J. Soc. Cosmet. Chem. **25** 355–366 (1974)

The chemistry of human hair cuticle—II: The isolation and amino acid analysis of the cell membranes and A-layer

J. A. SWIFT and B. BEWS*

Synopsis—Mixtures of papain and dithiothreitol have been used to effect the separation of the A-LAYER and CELL MEMBRANE COMPLEX and the cell membrane complex alone from human HAIR CUTICLE. The course of these ENZYMATIC DIGESTIONS has been followed gravimetrically and by the ELECTRON MICROSCOPE examination of digested hair sections, and it is evident that the components mentioned are isolated cleanly. The AMINO ACID compositions of the cell membrane complex and of the A-layer (obtained by difference) are quite different from the composition of the whole cuticle. The significance of the analyses is discussed.

INTRODUCTION

The previous paper of this series (1) described a method for the physical isolation of cuticle from human hair and emphasized the lamellar substructure of each cuticle cell sheet. Our interest is now in the further separation of these laminae for chemical analysis. Various chemical procedures have been described for isolating different fractions from keratin fibres (cf. reference 2). Many of these lead to uncertainties about the significance of the subsequent chemical analysis and most do not allow the

* Unilever Research Isleworth Laboratory, 455 London Road, Isleworth, Middlesex.

accurate specification of the morphological origin of the isolated fractions. Enzymic digestion procedures, by virtue of their high chemical specificity, are likely to lead to much cleaner fractionation and indeed Bradbury and Ley (3) have recently used pronase to dissolve wool endocuticular components selectively. Using mixtures of papain and dithiothreitol we have shown (4), by the electron microscope examination of digested hair sections, that a distinct layer remains undissolved in the vicinity of the cuticle cell membranes. The present paper is concerned with the further study of this digestion procedure which has enabled us to separate from isolated human hair cuticle the cell membrane complex and A-layer.

Methods and materials

The isolation of human hair cuticle

This material was prepared by shaking root-end pieces of brown Caucasian hair in water according to procedures described in the previous paper of this series (1). Cuticle fragments obtained after 2 h shaking (i.e. 5% by weight of the original hair) were separated and dried *in vacuo* over phosphorus pentoxide.

Digestion of cuticle fragments with papain/dithiothreitol

Preliminary gravimetric experiments, in which isolated cuticle was digested by dispersion in a solution containing 1 mg/ml papain (crude powder type 2, Sigma) and 2 mg/ml dithiothreitol (Calbiochem) in 0·1M phosphate buffer at pH 6.7, showed that 92% of the cuticle was dissolved after 18 h at 65°C. In addition, from observations in the transmission electron microscope of thin transverse hair sections digested under similar conditions, it was evident that some components were almost completely dissolved in as little as 15 min at 65°C. The rate of digestion could be reduced by working at 50°C and this lower temperature was used in all subsequent experiments. The course of the digestion was studied by using the following procedure. Weighed samples of cuticle (30 mg) were triturated with phosphate buffer (0.1M, pH 6.7) to form a uniform suspension. A solution of papain (2.5 mg) and dithiothreitol (10 mg) in buffer (2 ml) was then added and the suspensions incubated in a water bath at 50°C. After appropriate time intervals, samples were withdrawn and chilled to quench the enzyme reaction. The undigested cuticle residues were recovered by

centrifugation, washed twice with distilled water, dried *in vacuo* over phosphorus pentoxide and weighed.

Examination of morphological sites of digestion of human hair cuticle by papain/dithiothreitol

