# THE CHEMICAL COMPOSITION OF WOOL

## X.* MATERIAL DIGESTED BY TRYPSIN FROM FIBRES AND CORTICAL CELLS

By D. E. Peters†‡ and J. H. Bradbury†

[Manuscript received 5 May 1972]

*Abstract*

Samples of wool, wool pretreated with formic acid, and cortical cells produced by mechanical agitation in formic acid have been digested by trypsin. The evidence shows that the formic acid treatment is not degradative. It is found by electron microscopy that trypsin digests the endocuticle, part of the cell membrane complex, and the nuclear remnants and intermacrofibrillar material of the cortex. These digested components amount to about 3·6, 1·8, and 12·6% by weight of the fibre respectively.

All available evidence indicates that the amount of keratin dissolved by the enzyme treatment is small or negligible. Thus, as a first approximation the trypsin digest from the cortical cells corresponds to the nuclear remnants and intermacrofibrillar material. Its amino acid analysis is very similar to that of the trypsin digest of wool but differs somewhat from the analysis of endocuticle. Compared with wool the nuclear remnants and intermacrofibrillar material contain approximately twice as much lysine, three times as much methionine, and one-third as much cystine. There are also other smaller differences between the amino acid analyses.

## I. Introduction

Prolonged treatment of wool with enzymes has resulted in the dissolution of 10–20% of the material of the fibres. Elöd and Zahn (1946) dissolved 14% with pancreatin in 11 days; Crewther (1956) dissolved up to a maximum of 17% with trypsin depending on conditions of pretreatment of the fibres and the time of digestion; Springell (1963) dissolved 10–20% with pronase; and Leeder (1969) and Bradbury, Leeder, and Watt (1971) dissolved a maximum of 12% after 6 weeks treatment with pronase. During a sustained enzyme treatment cuticle and cortical cells are also released (Burgess 1934; Elöd and Zahn 1943; Mercer and Rees 1946; Springell 1963; Kulkarni, Robson, and Robson 1971) and their amount is increased by mechanical agitation.

This observation led Burgess (1934) to suggest that the enzyme dissolved an "intercellular cement" of low sulphur content; the latter point was confirmed analytically by Elöd and Zahn (1943). This early evidence indicates attack on the cell

* Part IX, *Text. Res. J.*, 1972, **42**, 471.

† Chemistry Department, Australian National University, P.O. Box 4, Canberra, A.C.T. 2600.

‡ Present address: Division of Protein Chemistry, CSIRO, Parkville, Vic. 3052.

membrane complex, but it is found that enzymes also attack the other non-keratinous material of the fibre, viz. endocuticle (Birbeck and Mercer 1957; Bradbury and Ley 1972) and the nuclear remnants and the intermacrofibrillar material within the cortical cells (Mercer, Farrant, and Rees 1956; Anderson and Lipson 1970; Kulkarni, Robson, and Robson 1971). In this paper we report an investigation by electron microscopy of the sites of removal of material by trypsin digestion of wool fibres and separated cortical cells and the amino acid analyses of this material. A further publication (Bradbury and Ley 1972) will describe similar studies on separated cuticle cells.

## II. Experimental

Merino 64's virgin wool (Fleece No. 3716 from Division of Animal Physiology, CSIRO, Prospect, N.S.W.) was cleaned as described by Bradbury *et al.* (1966) and cut into 0·5-cm lengths. In some cases the fibres were immersed for 2 hr in redistilled formic acid at room temperature to remove a small amount of material which originates from the cell membrane complex (Bradbury, Chapman, and King 1965*a*; Peters 1971). They were then washed for 24 hr in deionized water and air dried. Cortical cells were prepared by exposure of the keratin to formic acid for 1 hr at room temperature, which included dispersion by a Polytron stirrer for 10 min and subjection to ultrasonic disintegration for 50 min (Bradbury and Peters 1972). The trypsin used was a twice-recrystallised sample obtained from Worthington Biochemicals.

### (*a*) *Enzymic Digestions*

Weighed keratin samples (wool, 1 g, or cortical cells) were treated with trypsin in water (water : keratin : trypsin ratio of 10,000 : 100 : 1) at pH 8·5, controlled by the addition of 1M ammonium hydroxide by a Pye Autotitrator Controller connected to a Beckman Research pH-meter, which acted as a pH-stat. The digestions were made in a closed flask with mechanical stirring in an atmosphere of nitrogen at a temperature of 37°C. The amount of protein dissolved was sometimes checked during a digestion by measurements of the absorbance at 280 nm on a small sample of the solution, which was centrifuged before measurement in order to obtain a clear solution. At the end of the digestion the pH was reduced to 4 with HCl, the mixture centrifuged at 45000 *g*, and the solid material washed with water. The supernatant liquids were combined, concentrated to about 25 ml at the rotary evaporator, and then lyophilized. The small amount of ammonium chloride produced during the digestion was removed by the evaporation and lyophilization. The material was weighed and the amount of trypsin used was subtracted from this weight before the calculation of the percentage of material digested by the enzyme.

