THE BROMINE ALLWÖRDEN REACTION

by

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INTRODUCTION

Since the discovery by ALLWÖRDEN[1] that wool fibres treated with chlorine water develop bubbles on the surface layers, considerable attention has been directed to the mechanism of the formation of these bubbles and the nature of the membrane defining them. LINDBERG, PHILIP AND GRALÉN[2] were able to demonstrate with the electron microscope that the membrane defining the bubbles was extremely thin (*ca.* 100 A) and uniform. Bubbles which are superficially similar to those obtained with chlorine may be produced with bromine[3] and also, it is claimed, by the action of sodium bicarbonate on fibres oxidised with peracetic acid[4], although we have not been able to obtain an ALLWÖRDEN reaction by the latter method.

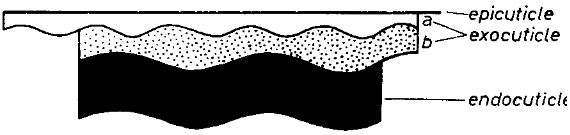

Fig. 1. The structure of the surface layers of wool according to LINDBERG *et al.*[2] and LAGERMALM[12] (transverse section).

The membrane isolated by LINDBERG *et al.*, termed the epicuticle, is a well defined histological component and its relation to the cuticle is shown in Fig. 1. The character of the membrane revealed by bromine is, however, not so well established. The difficulty in this case is due partly to the variable nature of the reaction obtained with different fibre types, but mainly to the lack, in the past, of a suitable method of studying the fine structure of the membranes. We have recently pointed out[5] that the method of shadow-casting in optical microscopy is a valuable technique in the study of the fine histology of wool fibres, enabling surface texture to be investigated with ease at the limit of resolution of the optical microscope. This technique has now been applied to the membranes obtained by the action of bromine on wool, and the results described below throw considerable light both on the character of the membranes and on their origin in the wool fibre. The special advantage of this technique is that fine structures normally seen only in the electron microscope are visible over large areas of specimen, allowing the correlation of fine histology with coarser features. The transparency of the fibre, normally a severe limitation in the optical microscope, is of great advantage in this respect as the permissible thickness of specimens is not limited as is the case with the electron microscope.

PREPARATION OF MEMBRANES

The bromine membranes were prepared from solvent-scoured 64's-quality merino wool essentially according to the method of SCHURINGA *et al.*[6] and shadowed with gold[5] at an angle of 35°

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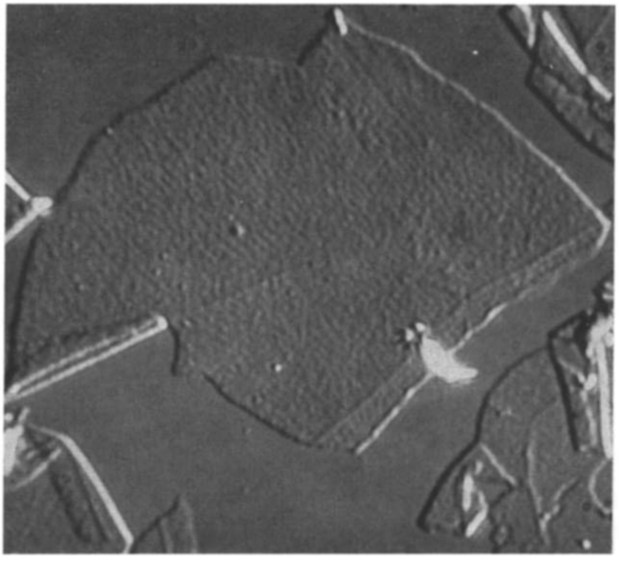
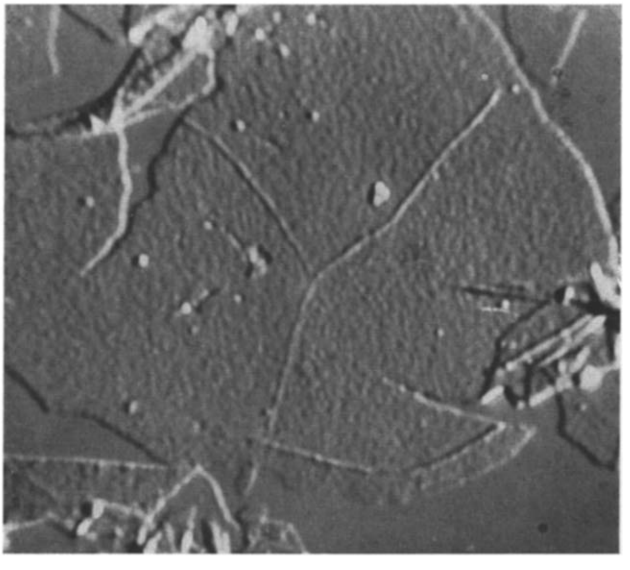
The shadowed preparations were mounted in Euparal and photographed with a × 95 oil immersion objective n.a. 0.95. The development of bubbles on various solvent-scoured wools and human hair was observed by mounting fibres in bromine water beneath a coverslip and the progress of the reaction followed using a × 42 objective n.a. 0.85.

