Biology. — *An Electron microscope Study of the Structure of the Woolhair.* By CHR.[e] J. GORTER and A. L. HOUWINK. (Communicated by Prof. G. VAN ITERSON JR.)

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Introduction.

During the past two years the former author has been associated to the Fibre Research Institute T.N.O. of the National Council for Industrial Research at Delft. Research of the structure of the wool fibre was part of her task. To enlarge her investigations with a study of the submicroscopical structure, the Direction of the Fibre Institute permitted her to cooperate with the Institute for Electron Microscopy at Delft. That is why a more detailed study of the wool was possible.

The elements of the woolhair, scales and cortical cells, have been studied by means of electron microscopy showing the finer structure as it is seen in very finely ground wool fibres, or as it is disclosed by the more or less prolonged action of chemicals and enzymes. Comparing our micrographs with the electron micrographs of wool fibres, which have been published by MERCER and REES (6, 7) we found a striking conformity. This is the reason why we feel justified in publishing some of our micrographs. They may help to confirm some of the views that MERCER and REES founded on the results of their investigations. Still we wish to emphasize the fact that the interpretation of electron micrographs is very difficult and permits only very cautious conclusions about submicroscopical structures.

As for the submicroscopical structure of natural cellulose fibres, we mention a study of FREY–WYSSLING and MÜHLETHALER (2), that gives support to the opinion of FREY–WYSSLING, who claimed already in 1935 for cellwalls of plants that the differentiation in fibrils, microfibrils and micellar strands is only a product of desintegration, dependent on the method of desintegration.

Electron micrographs of wool have been published a.o. by ZAHN (13), HOCK and MCMURDIE (4), MERCER and REES (6, 7, 8) and OLOFSSON (9).

These authors used various mechanical and chemical methods to desintegrate the fibres into very small particles, cells, or parts of cells. This is necessary because objects, which have to be examined with the E.M. should not be thicker than some tenths of a micron. Using thicker material, one may expect destruction by the electrons (HOCK and MCMURDIE (4) and OLOFSSON (9)).

Structure of the woolhair.

To understand the electron micrographs it is necessary to draw the

Fig. 1. Fragments of scale. Shadowcast. 5170 ×. 90 K.V. Mechanically loosened.

