THE CHEMICAL COMPOSITION OF WOOL

I. THE SEPARATION AND MICROSCOPIC CHARACTERIZATION OF COMPONENTS PRODUCED BY ULTRASONIC DISINTEGRATION

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Summary

A study is made of the disruption of wool by ultrasonic disintegration and the separation and identification by microscopy of the major components of the fibre. The ultrasonic disintegration process is investigated empirically in order to establish the conditions under which maximum rates of disruption occur. Of a large number of liquids examined it is found, in confirmation of earlier work, that the rate of disintegration of wool is very slow in water but usually increases in amount as the swelling of the fibre increases. The several exceptions to this rule show that there are other factors of secondary importance in determining the rate of disruption. The amount of disruption is sufficient for preparative purposes and increases in amount in the series dimethyl sulphoxide, formic acid, and dichloroacetic acid.

Cortical cells, cuticle, and disrupted cortical cells can be separated on the basis of their different sizes by a combination of screening and sedimentation methods. The histological purity of the fractions is established by light and electron microscopy. It is also shown that the random nature of the ultrasonic disruption process results in the production of components of a continuous range of sizes from cortical cells down to needle-like components of size approaching that of microfibrils.

I. INTRODUCTION

Wool consists of cortical cells which account for about 90% of the weight of the fibre and cuticle which amounts to about 10%. The spindle-shaped cortical cells are arranged side by side with their long axes in the direction of the fibre and are surrounded by a sheath of flat cuticle cells (Mercer 1961). Both the cortical and cuticle cells are structurally complex. The former consist of spindle-shaped components of decreasing size ranging from macrofibrils through microfibrils to protofibrils (Filshie and Rogers 1961), together with cell membranes and nuclear remnants. The cuticle cells consist of three layers called epicuticle, exocuticle (which includes the so-called *a* layer), and endocuticle (reviewed by Lundgren and Ward 1963; see also Rogers 1959*a*).

This complex structure has been identified by light and electron microscopy of the intact fibre and of the components separated by various means. Chemical analyses have been made of intact fibres (Simmonds 1954, 1955, 1956; Corfield and Robson 1955; Ward, Binkley, and Snell 1955; Bradbury 1960*a*) and of separated cuticle (Geiger 1944; Lustig and Kondritzer 1945; Lindley 1947; Elliott and Roberts 1957; Elliott, Asquith, and Rawson 1959; Bradbury 1959, 1960*a*) and

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cortical cells (Ward, Binkley, and Snell 1955; Ward and Bartulovich 1956; Simmonds and Bartulovich 1958). However, the results of analyses of separated components obtained by different workers have not shown good agreement for three reasons. Firstly, with one exception (Bradbury 1959, 1960*a*) the components were prepared by a chemical or enzymic treatment of the intact fibre which produced an unknown amount of chemical modification. Secondly, the microscopic examination has sometimes been inadequate to identify the component and show the absence of contamination by another component. Finally, most of the chemical analyses are either incomplete or less accurate than can be obtained with modern methods of ion-exchange chromatography.

A new approach to the problem of the separation and analysis of components of wool can be made by ultrasonic disintegration of the fibre under suitable conditions (Jeffrey, Sikorski, and Woods 1956; Andrews 1957; Bradbury 1960*b*; Bradbury, Rogers, and Filshie 1963; Ishikawa and Kishino 1963). In this paper a detailed study is made of the disruption of wool by ultrasonic disintegration and of methods of separation and identification of the major components of the fibre. The chemical analysis of the components will be the subject of a further paper.

II. Materials and Methods

(*a*) *Preparation of Wool*

The wool used was virgin Merino 64's (fleece No. SW284) from a pen-fed sheep kept at the Division of Animal Physiology, CSIRO, Prospect, N.S.W. The tips of the fibres were removed and the wool cleaned by Soxhlet extraction with petroleum ether for 8 hr. The fibres were air-dried and washed with a 1% aqueous solution of Gardinol BW* at 50°C (6×5 min) and water at 50°C (4×5 min). The aqueous treatment removed large amounts of dirt and skin flakes, but previous work (Bradbury, Rogers, and Filshie 1963) had shown that a few skin flakes still adhered to the surface of the fibres. The aqueous mixture of dirt and skin flakes was allowed to stand in a separating funnel when the dirt settled to the bottom and the supernatant suspension of skin flakes was removed. In this way the latter were readily obtained in a reasonable state of purity.

(*b*) *Ultrasonic Disintegration of Wool*

A known weight of air-dried wool fibres was cut into short lengths (*c.* 0·5 cm) with scissors (to facilitate even treatment) and immersed in the appropriate liquid in a round-bottomed glass beaker. The titanium probe of the 500-W Mullard-MSE ultrasonic disintegrator (Hughes 1961) was placed below the surface of the liquid in the beaker. The latter was surrounded by a stirred ice–salt-bath which usually maintained the temperature of the liquid at <40°C throughout the ultrasonic disintegration; higher temperatures are recorded in the text where they occur. The disintegrator was tuned to maximum cavitation and the wool sample treated at 20 kc/s for the desired length of time. The mixture was then poured through a

* "Built" anionic detergent, pH 6·5 (Pressley 1960), manufactured by Gardinol Chemicals, Box Hill, Vic.

coarse sieve which retained nearly all of the residual short lengths of fibres. The residual fibres were washed about three times with distilled water which was also poured through the sieve. Microscopic examination showed that the residual fibres were very unevenly treated both along the length of one fibre and also from one fibre to the next, thus showing that ultrasonic disintegration is unable to produce uniformly degraded fibres of the type used by Haly (1958) and Snaith (1960). The suspension of wool components in liquid was then either separated into its components as described in Section II(*c*) or an estimate made of the amount of disintegration as follows:

The suspension was normally filtered through a tared sintered-glass crucible (No. 3) containing a small amount of diatomaceous earth to prevent clogging of the pores of the crucible. The residue was washed three times with water and dried to constant weight at 105°C. In earlier work the solid was sedimented in a tared centrifuge tube, washed three times with water, and dried to constant weight at 105°C. The percentage disintegration of the wool by the ultrasonic treatment was calculated. In a few cases where an appreciable amount of disintegration of the probe occurred during ultrasonic treatment, the suspension was dark in colour and the titanium was removed by centrifugation (see below) before collection of the wool components.