RESULTS

The bromine membranes were found to be uniform in structure and Figs. 2 and 3 show the texture of the inner surface observed in all cases. It is at once apparent that the membrane is not pure epicuticle as obtained with chlorine, the epicuticle being





Figs. 2 and 3. Epicuticle with adhering exocuticle component, isolated from merino 64's-quality wool after treatment with bromine water. (Gold-shadowed, positive prints × 2,300)

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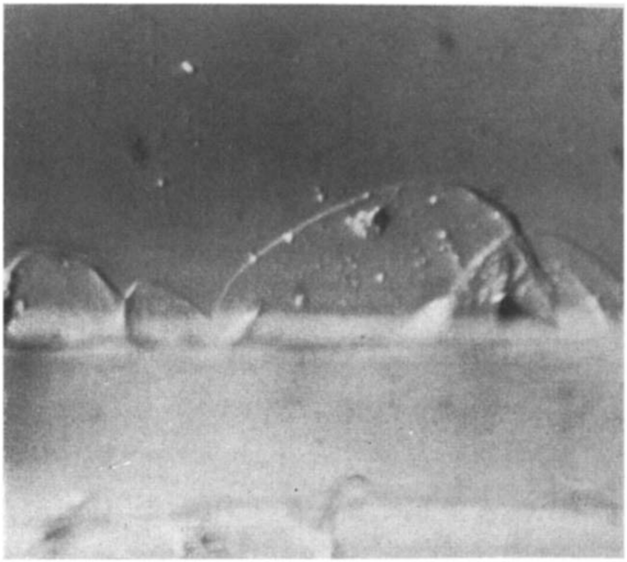

Fig. 4. Bubbles of epicuticle obtained by treating wool with chlorine water. (Gold-shadowed, positive print × 2,300.)

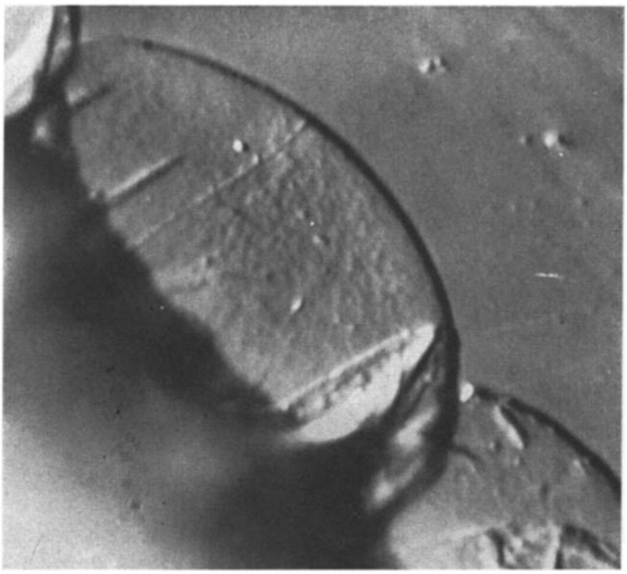

Fig. 5. Bubbles of epicuticle with adhering exocuticle obtained by treating wool with bromine water. (Gold-shadowed, positive print × 2,300.)