For this work, root-ends of brown Caucasian hair were embedded with Spurr's resin (5) in Beem capsules (LKB Produkter). It is noteworthy that these resins are unlikely to diffuse into the fibres, but nevertheless bonding of the resin with the fibre surface is sufficiently strong to provide the rigid support of the hair for subsequent thin sectioning. Transverse sections of the hair approximately 60 nm thick were cut on a diamond knife (Du Pont de Nemours) at a Porter-Blum MT-2 ultramicrotome (Sorvall) and collected on 100 mesh gold electron microscope grids (Polaron) previously covered with a thin collodion/carbon support film. Papain/dithiothreitol reagent, of the same composition as that used in the gravimetric experiments, was prepared and filtered immediately before use through 0.45 μm Millipore discs. The grids were immersed in the reagent at 50°C, and after various times of digestion, rinsed briefly in a solution containing dithiothreitol and buffer, then in two changes of distilled water each for 5 min and dried. Some of the grids were 'shadowed' by the oblique vacuum evaporation of carbon/platinum on to the upper side of the grids (subtending an angle of 30° with the grid surfaces). The shadowing technique did not permit the discrimination of structure within the cell membrane complex on subsequent examination in the electron microscope. The remainder of the grids were therefore stained with various heavy metal compounds according to the following scheme, designed to yield maximum structural information: grids were immersed at room temperature successively in 2% aqueous osmium tetroxide for 2 h, distilled water for 1 h, saturated aqueous uranyl acetate for 2 h, distilled water for 1 h, Reynold's lead citrate (6) for 10 min, 0·02 N sodium hydroxide for 10 s, distilled water for 1 h and then dried.

The various digested hair sections, shadowed or stained, were examined in a JEM 7 transmission electron microscope at 80 kV and using a 20 μm diameter objective aperture. In the case of the shadowed sections it is already known (7) that the surfaces of undigested hair sections are not flat and that the irregularities are related to the underlying morphological structure of the sections. On the other hand the present digestions so cleanly removed some of the structures of the hair cuticle that no confusion arose with the original irregularities of the hair section surfaces.

Amino acid analyses of isolated fractions

Insoluble material remaining after digestion of hair cuticle with papain/dithiothreitol reagent for 45 and 90 min was centrifuged at 30 000 *g*, the supernatant removed and the residue washed with three changes of distilled water, centrifuging after each wash. Material obtained by exhaustive digestion for a total of 3 days with two changes of reagent, was treated similarly. The residues were dried *in vacuo* over phosphorus pentoxide and their amino acid compositions determined by conventional autoanalysis.

Results and Discussion

Course of digestion of isolated cuticle with papain/dithiothreitol

A plot of the amount of residue remaining against time of digestion of isolated cuticle is shown in *Fig. 1* (A). From this curve it appears that at least two components are dissolving at different rates. That this is the case is revealed by the logarithmic plot of *Fig. 1* (B). The undissolved residue at long times of digestion is approximately 7% by weight of the original cuticle and by extrapolation of the log. plot to zero time it would appear that the fast dissolving components represent about 82% and the slow dissolving component about 11% by weight of the cuticle.

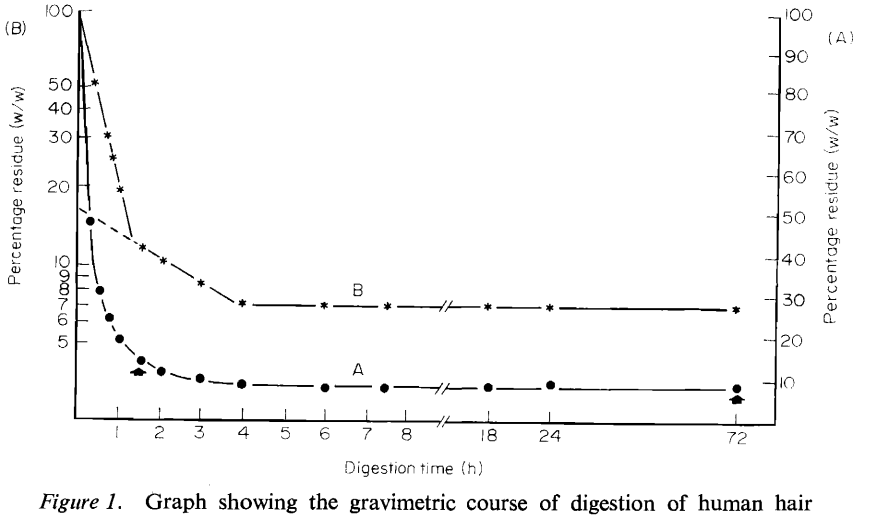

Figure 1. Graph showing the gravimetric course of digestion of human hair cuticle with papain and dithiothreitol. Curve A, axis A show the linear plot and curve B, axis B the corresponding logarithmic plot. Each point shown represents the mean of three separate determinations.