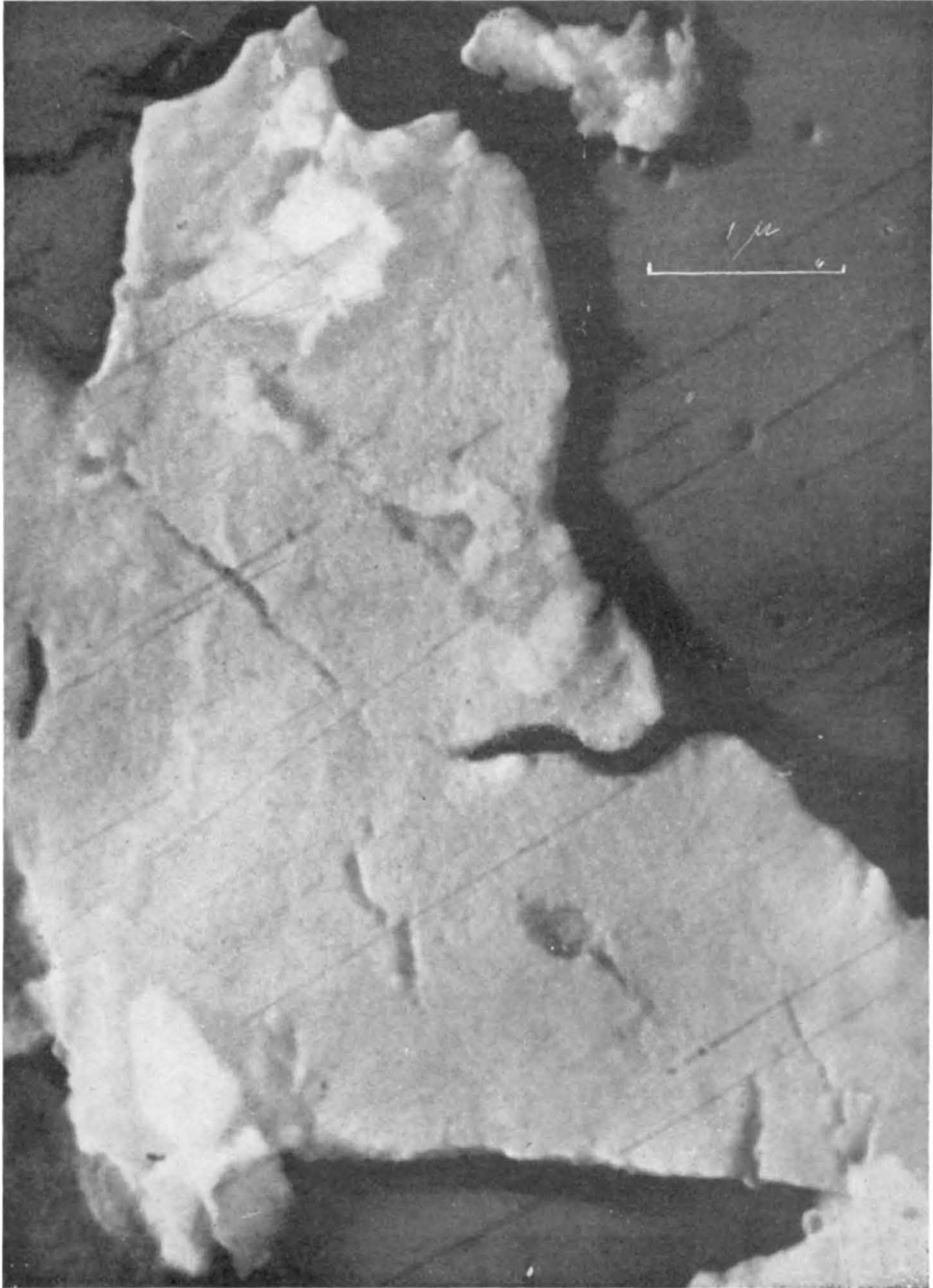


Fig. 1*a*. Detail of fig. 1. Shadowcast. 25.800 ×. 90 K.V.

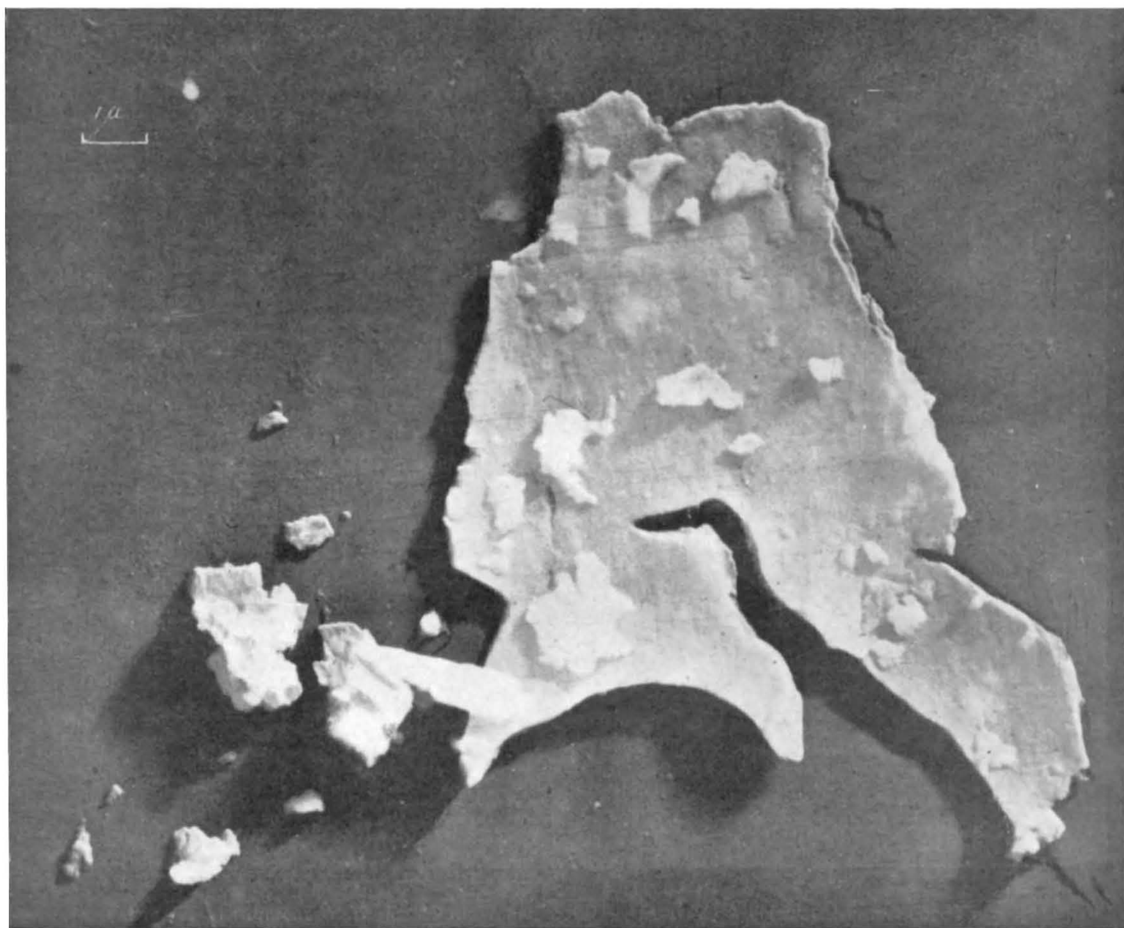


Fig. 2. Fragment of scale. Shadowcast. 8500 ×. 90 K V. Mechanically loosened.