(*c*) *Separation of Components of Wool*

The suspension of solid in the liquid (about 80% water, 20% liquid used for ultrasonic disintegration) usually contained the following components: cuticle, cortical cells, disrupted cortical cells, together with very small amounts of short lengths of fibres, skin flakes, dirt, and titanium chips. This mixture was sedimented and the pellet of material washed three times with distilled water and three times with ethanol, with centrifugation between each wash. This treatment effectively removed the liquid used for ultrasonic disintegration and suspension in ethanol prevented aggregation of particles, which occurred very readily in water. Four different techniques were investigated for the separation of the mixture of solid material.

(i) *Partition Method* (Albertsson 1960).—The suspension of solid material in ethanol was shaken with a mixture of an organic liquid (petroleum ether or benzene) and water. It was found that the aqueous layer contained a fairly pure sample of cortical cells but that the suspended solid in the organic layer was a mixture of cuticle and cortical cells. The latter could not be separated from the former even by repeated washing with water.

(ii) *Centrifugation in a Density Gradient.*—Linear density gradients were prepared by the method of Tung and Taylor (1955) in 6-ml and 50-ml glass tubes. The density gradient was checked by the use of glass balls of known density (Oster and Yamamoto 1963). Three different liquid systems were tried, viz. sucrose in water, carbon tetrachloride–petroleum ether, and carbon tetrachloride–ethanol. The suspension was layered on the top of the gradient and the centrifugation performed in a Servall RC-2 refrigerated centrifuge with a swing-out head. The aqueous system was unsuitable because of aggregation of the solid material and no appreciable

separation of cuticle and cortical cells occurred in the non-swelling carbon tetrachloride–petroleum ether mixture. The third system (carbon tetrachloride–ethanol, density 1·26–1·29) produced a band of short lengths of fibres and cortical cells at the top, a wide central band containing cortical cells, disrupted cortical cells, and cuticle (concentrated near the bottom of this band), and, at the bottom of the tube, dirt, titanium chips, and skin flakes. Repeated centrifugation of the central band on density gradients of the same type did not produce a clean separation of cuticle from cortical cells and macrofibrils. It appears that in carbon tetrachloride–ethanol mixtures the cortical cells have a range of densities which includes that of the cuticle. However, this seems to conflict with Fraser and Macrae (1957) who reported the complete separation of enzymatically produced cuticle and cortical cells by this method, but it is possible that the density of the cuticle and cortical cells were appreciably altered by the enzymatic digestion process. Ward and Bartulovich (1955, 1956), have obtained a wide range of densities of cortical cells in aqueous chloral hydrate.

(iii) *Sedimentation Velocity Method.*—The suspension of solid material in ethanol was layered on top of an ethanol–carbon tetrachloride mixture of density 1·1. Centrifugation for 15 sec at low speed sedimented the short lengths of fibres to the bottom of the tube whilst the rest of the solid remained suspended. After centrifugation for 30 sec the bulk of the cortical cells was at the bottom whilst the cuticle and disrupted cortical cells remained suspended. However, it was not possible to separate the cuticle and disrupted cortical cells by this method because of their similar rates of sedimentation. Also, it was necessary to repeat the sedimentation procedure several times on the cortical cell fraction in order to obtain complete removal of contaminating material (compare with Bartnicki-Garcia and Nickerson 1962).

(iv) *Differential Screening Method.*—Preliminary attempts at separation by use of coarse, sintered-glass crucibles and millipore filters were unsuccessful because particles of size much smaller than the rated pore size of the filter were held on the filter. This has been noted previously with millipore filters (T.D.C. Grace, personal communication) and is probably due to the tortuous passage along which the particle must move in order to pass through the filter, together with the sticky nature of the protein particle. However, it was found that a 40-μ screen held back the cortical cells and also the few skin flakes and a 18-μ screen filtered out the bulk of the disrupted cortical cells. On the basis of the foregoing results it was possible to devise a general scheme for the separation of the various components. This is shown schematically in Table 1.