The morphological progress of digestion in the hair cuticle

Examination of digested hair sections in the transmission electron microscope revealed significant progression in the dissolution of the various structural components of the cuticle. In addition, since we believe that the accessibility to enzyme attack of the sectioned hair cuticle and of the physically isolated fragments are probably similar, some correlation could be made between the removal of cuticle components observed in the electron microscope and the various stages of digestion revealed by the foregoing gravimetric results.

After digestion of the sections for only 15 min there was almost complete removal of the endocuticle and there was some loss of material from the exocuticle (*Fig. 2*). (It is pertinent to note that *Figs 2 and 3*, which are electron micrographs of 'shadowed sections', have been prepared from intermediate negatives so that shadow regions are dark. In achieving this normal consistency for shadows, regions of increasing electron opacity are depicted by increasing brightness in the photographs.) Even at 15 min digestion it is striking that a distinct layer approximately 100 nm wide associated with the cuticle cell boundaries exhibits an electron opacity similar to that of the sectioned epoxy resin, indicating that this component is unaffected by the digestion. After digestion for 45 min virtually all the exocuticle was removed leaving the cell boundary material still apparently unaffected (*Fig. 3*). At this stage it was necessary to examine metal-stained rather than shadowed sections to reveal the detailed substructure of the cell boundary layer. It was found that this consisted of the A-layer to which was attached, on the outer-facing aspect of the hair, the complete cuticle cell membrane complex (*Fig. 4*). With further increasing time of digestion the width and general opacity of the A-layer slowly diminished. For periods of digestion exceeding 24 h the A-layer had more or less disappeared still leaving a residue associated with the cell boundaries. Close examination of this revealed that the complete cell membrane complex was still present and bounded on either side by a layer approximately 80 nm thick of stainable material (*Fig. 5*). It is noteworthy that at this stage it was difficult to discern the laminated substructure of the membrane complex but this was probably because disorientation of the residue on the electron microscope grid occurs so that the membrane laminae are no longer parallel to the electron beam of the microscope. On the other hand, by examining the point where adjacent cuticle cells overlap to give a type of T-junction of cell boundaries, the initial orientation of the membranes was maintained

and the laminated substructure of the cell membrane complex could be seen (*Fig. 5*).

From the present results it is clear that the initial fast-dissolving component observed in the gravimetric studies consists of endocuticle and exocuticle, that the slowly-dissolving component consists of A-layer and that the final insoluble residue consists mainly of cuticle cell membrane material. Indeed there is good correspondence between the percentage areas occupied by the endocuticle + exocuticle + inner layer, the A-layer and cell membrane complex in hair cuticle sections (82–85%, 10–12% and 5–6% respectively) and the foregoing gravimetric determinations for what are apparently the same components (82%, 11% and 7% respectively). Little mention has been made of the fate of the cuticle inner-layer in the present work but it is assumed because of its similarity in structure and cystine content (1) that it behaves in a manner analogous to that of the exocuticle.

The progress of digestion leading to the isolation in one case of A-layer and cell membrane complex and in the other of the cell membrane complex alone is summarized in the schematic diagram of *Fig. 6*.

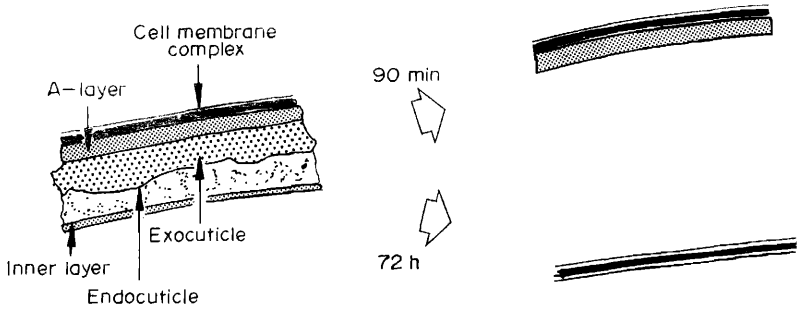

Figure 6. Schematic diagram illustrating the course of digestion of human hair cuticle with papain and dithiothreitol and leading to the separation of two major morphological components of the cuticle.