### (*b*) *Electron Microscopy*

This was carried out as described by Bradbury and Chapman (1964) except that sections were cut with an L.K.B. Ultrotome I and these were post-stained with lead citrate (Reynolds 1963). The electron microscope was a Hitachi model HU-IIC-S.

### (*c*) *Amino Acid Analysis*

Amino acid analyses were carried out using a two-column Technicon amino acid analyser essentially as described by Bradbury, Chapman, and King (1965*b*). Recorded analyses are the mean of duplicate runs and are corrected for the (small) amount of trypsin in the digest.

## III. Results

The amount of material digested from wool and cortical cells is given in Table 1. It is noted that there is an initial rapid removal of material from virgin wool and to a lesser extent from wool pretreated by formic acid. This effect is shown in greater detail

by the absorbance measurements given in Figure 1. It was shown in a separate control experiment that the concentration of dissolved material is proportional to the absorbance at 280 nm.

TABLE 1

AMOUNT OF MATERIAL DIGESTED BY TRYPSIN FROM WOOL AND CORTICAL CELLS

| Substrate | Time of digestion (hr) | Yield of digest (%) |
|---|---|---|
| Virgin wool | 1·67 | 1·4 |
| | 21·5 | 2·0 |
| | 42·5 | 2·9 |
| Wool pretreated by immersion in formic acid for 2 hr | 1·67 | 1·0 |
| | 30 | 4·3 |
| | 84 | 6·9 |
| Cortical cells | 36 | 15·1 |
| | 84 | 14·0 |

Electron micrographs of fibres and paracortical and orthocortical cells after digestion with formic acid are shown in Figures 2–4. The black spots and the black material localized in the cell membrane complex of Figure 2 are artifacts of the staining procedure. Electron micrographs of stained cortical cells produced by the normal formic acid treatment nearly always show the nuclear remnants to be full of material, but it is clear from Figures 3 and 4 that trypsin digests this material (see also Kulkarni, Robson, and Robson 1971).

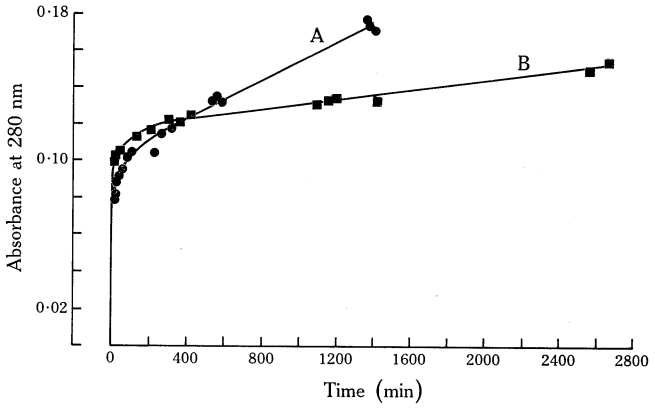



Fig. 1.—Graph of absorbance at 280 nm (corrected for trypsin present) against time of digestion by trypsin for: *A*, wool immersed in formic acid for 2 hr prior to treatment; *B*, virgin wool.

Amino acid analyses were carried out on trypsin digests from virgin wool after 1·67, 21·5, 42·5, and 44·5 hr of digestion and on wool pretreated with formic acid, after 1·67, 24, 30, and 84 hr of digestion with trypsin. It was found that the analyses obtained after treatment for 1·67 hr were somewhat different from those obtained after 20–84 hr digestion. The latter were all very similar in analysis and hence were averaged as shown in Table 2 separately for the virgin wool and formic acid-treated wool. Included in the table are the amino acid analyses of the 15·1% of material