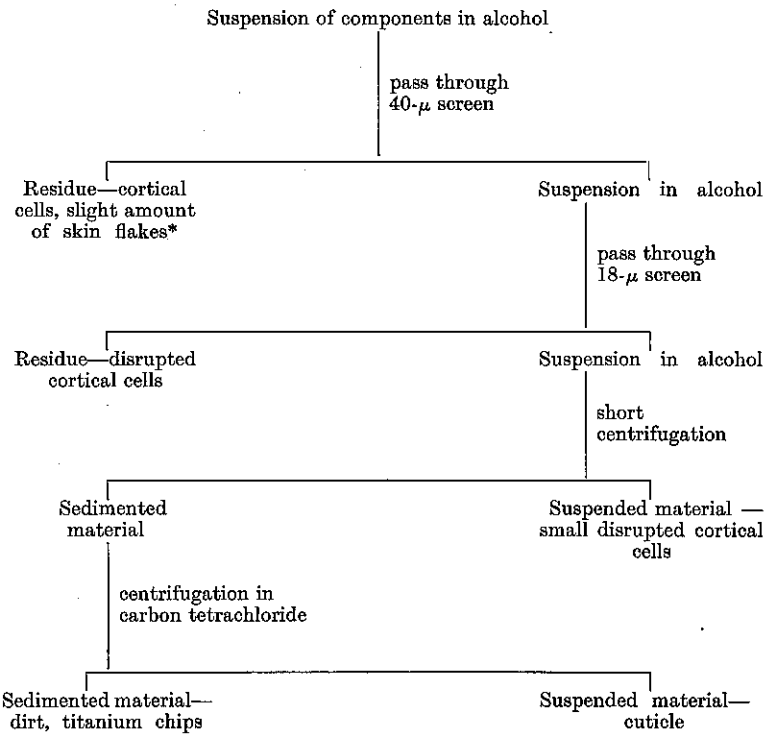
(*d*) *Light and Electron Microscopy*

The various suspensions of wool components were examined routinely by phase contrast with a Leitz Dialux microscope. Photomicrographs were made on Ilford N40 plates. Two methods were used to examine the wool components by electron microscopy. In the first method a drop of a suspension of the material in ethanol was placed on a grid coated with Formvar and the grid dried and examined in a J.E.M. electron microscope, model T-6. The second method consisted of staining the wool components by the thioglycollate–osmium tetroxide method (Rogers

1959*a*, 1959*b*) followed by embedding in Araldite. Thin sections were prepared with a Servall Porter–Blum microtome and were picked up on grids coated with Formvar. These were post-stained with lead hydroxide for 10 min (Rogers and Filshie 1962) and examined in the electron microscope.

TABLE 1

SCHEME FOR SEPARATION OF WOOL COMPONENTS PRODUCED BY ULTRASONIC DISINTEGRATION



* If short lengths of wool fibres are present in this fraction, these are removed by passage through a 100-μ screen or by a short sedimentation run.

III. Results and Discussion

(*a*) *Effect of Various Factors on Ultrasonic Disintegration of Wool*

It is now generally agreed that the ultrasonic disintegration of solid material is a mechanical disruption process (Ackerman 1962), brought about by the enormous pressures developed in the liquid on the collapse of the cavities produced by the ultrasonic waves (Hueter and Bolt 1955). Although the cavitation process has been the subject of a number of investigations (reviewed by Hueter and Bolt 1955) there are considerable gaps in our knowledge, particularly with regard to the mode of mechanical disruption of solid material. It is therefore necessary to make a systematic empirical study for each particular system.

Previous work on the ultrasonic disruption of wool (Bradbury 1960*b*; Bradbury, Rogers, and Filshie 1963) had shown that disruption is very slow in water but much faster in formic acid. For this reason 98% formic acid is used in all the initial studies.

(i) *Type of Vessel.*—The results are summarized in Table 2 from which it is seen that the rate of breakdown in the polythene beaker is far greater than in glass. This is not due to the use of flat-bottomed rather than round-bottomed vessels (see Table 2) but is partly explained by the increased temperature of treatment, since a treatment in a glass vessel at 67°C gave 12% disintegration. Hughes (1961) has found very little difference between nylon, polythene, and glass vessels. The polythene vessel was not used in further experiments because of the difficulty of

TABLE 2

EFFECT OF TYPE OF VESSEL ON AMOUNT OF DISINTEGRATION

Wool (1g, cut into short lengths) was immersed in 50 ml 98% formic acid and treated for 30 min. Probe placed *c.* 0·5 cm above bottom of vessel. Maximum power to transducer, output control on 8

Type of Vessel	Capacity (ml)	Diameter (cm)	Disintegration (%) with:	
			9 : 1 Probe*	4 : 1 Probe*
Pyrex glass, round-bottomed	300	6·5	6·5†	1·0
Pyrex glass, round-bottomed	100	4·5	1·5	1·7
Pyrex glass, flat-bottomed	300	6·5	7·0	2·9
Polythene, flat-bottomed‡	250	6·5	18·3	7·2§

* These are ratios of the cross-sectional area of the "step-down" type probe (Hughes 1961) at the transducer head as compared with its area at the other end.

† Mean of six determinations made over a period of 18 months, standard error = 0·37.

‡ Heating to 65°C occurred due to poor heat conduction of plastic compared with glass.

§ Probe placed 0·5 cm below surface of liquid.

keeping the temperature down to 40°C and also the fairly rapid disintegration of the polythene by the intense ultrasonic beam. In agreement with Hughes (1961) it was found that robust, round-bottomed glass beakers were best, in order to minimize shattering during treatment. The reasonable constancy of the results obtained over a period of 18 months shows that the ultrasonic disintegrator is maintaining a constant output.

(ii) *Type of Probe and Its Position in the Liquid.*—It is seen from Table 2 that the rate of breakdown is considerably greater with the 9 : 1 probe and this is further confirmed by the results of Table 3. This would be expected because the intensity of cavitation produced by the 9 : 1 probe is much greater than by the 4 : 1 probe and there is no appreciable amount of cavitation unloading (Hughes 1961). It is also noted that there is much more breakdown with the 9 : 1 probe when it is placed near the bottom of the vessel than elsewhere, as indicated in the operating manual with the instrument, although the placement of the 4 : 1 probe does not appear to be important. However, Hughes (1961) found that maximum rates of disintegration

of yeast cells are produced when the end of the probe just dips below the surface of the liquid.