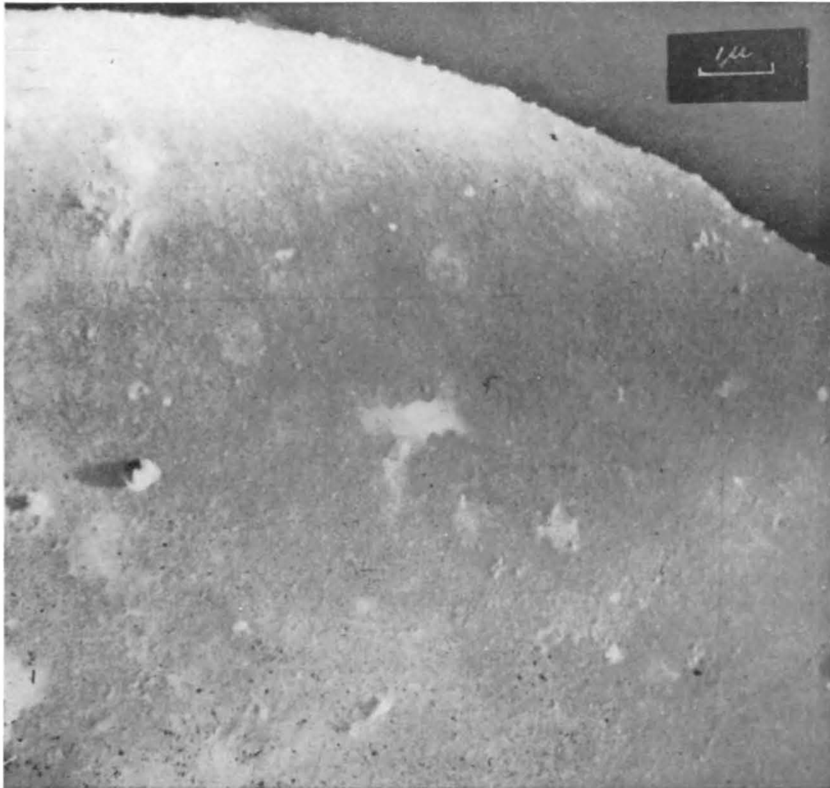


Fig. 3. Part of scale. Shadowcast. 10.000 ×. 90 K.V. Mechanically loosened.