Amino acid analysis

The first column of Table I contains the amino acid analysis for isolated cuticle and columns 2, 3 and 4, the analyses for the papain/dithiothreitol-insoluble fractions of the cuticle obtained after digestion for 45 min, 90 min and 3 days respectively. The analysis in column 5 was obtained by calculating the differences in amino acid composition between the '90-minute' and '3-day' residues.

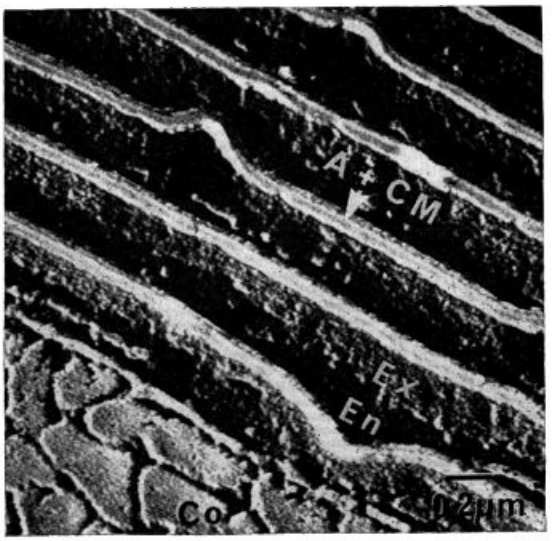

Figure 2. Transverse section of human hair cuticle digested for 15 min and then shadowed. Co, cortex; En, endocuticle; Ex, exocuticle, A+CM, A-layer+cell membrane complex.

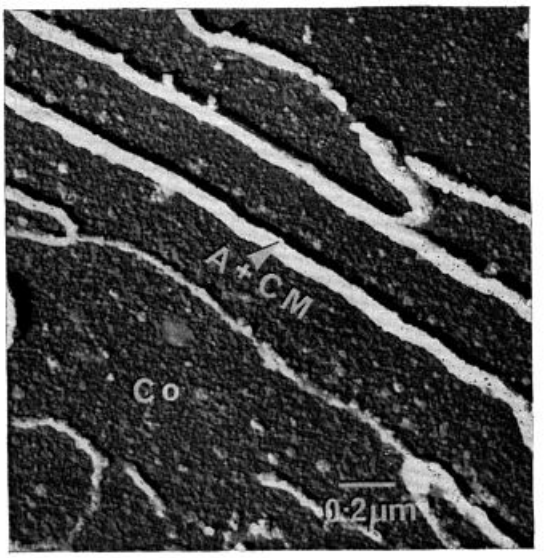

Figure 3. As Fig. 2 but digestion for 45 min.

(*Facing p.* 360)

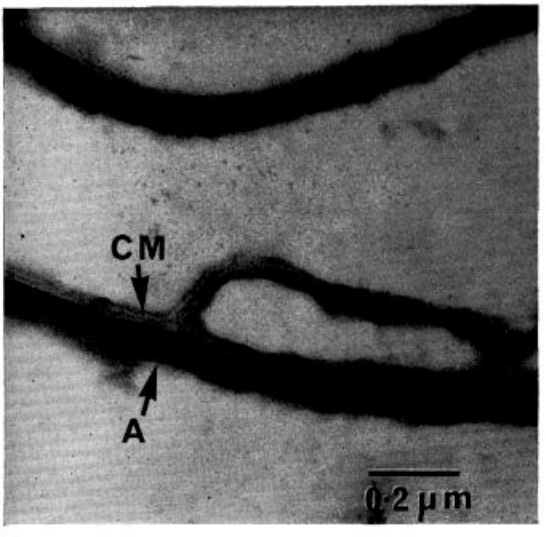

Figure 4. Transverse section of human hair cuticle digested for 45 min and stained with heavy metals. A, A-layer; CM, cell membrane complex.