(iii) *Power Input to Transducer.*—The output from the generator to the transducer can be increased continuously from zero to its maximum value when the output

TABLE 3

EFFECT OF TYPE OF PROBE AND ITS POSITIONING IN LIQUID ON AMOUNT OF DISINTEGRATION

Wool (1g, cut into short lengths) was immersed in 50 ml 98% formic acid contained in a 300-ml round-bottomed Pyrex vessel, and treated for 30 min. Maximum power to transducer used

Position of Probe	Disintegration (%) with:	
	9 : 1 Probe	4 : 1 Probe
0·5 cm above bottom	6·5	1·0
Middle of liquid	3·7	1·2
0·5 cm under liquid surface	3·3	1·1

control knob reads 8. When 1-g samples of wool were treated for 30 min in 98% formic acid with the 9 : 1 probe, placed 0·5 cm above the bottom of the 300-ml Pyrex vessel, it was found that the amount of disintegration increased as follows:

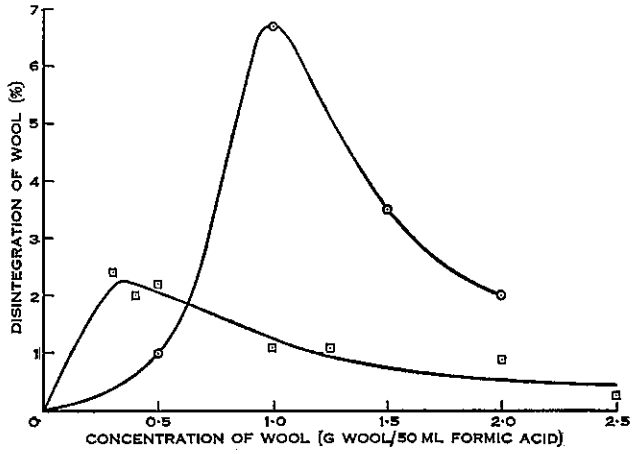


Fig. 1.—Disintegration of wool as a function of the amount of wool in 50 ml 98% formic acid when treated for 30 min at maximum power in the 300-ml round-bottomed Pyrex vessel with the 9 : 1 probe 0·5 cm above the bottom (⊙), or with the 4 : 1 probe 0·5 cm below the surface of the liquid (⊡).

0·7% (output control on 2), 1·7% (4), 6·3% (6), 6·7% (8) (Davies 1959). Microscopic examination of the various samples showed no appreciable differences between them in respect of the ratio of the amount of cuticle to cortical cells.

(iv) *Concentration of Wool in Liquid.*—The experimental results are shown graphically in Figure 1 for the 9 : 1 and 4 : 1 probes. As already noted, the amount of disintegration is much greater with the former than the latter, and the concentration which gives the maximum amount of disintegration is 1 g wool per 50 ml 98% formic acid.

(v) *Volume of Liquid.*—Both Davies (1959) and Hughes (1961) found a decrease in the percentage disruption of yeast cells as the volume of suspension was increased. Our experience with wool has been similar, as shown in Table 4. However, taking into consideration the tenfold range of volumes, the two different concentrations used, and the poor reproducibility of the method, the actual weight of wool disrupted in the 10 min treatment remains surprisingly constant. It is clear that the ultrasonic

TABLE 4

EFFECT OF VOLUME OF FORMIC ACID ON AMOUNT OF DISINTEGRATION

Wool was cut into short lengths and immersed in 98% formic acid in a 300-ml round-bottomed Pyrex vessel and treated for 10 min. The 9 : 1 probe used was placed 0·5 cm above bottom of vessel. Maximum power to transducer used

Concentration of Wool (g/100 ml)	Volume of 98% Formic Acid (ml)	Weight of Disintegrated Material* (mg)	Disintegration (%)
2·0	50	21	2·1†
2·0	100	14	0·7
2·0	150	18	0·6
2·0	500‡	30	0·3
4·0	50	20	1·0
4·0	100	16	0·4
4·0	150	24	0·4

* Mean weight = 21 mg.

† Mean of eight consecutive determinations, standard error = 0·15.

‡ Experiment carried out in a 600-ml round-bottomed Pyrex vessel.

energy is being utilized to approximately the same extent in each treatment; perhaps to the maximum extent. Nevertheless, this state of affairs can only exist over a limited concentration range since, as shown in Figure 1, the amount of wool disintegrated decreases when its concentration is reduced to a low value.

(vi) *Time of Treatment.*—As disintegration of wool in formic acid is prolonged so the rate of production of disperse protein increases, as shown in Figure 2. Microscopic examination of this material shows that the longer the time of treatment the smaller the particles become. Thus, the average size of the disrupted cortical cells decreases as the time of treatment is increased from 15 to 30 min. It is found that the disruption of yeast cells is a first-order rate process, i.e. the rate of cell breakage is proportional to the concentration of intact cells remaining (Davies 1959; Ackerman 1962), but the results obtained in this study do not fit a simple first-order rate equation. This is due to the fact that the mechanical breakdown of the wool fibre is a

complex process, which depends primarily on disruption of the cuticle followed by disintegration of the cortex.

(vii) *Type of Liquid.*—The results obtained on ultrasonic disintegration of wool in various liquids are summarized in Table 5. The liquids are arranged in order of increasing ability to swell wool and various relevant properties of the liquids concerned are also included. It is seen that there is only slight disruption in the alcohols, water, aqueous lithium bromide, and aqueous urea solutions, in agreement with previous work (Bradbury 1960*b*; Bradbury, Rogers, and Filshie 1963).