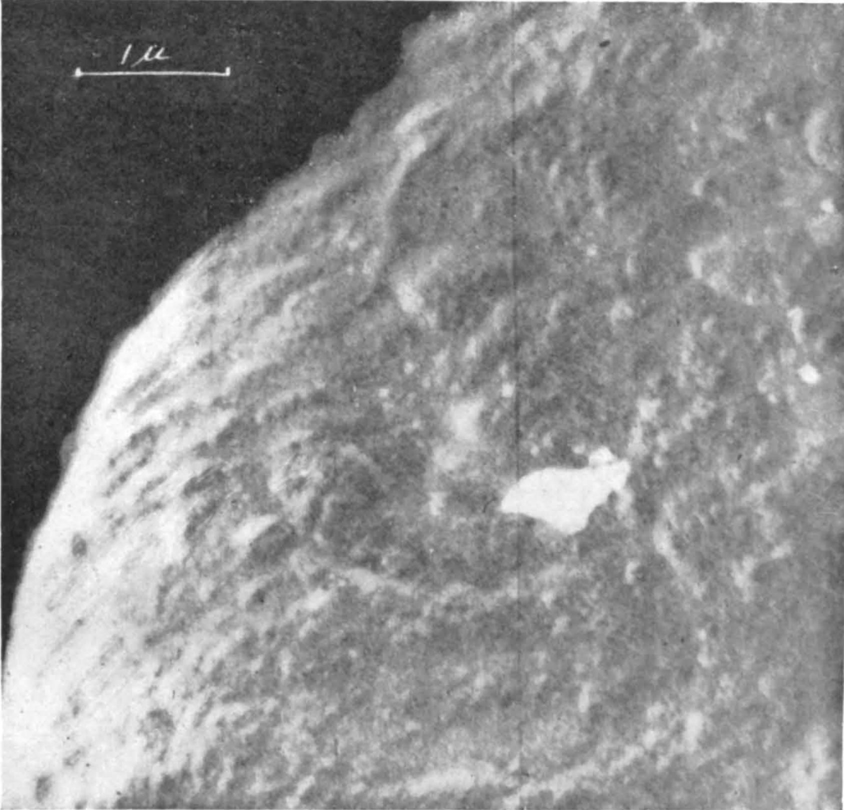
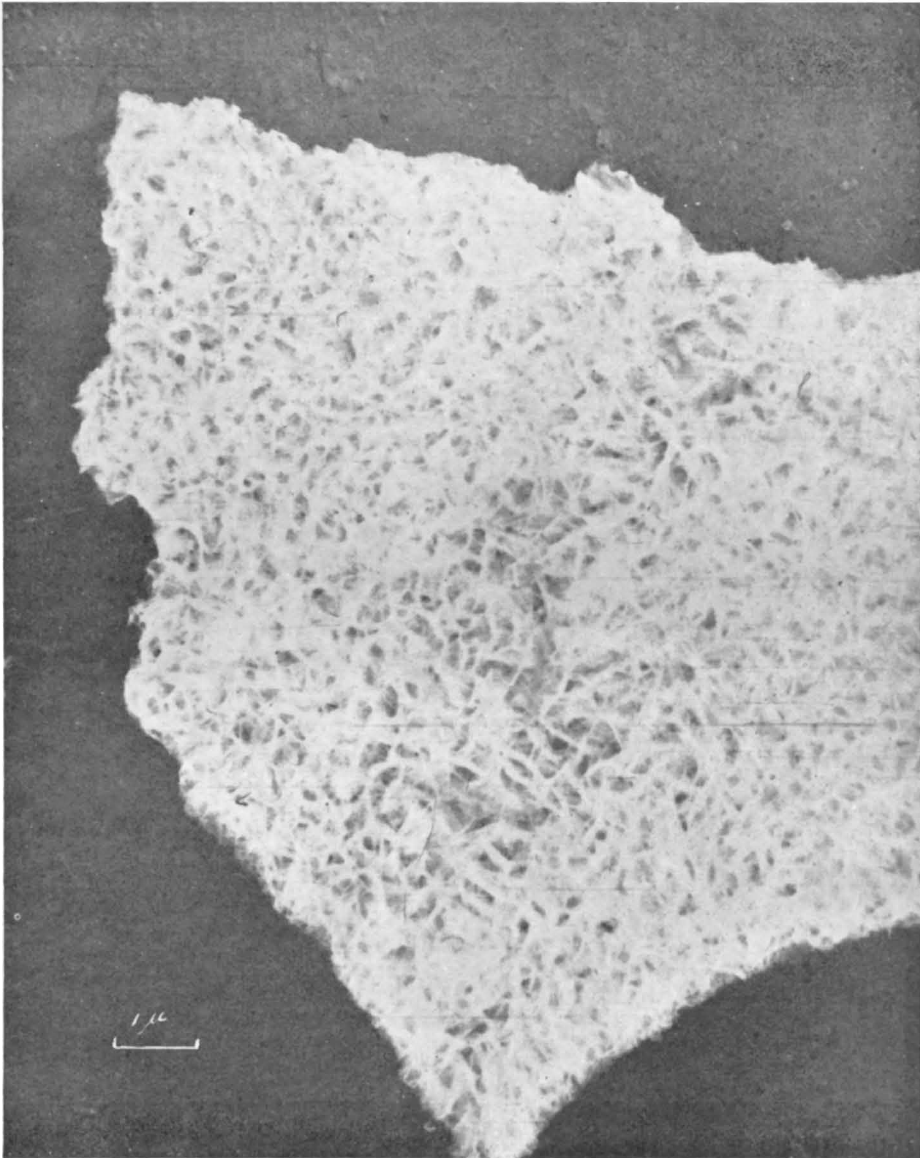


Fig. 4. Part of scale. Shadowcast. 20.000 ×. 90 K.V. Mechanically loosened.



(Cape wool)

Fig. 5. Part of scale. Shadowcast. 11.000 ×. 90 K.V. Trypsin preparation.