featureless both by this technique (Fig. 4) and in the electron microscope. The ribbed structure of the bromine membranes appears to be a definite component of the exocuticle adhering to the epicuticle. The ribs run parallel to the fibre axis and have a periodicity of about 0.35 μ, which is about half that of the furrows in endocuticle obtained by tryptic digestion[7]. Fig. 6 shows the surface of a fibre after the removal of the bromine membrane, the furrows showing a periodicity similar to that of the endocuticle, namely about 0.7 μ.

The fragments of membrane isolated using bromine water are in general much larger than those using chlorine water and are derived from an area of the fibre surface

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which is considerably greater than that of a single scale. Ridges of adhering exocuticle which closely resemble the scale pattern of an intact fibre are apparent in our preparations (Fig. 3). In a significant number of cases, the line of rupture of the membrane has closely followed the shape of a ridge and is displaced from it by a distance of about 1.5 μ.

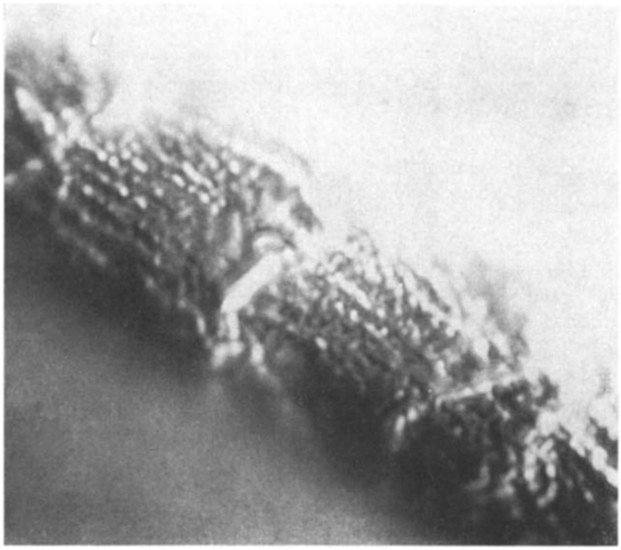

Fig. 6. Surface of bromine-treated fibre after detachment of epicuticle plus exocuticle component. (Gold-shadowed, positive print × 2,300)

INTERPRETATION OF SHADOWED MEMBRANES

It is clear that the membrane isolated from merino wool using bromine water is not pure epicuticle, but shows an additional adhering component of the exocuticle which is laid down in a perfectly uniform fashion. LEVEAU *et al.*[8] and PARISOT AND LEVEAU[9] have stated that in general the action of bromine water is to lift the entire cuticle, and MERCER AND GOLDEN[10] have suggested that the membrane is epicuticle thickened by more or less of the adhering protein of the cuticular cells. Our results indicate a membrane of clearly defined structure and very much thinner than the entire cuticle. Apart from the ridges mentioned previously, the exocuticle component adhering to the epicuticle appears to be continuous. This may account for the larger fragments of membrane obtained using bromine water rather than chlorine water.

In an electron optical investigation of the bromine ALLWÖRDEN reaction, SCHURINGA AND ALGERA[11] noted that a small percentage of the membranes in their preparation were striated. These striations appear to have a periodicity of about 0.3 μ and are presumably derived from the same layer of the exocuticle as those in our preparations. In a recent study of structural detail in the surface layers of wool using the electron microscope, LAGERMALM[12] observed two types of "streakiness" in the combined exocuticle and epicuticle layers isolated from phenol-trypsin digested wool. The outermost layer of exocuticle had a periodicity *ca.* 0.3 μ and the streakiness was parallel to the fibre axis as with the bromine membranes reported here, and it is reasonable to suppose that the

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exocuticle component of the bromine membranes is derived from the same layer, which is labelled "a" in Fig. 1.