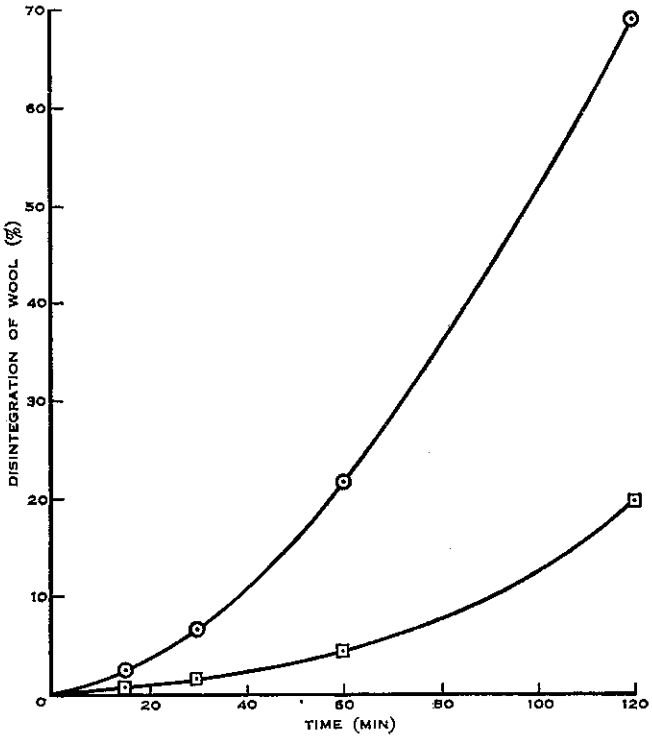


Fig. 2.—Disintegration of wool as a function of time of treatment in 50 ml 98% formic acid at maximum power in the 300-ml round-bottomed Pyrex vessel with the 9 : 1 probe 0·5 cm above the bottom of the vessel (⊙), or with the 4 : 1 probe 0·5 cm below the surface of the liquid (⊡).

Microscopic examination of the finely divided material shows that it consists mainly of skin flakes and cuticle. In general, it is found that the extent of disruption of the wool increases as the swelling of the fibres increases. This is in agreement with expectations, since it is well known that the strength of the fibre decreases as the amount of swelling increases. Thus, for a particular intensity of cavitation, the rate of disruption will increase with increased swelling (hence decreased strength) of the fibres. However, the intensity of cavitation varies from one liquid to another and is known to decrease with decrease in the surface tension of the liquid (Noltingk and Neppiras 1950, 1951) and also appears to be a complex function of the vapour pressure

and the viscosity of the liquid (Hueter and Bolt 1955). The comparatively low intensity of cavitation in trifluoroacetic acid is noted experimentally by the small amount of noise produced during ultrasonic disintegration, and the small amount of breakdown of the wool. There is little doubt that the low intensity of cavitation is due to the low surface tension of trifluoroacetic acid, although its abnormally high vapour pressure may also be a contributing factor (Hueter and Bolt 1955).

TABLE 5

DISINTEGRATION OF WOOL IN VARIOUS LIQUIDS

Wool (1g, cut into short lengths) was immersed in 50 ml liquid contained in a 300-ml round-bottomed Pyrex vessel and treated for 30 min. A 9 : 1 probe was used, with maximum power to transducer

Liquid	Disintegration (%)		Diameter Swelling* (%)	Surface Tension at 20 or 25°C (dyne cm^{-1})	Vapour Pressure at 25°C (mmHg)	Viscosity at 20 or 25°C (cp)
	Probe 0·5 cm above Bottom of Vessel	Probe 0·5 cm below Surface of Liquid				
Ethanol†	0·6	0·5	17	22	57	1·2
Water	0·1	0·2	17	72	24	0·89
Aqueous lithium bromide (100% w/v)‡	1·0	1·2	—	—	—	—
Dimethyl sulphoxide§	2·5	1·4	25	43	<1	1·6
Formamide	1·1	1·1	26	58	<1	3·3
Formic acid–water (40:60 v/v)	1·7	0·8	32	—	—	—
Acetic acid (100%)	1·0	Small	40	28	15	1·15
Formic acid (98%)	6·5	3·3	71	38	40	1·8
Dichloroacetic acid§	53·3	5·1	75	35	<1	*c.* 4
Trifluoroacetic acid	1·2	0·5	76	18	101	0·6

* Determined microscopically by Bradbury and Chapman (1963).

† Various other liquids which swell wool to about the same extent [viz: benzyl alcohol, ethyl formate, n-butanol–water (50 : 50 v/v)] produced similar amounts of disruption.

‡ Concentration of lithium bromide was increased gradually to produce equilibration at each stage (Barnard *et al.* 1954). The same technique used with water saturated with urea produced about the same extent of disruption.

§ Temperature during ultrasonic disintegration in dimethyl sulphoxide reached 50°C, in dichloroacetic acid 65°C.

It is not clear why the amount of disintegration in dimethyl sulphoxide is higher than expected or that of glacial acetic acid is less than expected (Table 5). It is noted that more disintegration occurs with the probe placed at the bottom than at the top of the liquid, although the ratio of these two quantities for any particular liquid varies markedly. The extraordinarily large amount of breakdown in dichloroacetic acid is also of importance.

(viii) *Use of Abrasives and Glass Balls.*—Additions of diatomaceous earth and carborundum before ultrasonic disintegration was tried but it was found impossible

after the treatment to separate the abrasive from the finely divided protein. The results obtained with glass (Ballotini) balls are given in Table 6. It is seen that there is a considerable increase in the amount of disruption in water (Hughes 1961) but it is still too low to be useful for the preparation of wool components. The increase in formic acid is also appreciable but from the experimental point of view this is offset by the extra step required to remove the balls from the protein material after the treatment.