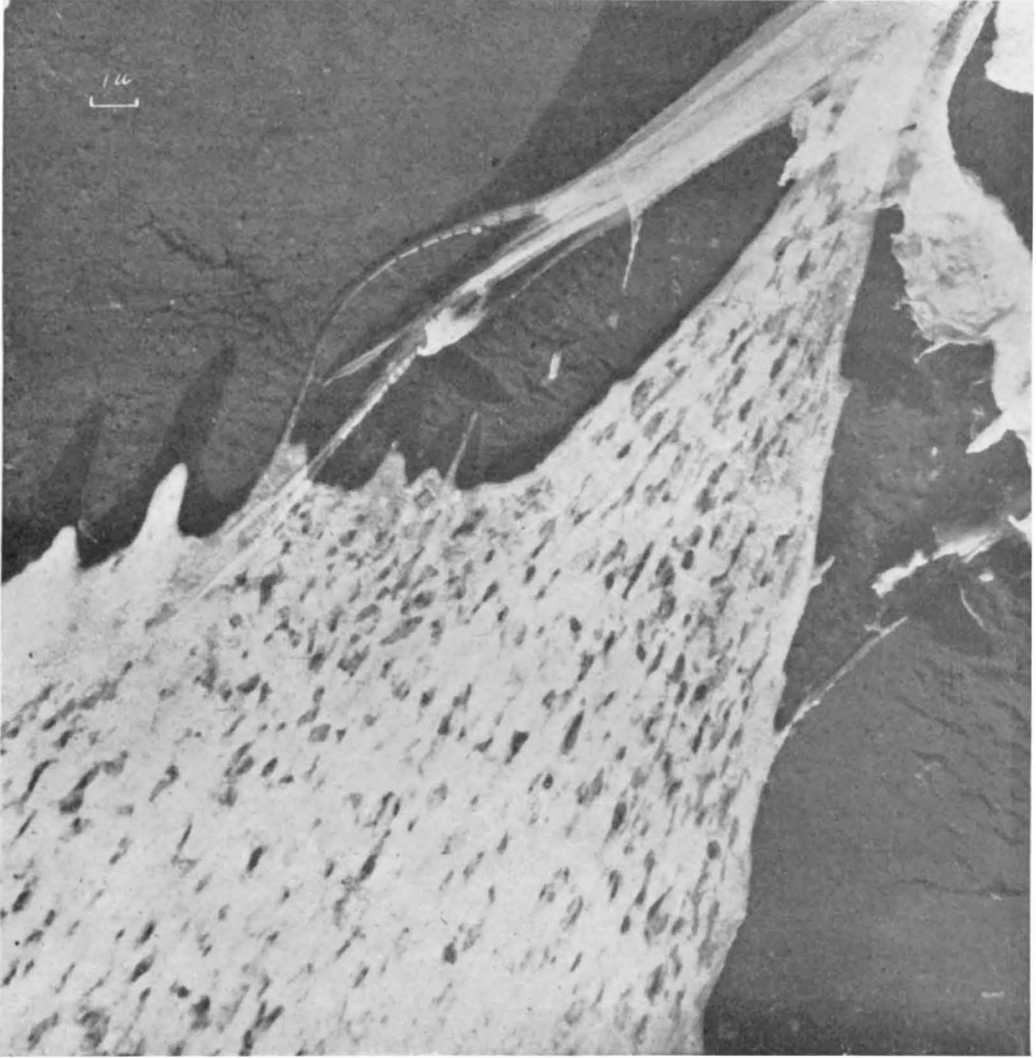


Fig. 6. Part of scale and tip of fibril. Shadowcast. 6240 ×. 80 K.V. Trypsin preparation. (The goldlayer is burst.)

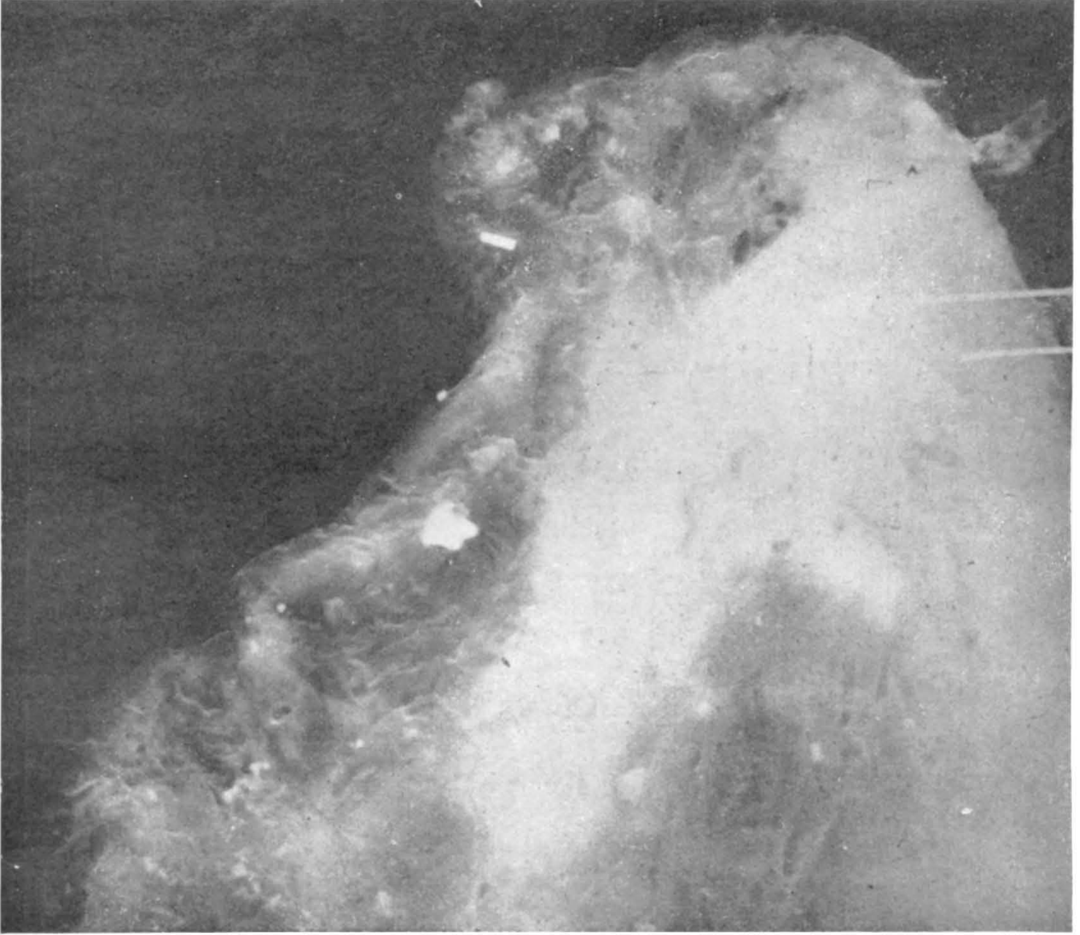


Fig. 7. Part of scale. Shadowcast. 9000 ×. 90 K.V. Trypsin preparation.