The marked difference in the periodicity of the exocuticle adhering to the epicuticle in bromine membranes and the furrows in the remaining fibre surface suggests that the "b" layer of the exocuticle is the more susceptible to bromine. Its degradation and dispersion during the reaction would account for both the adhesion of the "a" layer of the exocuticle to the epicuticle and the exposure of the endocuticle on the remaining fibre.

The fine ridges or "strings" adhering to the epicuticle, observed by LAGERMALM[12] in the electron microscope, and the ridges that we have observed in bromine membranes probably have a common origin. The distribution of the ridges in Fig. 2 and 3 according to a characteristic scale pattern immediately suggests that they originate from the junction of neighbouring scales. Their frequent parallelism to the ruptured edge of the membrane, presumably corresponding to a scale edge, tends to confirm this view. It is uncertain at present whether the scales are discrete structures or whether the cuticle consists of plates of endocuticle with a continuous exocuticle. No certain conclusion can be drawn from our results but they would lend support to the latter view. An alternative interpretation of the ridges might be that they originate from the distal edges of the scales, in which case the perfect continuity of the adhering exocuticle apart from the ridges would also point to a continuous exocuticle. The ridges noted by LAGERMALM were found to follow both scale edges and scale junctions.

The bromine Allwörden reaction in other wools and hair

LEVEAU, LANGLOIS AND PARISOT[8] have studied the bromine ALLWÖRDEN reaction in hairs and wools and have concluded that in general the entire cuticle is lifted to form the membrane of the ALLWÖRDEN bubble. We have confirmed this observation

(a)

(b)

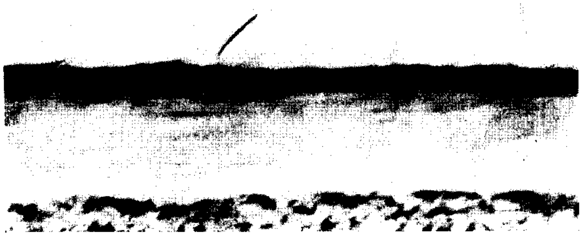

Fig. 7. Intermediate stages of the bromine ALLWÖRDEN reaction in (a) human hair and (b) Lincoln wool. The entire cuticle appears to lift in (a) and a scale flap in (b); (× 1,000)

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in the case of human hair (Fig. 7a) where the cuticle is seen to envelop the bubble. These authors also noted that in a sample of wool (of unspecified quality) the bubble was enclosed by a much thinner membrane which they believed to be the epicuticle, and that the scales lifted between neighbouring bubbles.

We have investigated the formation of bubbles in a variety of wools and find that there is a continuous gradation from fine merino wools, in which the bromine membrane consists exclusively of epicuticle with adhering exocuticle, to human hair in which the entire cuticle is lifted. The lifting of the entire cuticle is commonly observed in Lincoln fibres although not infrequently a flap of cuticle lifts and the major part of the bubble is of the merino type, as in Fig. 7(b). This latter behaviour is common in crossbred and Corriedale fibres which also, however, show bubbles of the merino type.

The varying character of the bromine ALLWÖRDEN reaction probably reflects differences in cuticle formation, both as regards the degree of overlap of the scales, which is very great in hair, and the nature of the cementing material between them. There is also good evidence, which is discussed in the following sections, that the keratinisation, or degree of disulphide crosslinking, in the cortex plays an important part in determining the exact nature of the reaction.