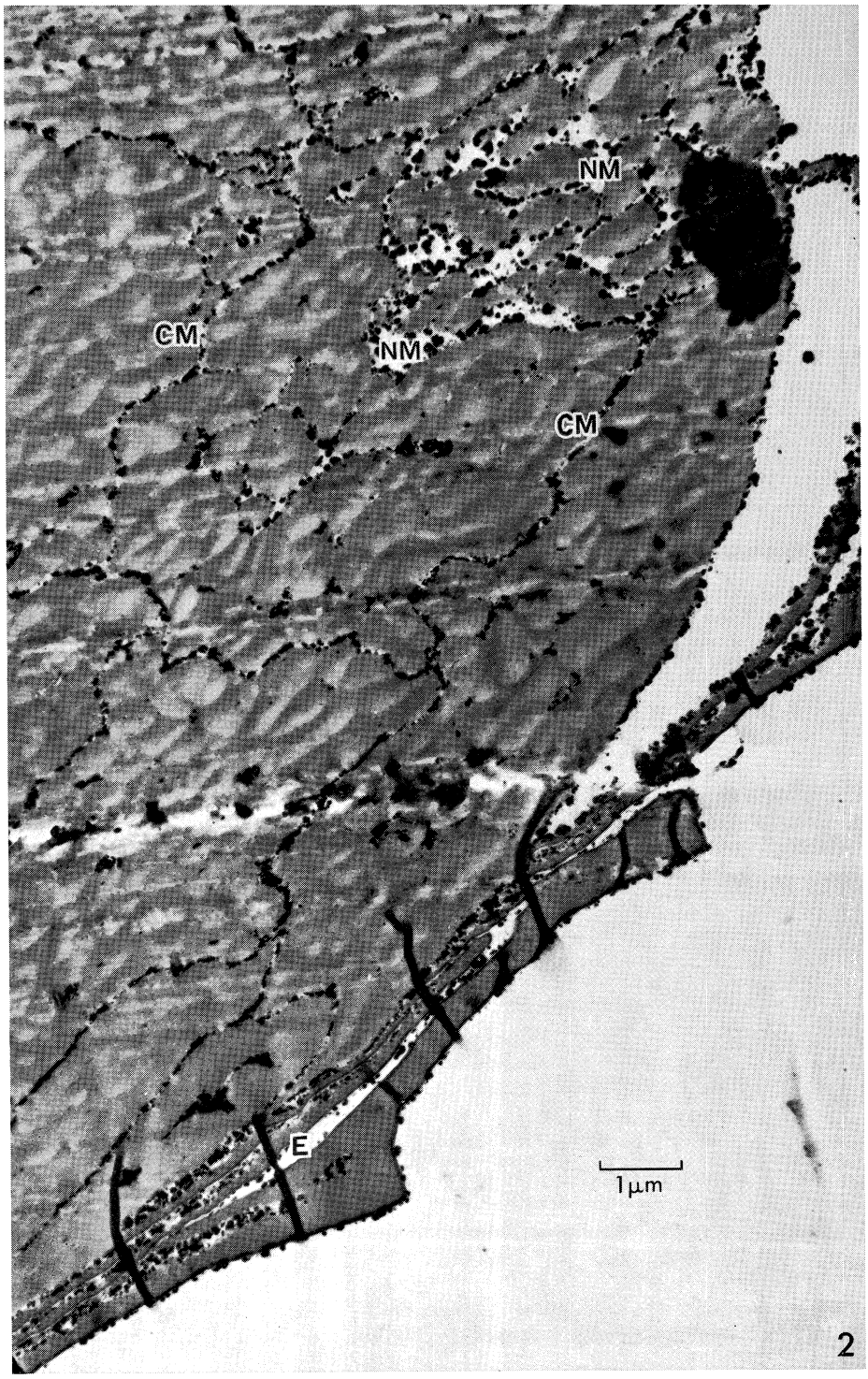
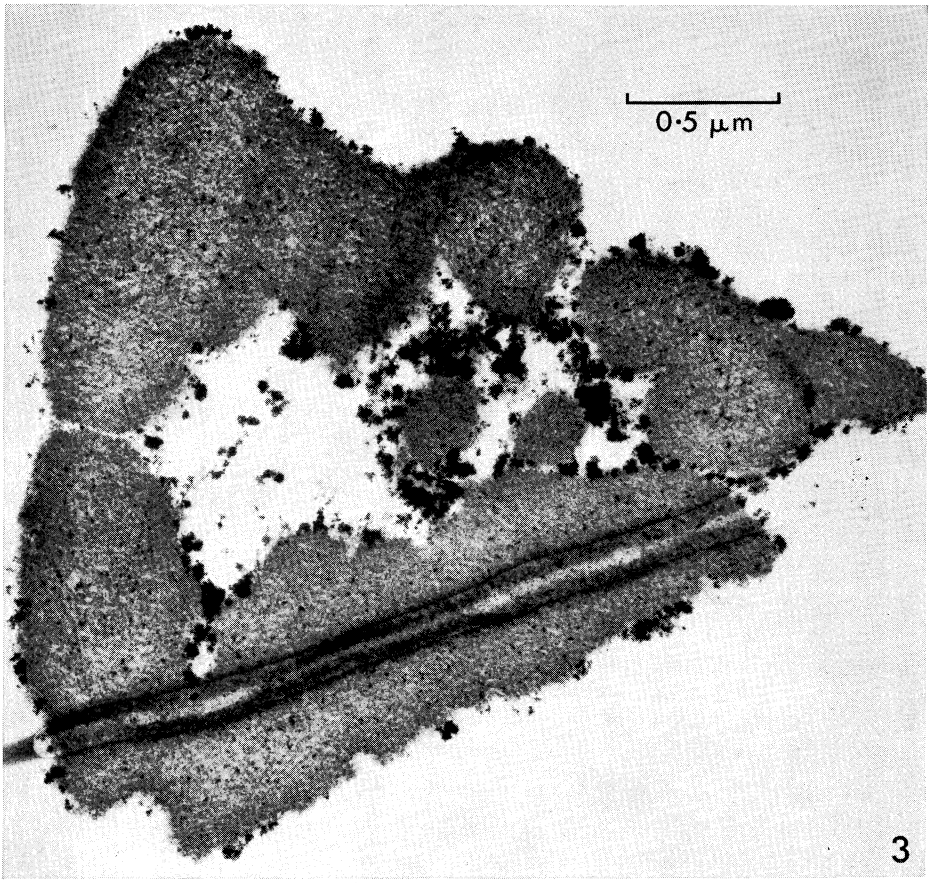