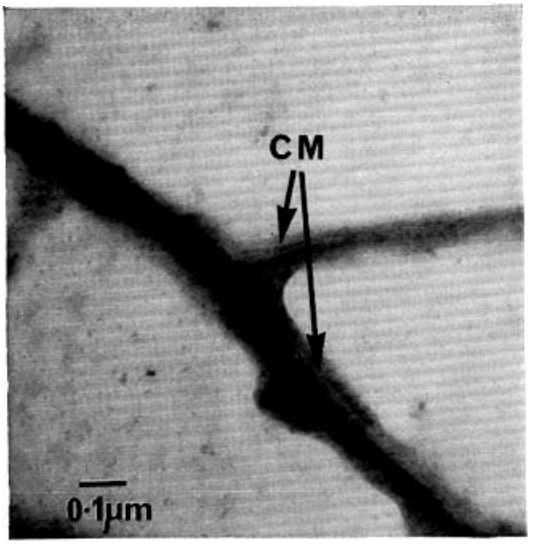

Figure 5. As Fig. 4 but digestion for 24 h.

Table 1. Amino acid compositions of whole cuticle and various fractions obtained in the present work. Also included for comparison, are other published analyses for wool cuticle and various membrane fractions obtained from wool .

	Amino acid composition (residues/1000)								Membrane fractions from wool-Ref. 8, 9		
	Cuticle	'45 min residue' *	'90 min residue' †	CV	Membrane fraction †	CV	'A-Layer' (Calculated)	Wool-Cuticle (ref. 3)	Performic acid ammonia residue	Performic acid urea residue	Epicuticle
Aspartic	32.4	32.5	68.4	13.7	71.9	11.0	67.5	34.6	57.9	50.2	58.4
Glutamic	103.9	96.0	93.2	5.6	77.8	4.7	95.6	86.7	104.3	102.5	106.6
Threonine	46.0	36.9	41.0	8.2	45.4	9.5	40.1	44.4	58.9	55.7	35.9
Serine	160.5	168.3	96.1	11.9	88.1	9.7	97.2	143.4	99.9	100.8	136.3
Proline	105.2	111.6	60.3	12.3	49.2	12.3	62.2	105.1	70.1	71.3	58.0
Glycine	88.4	138.4	125.1	2.9	141.8	9.8	121.8	81.7	139.0	144.3	153.3
Alanine	54.0	54.5	58.6	8.6	50.5	13.8	59.9	57.8	69.0	60.9	46.1
Valine	72.7	69.9	72.6	5.9	74.0	8.0	72.2	75.1	46.6	51.6	57.2
Isoleucine	22.2	14.5	35.3	18.4	46.6	10.2	33.2	26.7	26.5	24.6	25.2
Leucine	22.6	31.6	61.7	16.6	82.2	8.0	57.7	61.2	50.8	46.7	54.6
½ Cystine+Cysteic acid	193.8	117.0	99.5	10.7	99.2	5.9	99.8	156.3	116.0	144.3	119.1
Methionine	3.8	0.2	1.0	34.0	1.8	45.9	0.87	3.4	0	0	0.03
Tyrosine	21.0	17.2	38.4	12.8	31.1	11.9	39.6	28.3	0	0	20.7
Phenylalanine	9.1	4.2	28.0	13.8	15.2	4.5	30.2	16.9	15.3	15.2	18.5
Histidine	5.2	4.8	10.5	17.9	16.7	5.7	8.9	8.1	12.9	13.7	10.3
Lysine	34.4	57.0	59.8	12.0	78.0	7.1	56.6	27.4	90.0	76.9	48.3
Arginine	25.1	37.8	51.7	13.9	32.8	21.5	55.0	42.9	42.6	41.2	42.7
% total amino acid	86.8	65.4	70.7		24.1		—	93	77	86	78
% by weight of cuticle	100	27.0	13.0		5.5		11–12		—	—	—

CV, Coefficient of variation expressed as percentage.
*, Single analysis.
†, Mean triplicate analyses.