1 μ

Fig. 8. Fragment of scale. Shadowcast. 6400 ×. 80 K.V. Trypsin preparation. Shrinkage of the scale.

1 μ

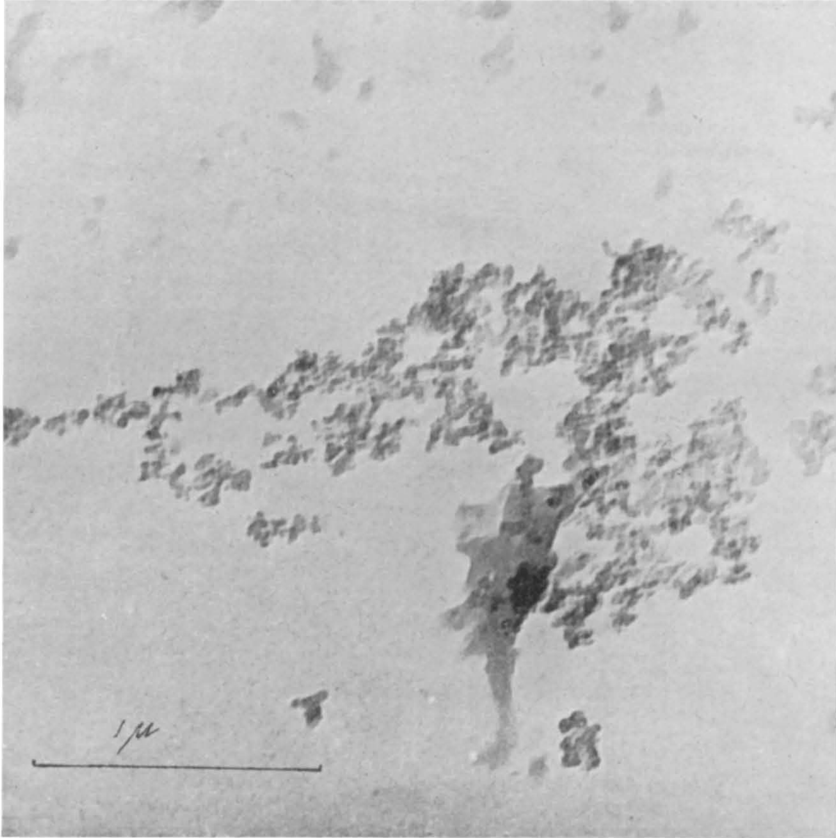


Fig. 9. Particles of wool. 38.000 ×. 90 K.V. Mechanically ground.
Diameter of particles — ± 200 Å.

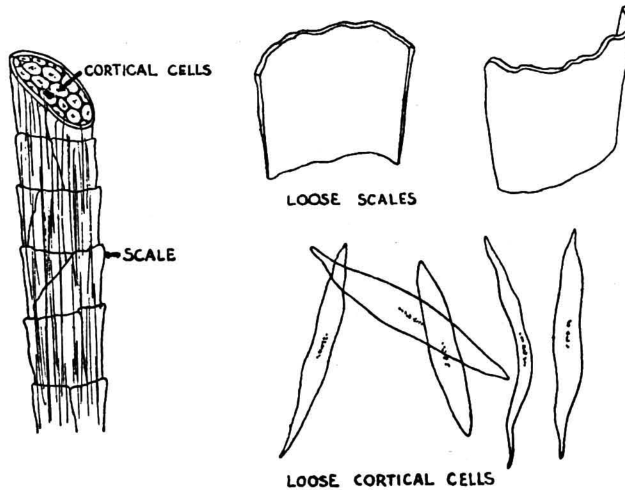
Fig. 10. Tip of cortical cell. Not shadowcast. 2000 ×. 90 K.V. Loosened by scraping across edge of razorblade.

Fig. 11. Tip of fibril. Shadowcast. 19.000 ×. 90 K.V. Trypsin preparation of fiber, pretreated with acid.

N o t e. In the original micrograph a microfibril with branch can be seen by — *.

attention to some main things about the structure of woolhairs; see scheme. (Comprehensive literature: KRONACHER und LODEMANN (5), REUMUTH (11)).

SCHEME OF THE ELEMENTS OF A WOOLHAIR



Wool is built of two or three elements:

1. The cuticle (also called epidermis), a layer of thin, flat cells [1]) (scales) which overlap each other. The apical margin of the scales is thickened.
2. The cortex, consisting of spindle-formed cells (cortical cells), that fibrillisate under special conditions. In thin fibres it forms the bulk of the fibre. Its physical and chemical constitution is responsible for most of the typical properties of the wool fibre.
3. The medulla, a mass of degenerated cells in the central part of the fibre, often absent (especially in thin fibres).

