. The presence of these membranes was confirmed.

After treatment with bromine, similar membranes were obtained. This proves:

1. that every ALLWÖRDEN sac is surrounded by a membrane;
2. that these membranes are identical with those found by LINDBERG, PHILIP AND GRALÉN.

A small percentage of membranes showed a striation caused by adhering interfibrillar keratin of the underlying scale material.

It is proposed to call the scale layer of the hair the *epidermis* and to use the name *cuticle* for the membrane.

RÉSUMÉ

Afin d'obtenir des membranes fines telles que celles décrites par LINDBERG, PHILIP ET GRALÉN nous avons traité de la laine par deux méthodes différentes, c'est à dire par une solution 0.3 *M* de sulphure de sodium et par une solution d'hypochlorite de sodium acidifiée, contenant 2% de chlore actif. Nous avons pu ainsi confirmer la présence des membranes décrites par LINDBERG *et al.*

Nous avons obtenu des membranes semblables par traitement au brome. Ceci prouve que:

1. chaque sac de ALLWÖRDEN est entouré d'une membrane;
2. ces membranes sont identiques à celles découvertes par LINDBERG *et al.*

Un faible pourcentage de membranes montrait un système de stries provenant de la kératine interfibrillaire de la couche de tissu écailleux située sous les membranes.

Nous proposons d'appeler *épiderme* la couche écailleuse du poil et de réserver le nom de *cuticule* aux membranes.

ZUSAMMENFASSUNG

Um dünne Membranen zu erhalten, wie sie von LINDBERG, PHILIP UND GRALÉN beschrieben worden sind, wurden Wollproben nach zwei verschiedenen Methoden behandelt und zwar mit einer 0.3 *M* Natriumsulphidlösung und mit einer sauren Natriumhypochloritlösung, die 2% aktiven Chlor enthielt. Das Vorhandensein der Membranen konnte bestätigt werden.

Ähnliche Membranen wurden nach Behandlung mit Brom erhalten. Dies beweist:

1. jeder ALLWÖRDEN'sche Sack ist von einer Membrane umgeben;
2. diese Membranen sind identisch mit den von LINDBERG *et al.* beschriebenen.

Ein kleiner Prozentsatz dieser Membranen wies eine Streifung auf, die von anhaftendem Interfibrillar-Keratin des unter den Membranen gelegenen schuppenartigen Gewebesherrührt.

Es wird vorgeschlagen, die Schichte schuppenartigen Gewebes des Haares *Epidermis* zu nennen und den Namen *Cuticula* (Häutchen) für die Membrane zu gebrauchen.

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