Membranes

As discussed previously, the insoluble material remaining after digestion for 3 days consists mainly of cell membrane complex and contains approximately 39% lipid material. The amino acid analysis for this fraction probably represents that for the membrane-associated proteins and it is interesting in that it differs considerably from that for intact cuticle. The last three columns of Table I show the amino acid compositions of membrane fractions obtained from wool by Bradbury and co-workers (8, 9) while column 6 shows the composition of wool cuticle (3). In each case the membrane fractions show substantially higher concentrations of aspartic acid, glycine and lysine, and lower concentrations of serine, proline and cystine than the corresponding cuticle samples. The cuticular membrane fraction from human hair also contains higher proportions of isoleucine, leucine and the aromatic amino acids than does intact cuticle. Closer comparison of the human hair and wool membrane fractions is not possible since those from wool are prepared from whole wool rather than isolated cuticle.

The presence of high concentrations of basic amino acids in the cuticle membrane complex is consistent with the intense staining which is seen under the transmission electron microscope, in the intermembranous cement (δ-band) and the thin layers bounding the complex in hair sections stained with dodecatungstphosphoric acid (1). This heteropolyacid exists in solution as a trivalent anion and is generally believed to bind readily to the basic groups of proteins (10). Electron histochemical staining of hair sections for cystine (7, 11) indicates that this amino acid is absent from the cuticle cell membrane complex. Since some cystine is present in our membrane fraction, at least part of the fraction is probably derived from the protein on either side of the lamellated membrane complex proper and indeed this is consistent with our present electron microscope observations (*Fig. 5*).

The main advantage of the present method for preparing cell membrane fractions from human hair compared with the previously used oxidative procedures (8) is that the destruction of sensitive amino acids such as tyrosine and methionine is minimized. Furthermore the lipid components of the membranes are well preserved so that further study of these materials is possible.

The A-layer

The fractions obtained after digestion for 45 and 90 min evidently

contain mainly A-layer and cell membrane complex, with the 45-min fraction containing a small amount of undissolved exocuticle and the 90-min fraction having had some A-layer removed (cf. *Fig. 1*). As in the case of the membrane fraction, the accuracy of the amino acid analyses is limited by the presence of non-proteinaceous components. A more serious limiting factor is the difficulty of obtaining a well-characterized sample of the A-layer and cell membrane complex free from other contaminants such as exocuticle but not having lost any of the A-layer. Since digestion of the A-layer is essentially complete in less than 7 h, small errors in sampling time or inefficiency of quenching the enzyme reaction result in substantial variations in composition. These difficulties are reflected in the coefficients of variation shown in the third column of Table I. In spite of these limitations the calculated differences in amino acid composition between the '90-minute' and '3-day' fractions shown in the fifth column of Table I, approximate to the composition of the A-layer, and show major differences from the composition of the intact cuticle. The A-layer thus contains higher concentrations of aspartic acid, basic and aromatic amino acids and lower concentrations of serine, proline and cystine than the whole cuticle. The low content of sulphur-containing material in the A-layer/membrane residues was confirmed semiquantitatively by X-ray microanalysis where the sulphur peak/background ratio for the '90 minute residue' was substantially lower than that obtained from intact cuticle or from exocuticle isolated by pronase digestion according to the methods of Bradbury and Ley (3).

The A-layer is probably a complex mixture of different types of proteins. Analysis of residues remaining after various times of digestion between 45 min and 3 h showed that the relative concentration of aromatic amino acids increased as digestion proceeded, as indicated in Table II. No such trends were shown by the other amino acids.

Table II

Residue as % by weight of cuticle	27	14.3	13.3	12.4	9.8
Moles/1000 tyrosine	17.2	18.4	33.7	41.9	48.9
Moles/1000 phenylalanine	4.25	11.6	23.5	30.9	40.7

These results indicate the presence of a component rich in aromatic amino acids and more resistant to proteolytic enzymes than the rest of the A-layer, though whether or not this component forms a discrete lamella close to the membrane is not yet clear.