The relation of bromine membranes to bilateral structure

It is now well established that the cortex of highly-crimped wool fibres, such as fine merinos, has a bilateral structure with two segments which differ markedly in chemical composition, resistance to alkali, and dyeing characteristics[13,14,15,16]. It is to be expected therefore that the action of bromine water on the segments will differ. LEVEAU, CEBE AND PARISOT[17] have noted a preferential development of bubbles around the S (alkali susceptible) segment and have concluded from this that the cuticle also possesses a bilateral structure. The nature of the cuticle in a fine merino fibre, where only one or two scales encircle the cortex at any point, is such that this is extremely

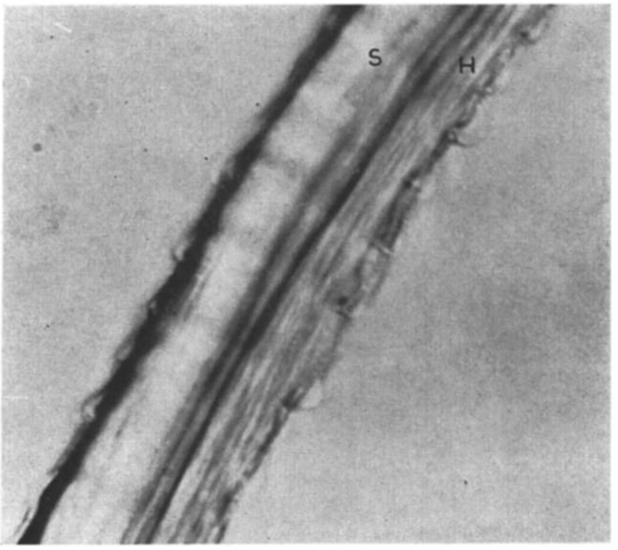

Fig. 8. Stages in the bromine ALLWÖRDEN reaction in merino 64's-quality wool (× 1,000).

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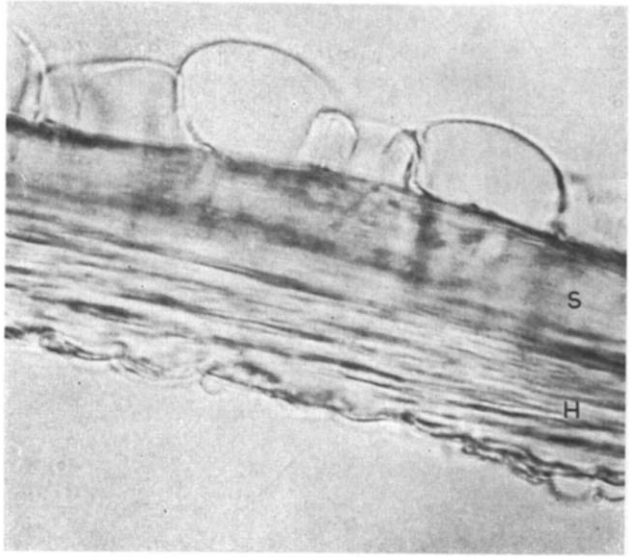
Fig. 9. Stages in the bromine ALLWÖRDEN reaction in merino 64's-quality wool (× 1,000).



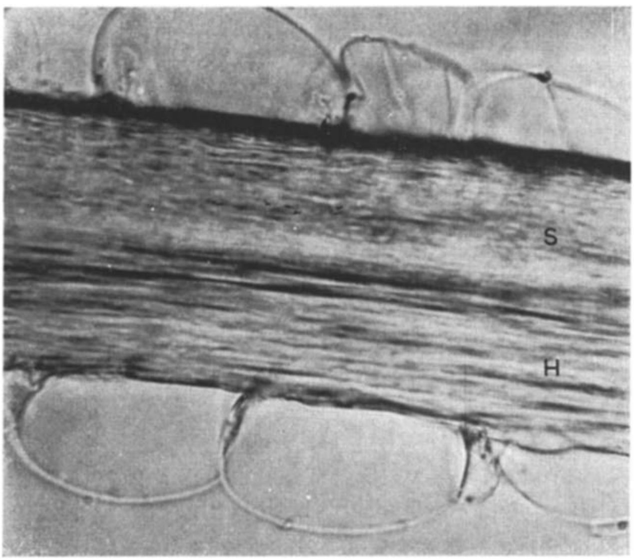


Fig. 10. Stages in the bromine ALLWÖRDEN reaction in merino 64's-quality wool (× 1,000).