(ix) *Special Treatments.*—Bradbury and Chapman (1963) found that the cuticle can be cracked by swelling the wool fibre by more than 30% of its diameter. Fibres were cracked by immersion in 98% formic acid for several minutes, the acid removed

TABLE 6

DISINTEGRATION OF WOOL IN WATER AND FORMIC ACID CONTAINING GLASS BALLS

Wool (1g, cut into short lengths) was placed in 50 ml liquid in a 300-ml round-bottomed Pyrex vessel containing 2·5 g glass (Ballotini) balls, and treated for 30 min. A 9 : 1 probe was used, with maximum power to transducer

Liquid	Diameter of Balls (cm)	Disintegration (%)	
		Probe 0·5 cm above Bottom of Vessel	Probe 0·5 cm below Surface of Liquid
Water	No balls	0·1	0·2
Water	0·06–0·08	0·6	0·4
Water	0·01–0·02	0·7	0·6
Water	0·0025–0·0044	0·5	0·2
Formic acid (98%)	No balls	6·5	3·3
Formic acid (98%)	0·01–0·02	10·7	9·6

by prolonged washing with water, and these fibres were then subjected to ultrasonic disintegration in water, the conditions described in Table 6 being followed. The amount of disintegration with the probe near the bottom was 1·0% in the absence of glass balls and 1·1% in the presence of glass balls.

Microscopic examination of material produced by ultrasonic disintegration of wool for only 30 sec in 98% formic acid showed the presence of cuticle, a few skin flakes, and cortical cells. On the other hand, a similar ultrasonic disintegration for up to 5 min in dichloroacetic acid produced almost entirely cuticle, with only a very slight amount of skin flakes and cortical cells. Initially, it was thought that the rapid swelling of the fibre by formic acid weakened the whole structure thus allowing the ready liberation of cortical cells, whereas the slow swelling by dichloroacetic acid (Bradbury and Chapman 1963) weakened only the cuticle which was thus preferentially removed. However, this explanation cannot be correct because when the fibres are swollen to saturation with dichloroacetic acid and then subjected to ultrasonic disintegration for 5 min the result is the same as in the absence of the pre-swelling treatment.

(*b*) *Microscopy of Components*

In Plate 1, Figures 1 and 2, are shown light micrographs of cortical cells and cuticle separated from wool treated in formic acid for 30 min and subjected to the screening process described in Table 1. It is found that there is a continuous range of sizes of needle-like cells from cortical cells (diameter 5–10 μ) down to diameters of 100–200 Å, which approaches that of microfibrils (diameter about 70 Å). Similarly, it is found that the cuticle cells show a wide range of sizes from those which are largely intact down to small fragments of thin, flat cells. This type of behaviour would be expected in view of the random nature of the ultrasonic disintegration process.

Plate 2, Figure 1, is a cross-section of cuticle cells at low magnification in the electron microscope. The dark line marked *a* is the so-called *a* layer of the cuticle and the exocuticle and endocuticle regions are also indicated (reviewed by Rogers 1959*a*) It is noted that the endocuticle shows signs of erosion in several cases. This is shown more clearly in Plate 2, Figure 2, where all the endocuticle appears to have been removed by the ultrasonic process. In this section the shape of the scale edges can be seen clearly at *SE* and it is noted that the single scale cell has two scale edges, as has been previously observed by Bradbury and Rogers (1963). The second cuticle cell is probably joined to the first at *J* and, if so, it forms an underlying or second cuticle cell in the manner shown in Plate 2, Figure 3, except that all the endocuticle is removed from the areas marked *R*. A double cuticle cell is shown in cross-section in Plate 2, Figure 3, in which the exocuticle and endocuticle of each layer can be clearly seen.

Plate 3, Figure 1, shows part of a disrupted cortical cell which is in the process of disintegrating into a long particle *P*, of diameter about 0·1 μ. The latter is still attached to the disrupted cortical cell at one end and elsewhere by thin sheets of material, which may be matrix protein. The twisting together of two small disrupted cortical cells is shown in Plate 3, Figure 2, and the shape of an untwisted disrupted cortical cell in Plate 3, Figure 3. This type of behaviour is observed only in a minority of cases and has been observed for intact cortical cells by McMurtrie (1886) and subsequently by other workers (W. von Bergen, personal communication). In Plate 3, Figures 2 and 3, thin sheets of material are noted projecting from the macrofibrils at *S*.

The light and electron microscopy of the three main fractions show that the separation procedure summarized in Table 1 is eminently suitable for the preparation of components which are almost histologically pure. Thus, the cortical cell fraction is contaminated with only a very small amount of skin flakes. The "disrupted cortical cell" fraction contains needle-like components of size between 18 and 40 μ and is virtually free of cuticle or skin flakes. The cuticle fraction is sometimes contaminated with up to 10% of small disrupted cortical cells, which sediment at about the same rate as the cuticle material.