The presence of a cementing material between the cortical cells and also between the cortical cells and the scales is generally accepted. The scales too are believed to be cemented together. We will not discuss whether these cementing substances are identical.

Investigations with the optical microscope made evident that scales and cortical cells have a different structure: the cortical cells are fibrous while the scales are not.

Experimental procedure.

The electron microscope used was that of the Institute of Technical Physics of the Technical University at Delft, built by Ir J. B. LE POOLE.

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[1]) We use the term "cell" without discussing whether this term is right in this case; the genesis of the scales from cells being as yet obscure.

A description of this instrument is published in Philips Technical Review, Vol. 9, 1947. It has the advantage over other similar instruments of giving the possibility to vary continuously the magnification from 1000—80.000 diameters and the accelerating power from 50—150 K.V. So one can work with relatively dense objects. The instrument has four electromagnetic lenses.

For our specimens (parts of the woolhair) we mostly used a voltage of 80—90 K.V.

The wool used was a New-Zealand Crossbred wool [2]) and in one case a Cape-wool. The methods of preparation were mechanical or chemical ones:

1. Dry wool fibres were ground in a mortar or between two rough glassplates (roughened with emery of a particle size of $\pm 50\ \mu$) until the desired fineness was obtained.
2. Loose scales or fragments of scales were prepared by scraping the fibre across the edge of a razor blade; this method proved to be very satisfying.
3. By the action of trypsin or of acid. The woolhair breaks up into the cortical and cuticle cells.

0,03 g wool was treated with 10 ml. of a solution of trypsin (0.75 % trypsin — 0.3 % $NaHCO_3$) at p_H 8 and 36° C. In some cases the wool was pretreated with acid (boiled 30 min. with 0,5 n sulfuric acid, washed and dried), because a wool pretreated in such a manner is more readily attacked by trypsin.

A droplet of a suspension of desintegrated woolhair elements was placed on the very thin collodion or Geisseltallack film, which in the E.M. takes the place of the slide in the ordinary microscope (the penetrating power of electrons being much less than that of visible light, even cover glasses being much too thick). In the Delft E.M. this film is mounted over a hole, one third of a mm. in width, in the centre of a metal specimen holder. The electron beam passes through the hole. The air-dried preparation [3]) was in some cases examined directly in the electron microscope. Most of our preparations, however, have been shadowed according to the method of WILLIAMS and WYCKOFF (12). The object holders are placed under a bell-jar, the air is evacuated, a small quantity of gold is then electrically heated and evaporated. The gold to be evaporated is placed about 2.5 cm. above the level of the — horizontally placed — specimen holder, at a distance of 10 cm. As all evaporated gold atoms move through the high vacuum along rectilinear paths just as light, on one side of the object everything is golded, on the other side there is a "shadow". The whole collodion film with all horizontal parts of the preparation is also covered

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[2]) Degreased with ether and thoroughly washed with water.

[3]) An E. M. can as yet only be used for examination of dry preparations.

with a thin layer of gold. During examination in the E.M. the electrons pass relatively unhindered through the "shadow", which becomes a dark region on the E.M.-graph negative. On the other hand the golded side of the object becomes bright. So on the negative film the object looks like something laying on sandy soil in a setting sun. The micrographs in this paper, which represent shadowcast objects, are therefore all printed in negative. Most of the details of the E.M.-graphs may indeed be interpreted in this way.

However, one should never forget that the objects are not observed by means of reflected radiation, but by means of the electrons, which pass through the objects. Something may appear white on the negative-printed electron micrograph because it has been coated with the evaporating gold, but also because it was thick or dense. As density depends on the atom-weight, a thin layer of gold causes much scattering of electrons.

The Scales.

MERCER and REES (6) suppose the cuticle cells or scales of the wool fibre to contain two varieties of keratin, K_1 and K_2, K_1 being more readily dissolved by trypsin and by various chemicals than K_2. K_2 would form the inner, more resistant part of the scales, lying in "roughly parallel folds", whereas the flat, very thin outer layer would consist of K_1. K_1 might be identical with the cementing material between the cortical cells (and cortical cells and scales).

The inside structure of the scales is disclosed when the K_1 is etched away by a trypsin treatment.

MERCER and REES (6) present micrographs to support their views (1, 2 and 4 of their publication 6). With these micrographs our figures 1—11 should be compared.

E.M.-graphs of scales loosened by mechanical action.

Fig. 1, 1A and 2. Fragments of scales, loosened by grinding the untreated wool fibres in a mortar. No specific surface structure. At several places one observes a double wall, indicative of the thickness of the scales. This indicates the scale not to be homogeneous, but to consist of at least two different layers. It is impossible to say, whether the outer surface or the inner surface of the scale is shown in the E.M.-graphs. It seems remarkable that the scale fragments are bent upwards as shown in these micrographs.

Fig. 3 and 4. Fragments of scales, loosened by scraping the fibre across the edge of a razor blade. In Fig. 3 no specific structure is visible. The surface is seen as a more or less flat layer, in accordance with the results of MERCER and REES (6; Fig. 1). In Fig. 4 (a higher magnification of a detail of this specimen) a rougher surface is found, but there too is no indication of a specific structure.