The low cystine content of the A-layer is surprising and contrary to electron histochemical evidence (7, 11). It is possible that a cystine-rich component has been removed specifically from the A-layer during the enzyme digestion but this is considered unlikely since our electron microscope observations indicate that the overall structural integrity and electron opacity of this layer is maintained for the analysed specimens. A further possibility is that the electron histochemical methods are not as specific in their staining of cystine or cysteine residues as has been claimed. Using an organomercurial method, Dobb, Murray and Sikorski (11) have shown a near-stoichiometric uptake of mercury by cysteine in reduced wool. On the other hand the uptake of mercury in such minor components as the A-layer may not be necessarily related to the presence of cysteine, for other amino acids or non-proteinaceous material could be involved in the reaction. In this respect it is interesting to note that Levy (12) has demonstrated reaction between an organomercurial halide and the amino groups of insulin. Indeed this reaction may explain the existence of the thin layer stained by mercury adjacent to the cuticle cell membranes of wool and described by Dobb *et al.* (11). Such a reaction would be consistent with our observations of high lysine concentrations in the membrane-associated fractions and the staining of a thin layer either side of the cuticle cell membranes complex by dodecatungstophosphoric acid. With respect to the silver-methenemine method for the electron histochemical demonstration of cystine, anomalous staining of the A-layer of human hair cuticle has been described already (7). The silver globules used in the latter method as a criterion for the presence of cystine (7, 13) have a physical appearance which is quite different in the A-layer than in the other components of the hair, perhaps indicating that the histochemical reaction is modified by groups other than cystine. In the light of these discrepancies in the electron histochemical demonstrations of cystine in the A-layer, we believe that our analyses showing the low cystine content of this component are realistic.

The chemistry of papain/dithiothreitol digestion

One striking feature of the present work is the rapidity with which human hair cuticle dissolves in mixtures of papain and dithiothreitol compared with papain alone (usually negligible) or mixtures of papain and sodium bisulphite (55% dissolves after 8 h rising to 70% after 28 h). One of the main reasons for this is undoubtedly the high percentage reduction of the keratin cystine by dithiothreitol (14, 15). Whereas complete reduction of

cystine in keratins by thiols is difficult to achieve because the reaction is determined by the law of mass action, dithiothreitol reduction readily splits 85% of the disulphide bonds in wool under mild conditions with only a small excess of dithiothreitol (14, 15) owing to the formation of a stable cyclic disulphide (4,5-dihydroxy-1,2-dithiane). After reduction of the disulphide bonds the proteins, previously resistant to attack by proteolytic enzymes, are rapidly digested under mild conditions. Borenfreund, Fitt and Berdich (17) have used combinations of trypsin and reducing agents including 2-mercaptoethanol and 2,3-dimercaptopropanol to degrade 'proteolytic enzyme resistant, keratin-like' components of sperm cells and similarly Pfau and McCrea (18) have used pronase and 2-mercaptoethanol to release DNA from vaccinia virus. Preliminary experiments by us have shown that a combination of pronase and dithiothreitol rapidly digests human hair cuticle (80% being dissolved in $1\frac{1}{2}$ h at 37°C pH 8.0 and over 91% within 16 h) indicating that pronase may prove to be a valuable alternative to the use of papain at 50°C.

The activation of papain is dependent upon the existence of cysteinyl residues at the prosthetic site (16) so that the presence of dithiothreitol will give maximum yield of the groups and thereby maximum proteolytic activity.

Acknowledgments

We are grateful to Mr F. J. Bailey of Unilever Research Laboratory, Colworth House for undertaking amino acid analyses for us. Thanks are also due to Mrs S. J. Smith for her valuable assistance with the electron microscope work.

(*Received: 28th February 1974*)

References

(1) Swift, J. A. and Bews, B. The chemistry of human hair cuticle. Part 1. A new method for the physical isolation of cuticle. *J. Soc. Cosmet. Chem.* **25** 13 (1974).
(2) Fraser, R. D. B., Macrae, T. P. and Rogers, G. E. *Keratins: Their Composition, Structure and Biosynthesis* (1972) (Charles C. Thomas, Springfield, Illinois).
(3) Bradbury, J. H. and Ley, K. F. Separation and analysis of exocuticle and endocuticle. *Aust. J. Biol. Sci.* **25** 1235 (1972).
(4) Swift, J. A. and Bews, B. The isolation of membranes from keratin fibres with papain and dithiothreitol. *J. Text. Inst.* **65** 222 (1974).
(5) Spurr, A. R. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26** 31 (1969).