unlikely from a histological standpoint and we have accordingly reinvestigated the bromine ALLWÖRDEN reaction in this type of wool. The course of the reaction is depicted in Fig. 8 to 11 which were photographed without the use of phase contrast which, though it assists observation of epicuticle, only serves to complicate the appearance of the thicker bromine membranes.

The addition of saturated bromine water to the fibres caused the rapid development of small bubbles on the tips of the scales around all parts of the fibre and gradual differentiation of the H (alkali resistant) and S segments in the cortex as in Fig. 8. No differentiation of the cuticle was observed at this stage either as regards bubble formation or swelling. The rapidity of bubble formation at the scale tips suggests that

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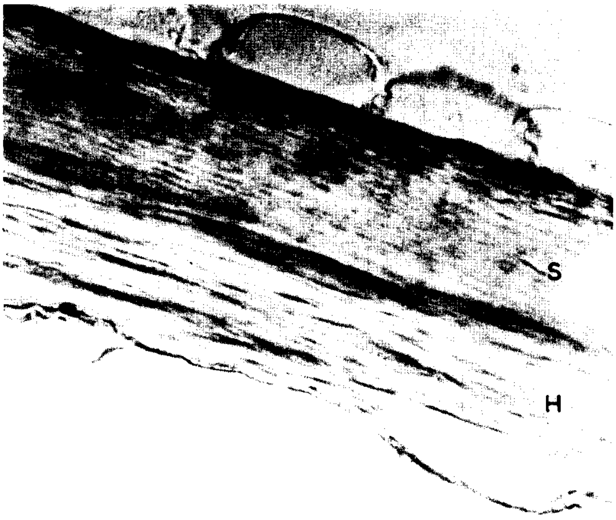

Fig. 11. Stages in the bromine ALLWÖRDEN reaction in merino 64's-quality wool (× 1,000).

the protein responsible for the initial osmotic effects originates from the cuticle, possibly in the exocuticle-thickened scale tip. When the differentiation of the segments was well advanced, larger bubbles developed rapidly around the S segment. At the same time the cuticle around the H segment appeared to loosen, much in the same manner as was observed in the initial stages of the reaction with hair and coarse wools. These features are seen in Fig. 9.

As the reaction progressed, the bubbles around the S segment spread around the fibre until it was uniformly covered. The exclusive development of bubbles around the S segment reported by LEVEAU *et al.*[17] appeared in our experiments only as a transient phase in the reaction which invariably gave way to the complete encirclement of the fibre as in Fig. 10. In a number of instances the cuticle lifted slightly around the H segment as in Fig. 11, although the combined epicuticle and exocuticle membrane still developed normally on the lifted surface.

The mechanism of bubble formation

From the evidence presented in the previous sections it is possible to postulate a mechanism for the bromine ALLWÖRDEN reaction which accounts for much of the observed data. Fig. 12a shows diagrammatically the disposition of the various layers in the cuticle of wool. The initial attack of bromine appears to be concentrated at the tip of the scale, leading to incipient bubble formation as in Fig. 12b. This is in accord with the notion of a thickened deposit of exocuticle at the scale tip[12] and the "b" layer appears to be the component involved. From the delay between the incipient bubble formation and full development it seems that proteins derived from the cortex are chiefly responsible for the bromine ALLWÖRDEN reaction. LEVEAU, LANGLOIS AND PARISOT[8] arrived at a similar conclusion from the fact that the entire cuticle was raised in most of their experiments.

In the case of Lincoln wool, the bubble formation is depicted diagrammatically in Fig. 12c. It is supposed that proteins released from the cortex by the action of bromine are unable to penetrate the cuticle, which therefore acts as a semi-permeable membrane. When sufficient osmotic pressure has developed, the cuticle detaches from the cortex and a cuticle-limited bubble is formed.