The influence of trypsin on scales.

Fig. 5 and 6. Fragments of scales, exposed to the action of trypsin for 24 hours. Fig. 5 (Cape wool). Only the more resistant inner part of the scale is left. It shows a "spongelike" or vesiculate structure. According to the views of MERCER and REES this vesiculate mass is K_2, whereas all K_1 has been removed. The same spongelike structure is seen in Fig. 6, which represents a part of a scale of a New-Zealand Crossbred woolhair, which was much thicker than the Cape woolhair. The inner structure of this scale appears to be rougher and some K_1 has remained.

We wish to emphasize that there are two possible explanations for the vesiculate appearance of the interior of the scale: 1. the spongelike substance which still remains after the trypsin treatment is K_2, whereas cavities are found where K_1 has been dissolved. This would mean that in the intact scale the interior part consists of two components. 2. The spongelike structure might be due to the manner in which E.M. preparations are made. All specimens are dried out. It might be possible that the K_2 inside the scale is homogeneous, that it is transformed into a gelatinous mass by trypsin treatment, and that this, on being desiccated in vacuo, becomes vesiculate. Micrographs of gels (vanadin pentoxyd e.g.) showing a vesiculate structure are given by FREY–WYSSLING and MÜHLETHALER (3).

In Fig. 6 is shown an all too common feature in electron micrographs of wool. By desiccation in vacuo the wool shrinks. When the material is only loosely attached to the collodionfilm the layer of gold is left intact. On the other hand, when the fibrils or the scales stick to the film, the latter, being pulled together by the shrinking specimens, is thereby stretched all around the wool fragment; the goldlayer, however, not being elastic, cracks and becomes discontinuous around the objects. Moreover, too strong irradiation may damage objects as well as gold layer. These phenomena can give rise to misinterpretation of electron micrographs.

Fig. 7. Trypsin preparation of a scale. This E.M.-graph shows a striking similarity with Fig. 4 of MERCER and REES (6). According to these authors the K_1 layer is etched away by the trypsin and the underlying K_2 is visible.

Fig. 8. Trypsin preparation of a scale. Longitudinal folds are to be seen. The scale is bent. According to the opinion of MERCER and REES (6), part of the K_1 is etched away and the "roughly parallel folds" of the K_2 are shown.

Fig. 9. We found this material together with larger fragments in preparations we made by grinding wool between emery roughened glassplates. It seems identical with that shown in fig. 5 of MERCER and REES (6). The authors take it for "cell contents of scales".

The smallest particles shown are of a diameter of 200 Å. In ground wool we found this material together with larger wool fragments, so that, when MERCER and REES are right, it is probable that it is indeed scale material next to cortical cell material.

The cortical cells.

In samples which had been prepared by grinding as well as in those which had been exposed to the action of trypsin the cortical cells show a subdivision in fibrils and microfibrils [4]).

Fig. 10. A fibril, isolated by scraping an untreated wool fibre across the edge of a razor blade. The subdivision is apparent. Compare with fig. 6 of MERCER and REES (7). The micrograph shows that a mechanical action is sufficient to loosen the microfibrils.

Still such microfibrils are rare in ground wool material. Usually we found more or less small pieces of material which do not show subdivision (see fig. 1).

Fig. 11. Tip of a fibril, liberated by sulfuric acid and trypsin. Clear fibrillisation. The microfibrils consist of (more or less parallel) still finer microfibrils. A subdivision of these microfibrils is not to be seen.

In most cases the microfibrils lie parallel, embedded in an amorphous substance. This micrograph gives support to the view of FARRANT, MERCER and REES (8) that there are: *a.* an amorphous matrix and *b.* a fibrillar structure. Nevertheless we emphasize that the amorphous substance might be due to the dissolving action of the trypsin and is not necessarily a part of the untreated fibre.

The finest microfibril (protofibril?) observed can be seen as a branch of the microfibril indicated in the micrograph by *x*. The diameter of this fibril is 200 Å.

The microfibrils are formed of rows of little irregular fragments which can be called "micellar strands". We have no indication that the microfibrils are formed by rows of regular particles as in the micrograph of MERCER and REES (8), which particles are keratin-molecules according to these authors.

Summary.

1. Mechanically loosened scales, observed with the E.M., show no specific structure, the inside is a solid mass.
2. The scales, exposed to the action of trypsin, show a "spongelike" interior.
3. Cortical cells, desintegrated by the action of trypsin, show fibrillisation.
4. Microfibrils of a diameter of about 200 Å have been observed.
5. The smallest particles, obtained by grinding wool, have a diameter of about 160—200 Å.
6. Many of our E.M.-graphs show a striking conformity with those of MERCER and REES (6, 7, 8).

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[4]) FREY-WYSSLING and MÜHLETHALER (2) use the terms: fibrils, microfibrils and micellar strands for cellulose fibres. It might be suitable to use the same terms for the woolfibres, though these terms are not well defined.

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