IV. Acknowledgments

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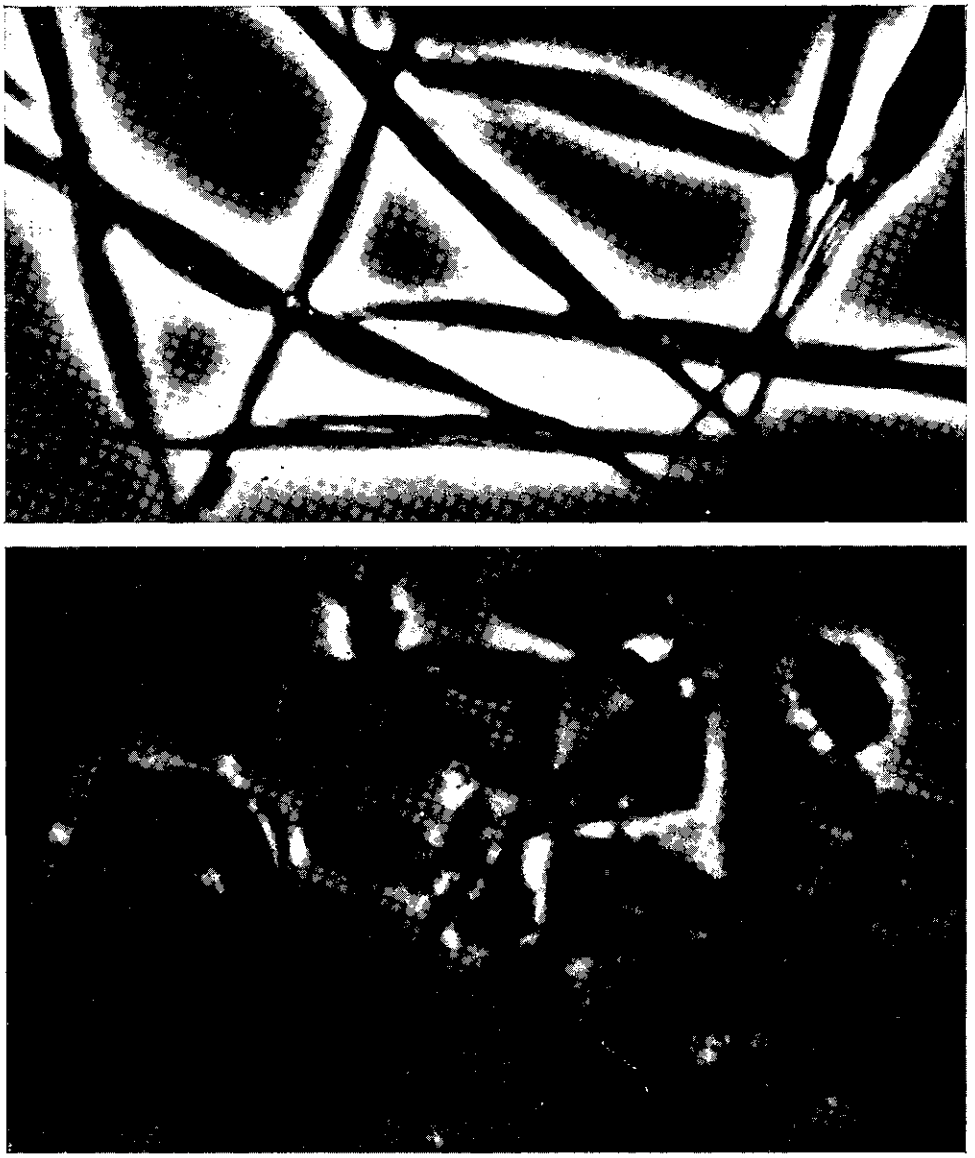

Fig. 1.—Light micrograph (phase-contrast) of cortical cells produced by ultrasonic disintegration in formic acid for 30 min followed by separation according to the scheme set out in Table 1.
Fig. 2.—Phase-contrast light micrograph of cuticle cells produced as in Plate 1, Figure 1.