(6) Reynolds, E. S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17** 208 (1963).
(7) Swift, J. A. The electron histochemistry of cystine-containing proteins in thin transverse sections of human hair. *J. Roy. Microsc. Soc.* **88** 449 (1967).
(8) Bradbury, J. H. and Leeder, J. D. The cell membrane complex of wool. *Appl. Polymer Symp.* **18** 227 (1971).
(9) King, N. L. R. and Bradbury, J. H. The chemical composition of wool V. The epicuticle. *Aust. J. Biol. Sci.* **21** 375 (1968).
(10) Kühn, K., Grossman, W. and Hofman, U. Die elektronenmikroskopische anfärbung des kollagens und die ausbildung einer hochunterteilen querstreifung. *Z. Naturforsch.* **136** 154 (1958).
(11) Dobb, M. G., Murray, R. and Sikorski, J. Specific labelling of thiol groups in mammalian keratin suitable for electron microscope studies. *J. Microsc.* **96** 285 (1972).
(12) Levy, D. The selective chloromercuration of insulin. *Biochim. Biophys. Acta* **317** 473 (1973).
(13) Swift, J. A. The electron cytochemical demonstration of cystine disulphide bonds using silver-methenemine reagent. *Histochemie* **35** 307 (1973).
(14) Weigmann, H-D. and Rebenfeld, L. Reduction of wool with dithiothreitol. *Text. Res. J.* **36** 202 (1966).
(15) Weigman, H-D. Reduction of disulphide bonds in keratin with 1,4-dithiothreitol. Part 1. Kinetic investigation. *J. Polym. Sci.* **6** (AI) 2237 (1968).
(16) Glazer, A. N. and Smith, E. L. Papain and other plant sulphydryl proteolytic enzymes. In: *The Enzymes Part* 3, ed. P. D. Boyer, 501 (1971) (Academic Press, New York).
(17) Borenfreund, E., Fitt, E. and Berdich, A. Isolation and properties of deoxyribonucleic acid from mammalian sperm. *Nature,* **191** 375 (1961).
(18) Pfau, C. J. and McCrea, J. F. Release of deoxyribonucleic acid from vaccinia virus by 2-mercaptoethanol and pronase. *Nature,* **194** 894 (1962).

J. Soc. Cosmet. Chem. **25** 367–377 (1974)

Use of a laboratory model to evaluate the factors influencing the performance of depilatories

T. J. ELLIOT*

Presented in part on the 14th November 1973 in Nottingham, at the Symposium on 'Evaluation of Product Performance', organized by the Society of Cosmetic Chemists of Great Britain.

Synopsis—The use of laboratory MODELS to evaluate the performance of COSMETIC and TOILETRY products is full of pitfalls, and subjective assessments nearly always give more accurate results.

However, the investigation of the comparatively large number of variables which influence the speed of action of DEPILATORIES cannot conveniently be carried out on a subjective assessment basis and requires the use of a suitable laboratory model.

The particular model developed (referred to as a DEPILOMETER) was designed to simulate practical use conditions as closely as possible and it enabled the more important formulation variables to be studied quickly. In-use tests on final depilatory formulations gave good correlation with the DEPILOMETER findings.

INTRODUCTION

The main problem of testing depilatories with a users panel on a day-to-day basis is the practical difficulty of the necessary time required to re-grow the hair in between tests.

Another problem is the known variability of hair coarseness between individuals so that in extreme cases this can be a more significant factor in speed of depilation than changes in formulation.

There have been several attempts in the past to develop an *in vitro* test for measuring the effectiveness of depilatories and these have mostly been

* Beecham Products, Beecham House, Brentford, Middlesex TW8 9BD.