In the case of Corriedale wool (Fig. 12d), it is supposed that the inter-scale cementing substance is either more susceptible to bromine or is intrinsically weaker than in Lincoln wools. This leads to the separation of individual scale flaps which lift to form part of the bubble surface, the detachment of the epicuticle and exocuticle component

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"a" resulting from the leakage of protein between the scales. It is of interest that merino fibres which have been extended 100% in steam give a reaction similar to Corriedale wool, presumably due to the rupture of the inter-scale cementing material.

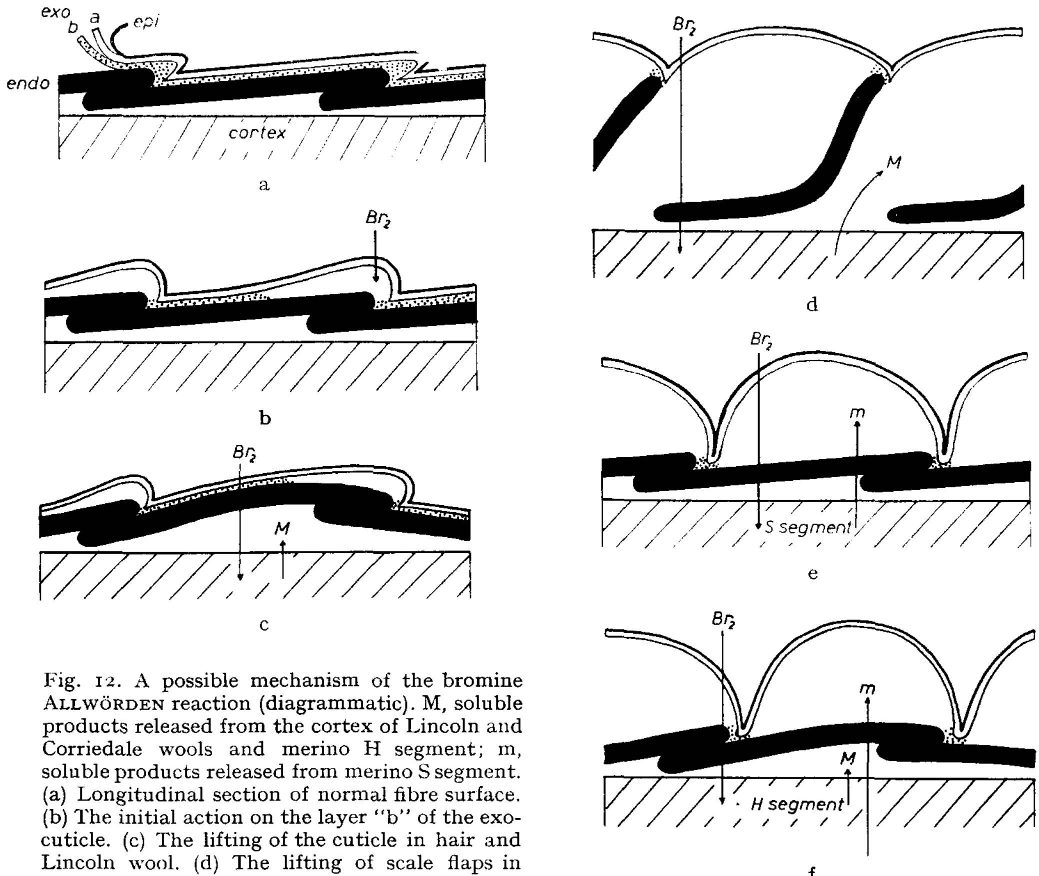

Fig. 12. A possible mechanism of the bromine ALLWÖRDEN reaction (diagrammatic). M, soluble products released from the cortex of Lincoln and Corriedale wools and merino H segment; m, soluble products released from merino S segment. (a) Longitudinal section of normal fibre surface. (b) The initial action on the layer "b" of the exocuticle. (c) The lifting of the cuticle in hair and Lincoln wool. (d) The lifting of scale flaps in Corriedale fibres. (e) The action on the S segment of merino wool. (f) The action on the H segment of merino wool.