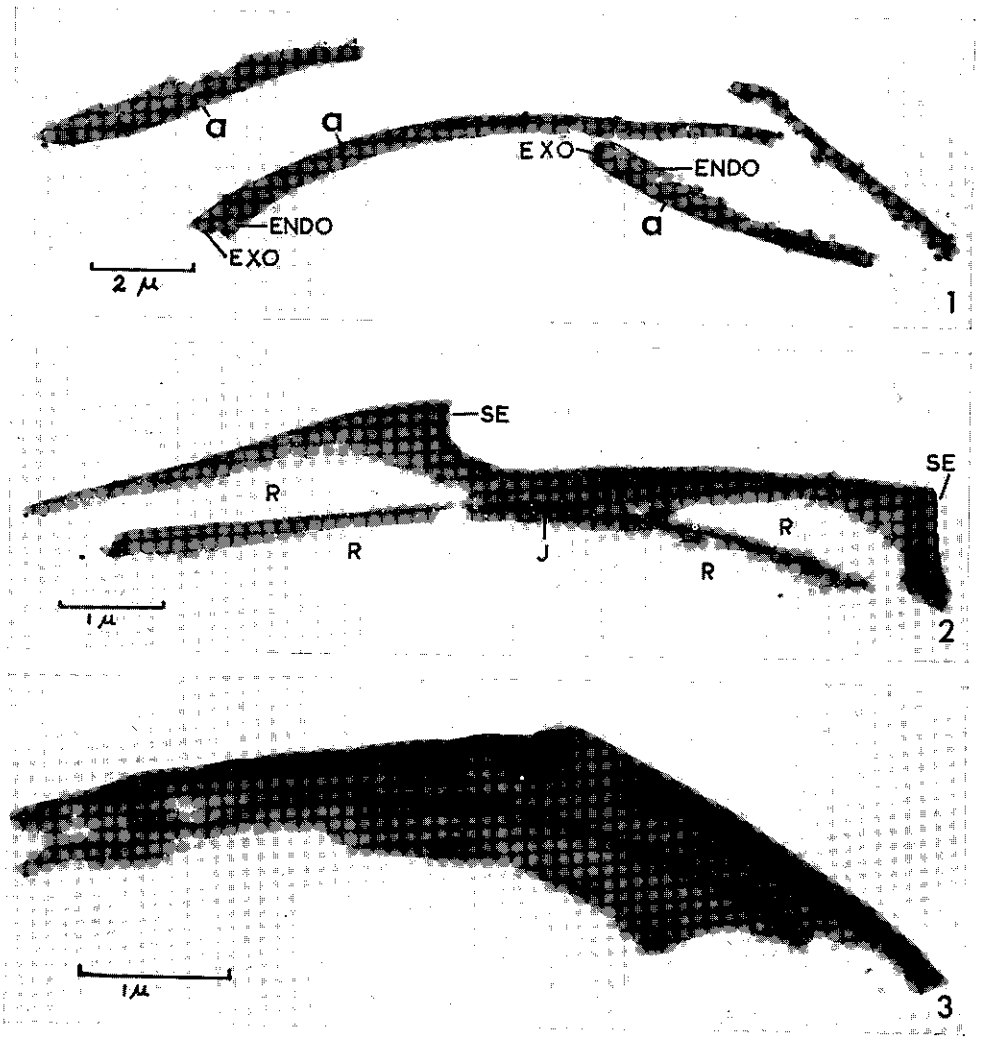

Fig. 1.—Electron micrograph of cuticle cells, prepared as in Plate 1, Figure 1, and stained and observed in cross-section as described in the text. The black spots are produced by post-staining with lead hydroxide.

Fig. 2.—Electron micrograph of cross-section of cuticle cell prepared as in Plate 2, Figure 1.

Fig. 3.—Electron micrograph of cross-section of double cuticle cell prepared as in Plate 2, Figure 1.

CHEMICAL COMPOSITION OF WOOL. I

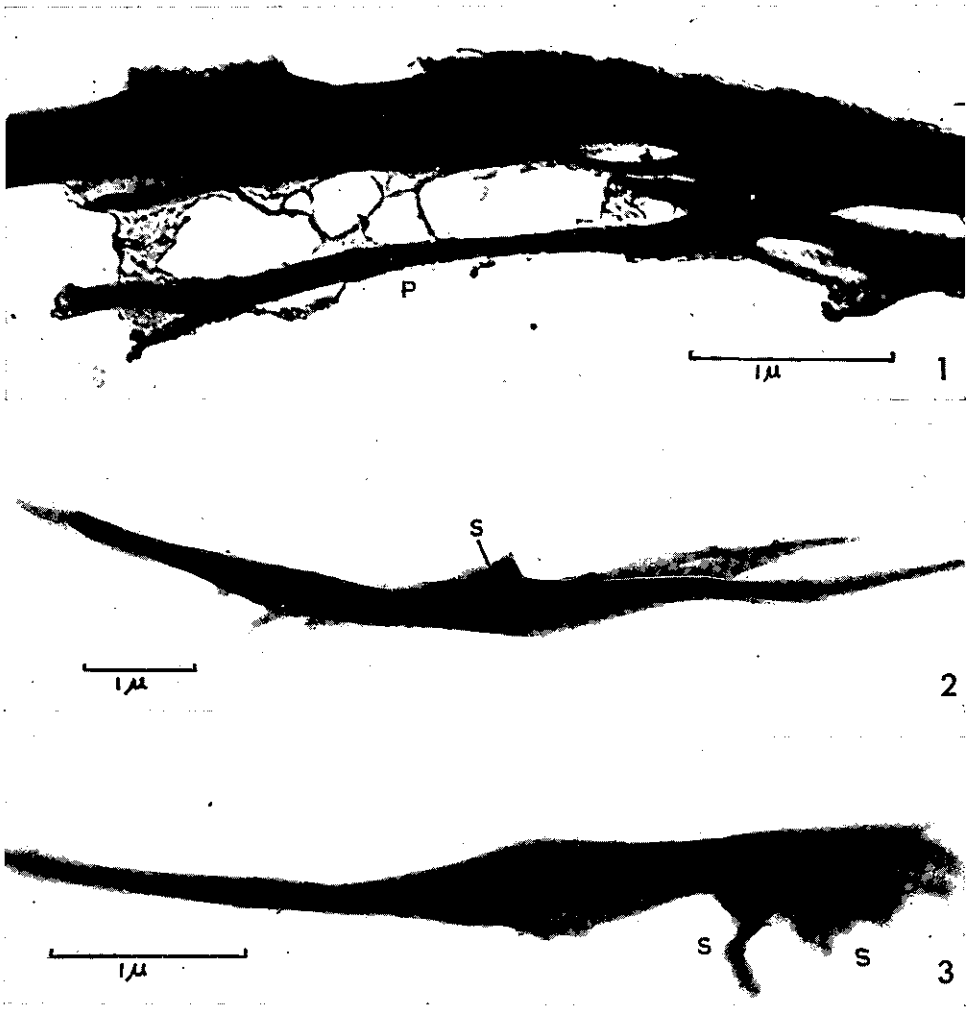

Fig. 1.—Part of a disrupted cortical cell prepared as in Plate 1, Figure 1, and deposited directly on Formvar-coated grid and examined by electron microscopy.

Fig. 2.—Electron micrograph of two small disrupted cortical cells (about 8 μ long) twisted about one another. Material deposited directly on grid.

Fig. 3.—Small disrupted cortical cell (about 5 μ long) showing twisted structure observed also in Plate 3, Figure 2. Electron micrograph of material evaporated onto the grid.