The development of bubbles in merino fibres is much more rapid than with coarser wools and hair. The lifting of the epicuticle plus layer "a" of the exocuticle is probably due to the release, from the S segment of the cortex, of soluble protein which is able to penetrate the cuticle but not the epicuticle, and this mechanism is depicted in Fig. 12e. The S segment of the merino cortex has a lower sulphur content[16] and therefore decreased disulphide crosslinking as compared with the H segment, coarser wools and hair. It is possible therefore that the soluble products resulting from bromine attack on the S segment, labelled "m" in Fig. 12, are of lower molecular weight than those released from the H segment and coarser wools, labelled "M" in Fig. 12. If this is in fact the case it is possible to postulate a mechanism for the unsymmetrical development of bubbles without supposing that the cuticle has a bilateral structure[17]. This mechanism is illustrated in Fig. 12c, which would correspond to the incipient lifting of the cuticle around the H segment by soluble products (M) derived from the H segment to which

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the cuticle is impermeable, and Fig. 12e where the cuticle is permeable to the soluble products (m) derived from the S segment. The gradual development of bubbles, similar to those around the S segment, over the entire fibre could be due to the diffusion of soluble products derived from the S segment throughout the fibre as in Fig. 12f.

SUMMARY

The nature of the membranes defining the bubbles in the bromine ALLWÖRDEN reaction has been studied using the gold shadowing technique in optical microscopy. In merino wool the membrane is shown to consist of the epicuticle with an adhering component of the exocuticle. The unsymmetrical development of bubbles in merino wool has been reinvestigated and shown to be a transient phase. A possible mechanism of the bromine ALLWÖRDEN reaction which explains this phenomenon without assuming a bilateral cuticle structure is presented. The variable reaction obtained with coarse wools and hair and the course of the reaction in merino wool is discussed in terms of this mechanism.

RÉSUMÉ

La nature des membranes qui limitent les bulles dans la réaction au brome d'd'ALLWÖRDEN a été étudiée, en utilisant l'ombrage à l'or et le microscope optique. Dans la laine de mouton, la membrane est formée de l'épicuticle et d'un constituant de l'exocuticle qui y adhère. Le développement disymétrique des bulles dans la laine de mouton a été étudié à nouveau et s'est révélé être une phase transitive. Un mécanisme possible de la réaction au brome d'ALLWÖRDEN qui explique ce phénomène sans faire intervenir une structure bilatérale de la cuticule est exposé. La réaction variable obtenue avec des laines et des cheveux bruts et la marche de la réaction dans la laine de mouton sont discutées par rapport à ce mécanisme.

ZUSAMMENFASSUNG

Die Natur der Membran, die die "bubbles" der Brom-ALLWÖRDEN-Reaktion begrenzt, wurde unter Benutzung der Goldbeschattungstechnik im Lichtmikroskop untersucht. Bei der Merinowolle wird gezeigt, dass die Membran aus einer Epicuticula mit anhaftender Komponente von Exocuticula besteht. Die unregelmässige Entwicklung der "bubbles" bei der Merinowolle wurde noch einmal untersucht, und es wurde gezeigt, dass sie eine Übergangsphase ist. Ein möglicher Mechanismus der Brom-ALLWÖRDEN-Reaktion, der dies Phänomen erklärt, ohne eine zweiseitige Cuticula-Struktur anzunehmen, wird angegeben. Die verschiedene Reaktion, die mit gewöhnlicher Wolle und Haaren erhalten wird, und der Verlauf der Reaktion in Merinowolle wird diskutiert in Bezug auf diesen Mechanismus.

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Received October 15th, 1954