Fig. 2.—Electron micrograph of cross-section of a wool fibre pretreated with formic acid and digested with trypsin for 84 hr (6·9% material extracted). Note the removal of the endocuticle at *E*, and of material from the cell membrane complex (*CM*), and the nuclear remnants (*NM*).



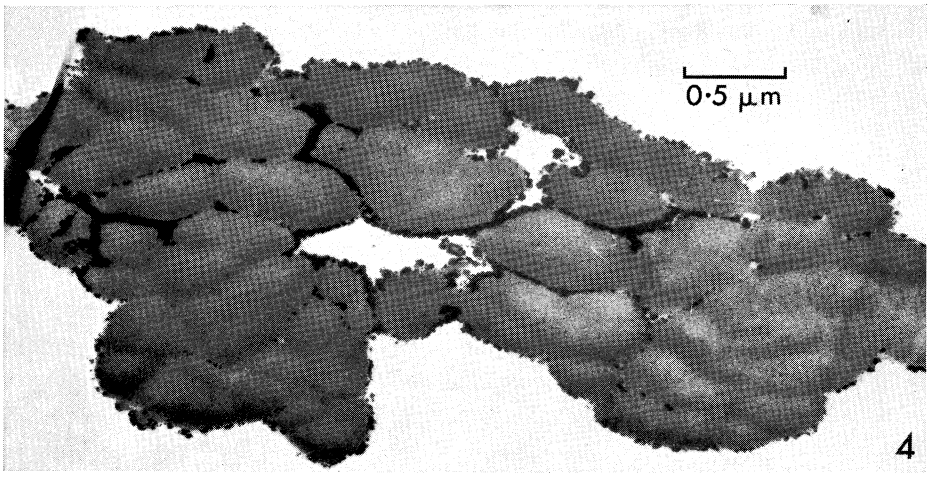




Fig. 3.—Cross-section of a paracortical cell after digestion with trypsin for 36 hr. The dark lines across the micrograph are due to folds in the supporting film. Note the centrally placed holes in the structure due to the digestion by trypsin of the nuclear remnant of the cell.

Fig. 4.—Cross-section of an orthocortical cell after digestion with trypsin for 36 hr. The holes are due to the digestion by trypsin of the nuclear remnants and intermacrofibrillar material of the cell.

extracted from cortical cells by digestion with trypsin and of endocuticle (Bradbury and Ley 1972).

## IV. Discussion

### (*a*) *The Digestion Process*

The digestion of wool by trypsin at pH 8·5 and 37°C involves an initial rapid process as shown in Figure 1, followed by a much slower process in which the rate of digestion of material is constant over the 42·5 hr of the experiment. This zero-order kinetics is not unexpected because after 42·5 hr only 2·9% of material is extracted from the fibre (Table 1) and the total amount which is available for digestion by enzymes is 10–20%. However, after much longer times of digestion the graph should level off as has been observed by Leeder (1969) for pronase digestion of wool, where no further extraction occurs between 4·5 and 6 weeks. A similar result is shown in Table 1 for digestion of cortical cells where the trypsin treatment is complete after 36 hr, and for cuticle where pronase digestion is complete after about 7 days and there is no further digestion after the addition of a second amount of enzyme (Bradbury and Ley 1972). This result is in contrast to the very slow attack of keratin by formic acid at room temperature (Bradbury and King 1967) and shows that the enzymatic attack is limited to certain components of the fibre.

In Figure 1 and Table 1 it is noted that the *initial* rate of digestion is faster from virgin wool than from formic acid-pretreated wool. This is probably because the formic acid pretreatment removes some lipid and protein material from the fibres (Bradbury, Chapman, and King 1965*a*; Bradbury and King 1967; Bradbury, Leeder, and Watt 1971), which would normally be extracted by the enzymatic treatment. The low recovery of anhydroamino acids in Table 2 for material obtained after digestion for 1·67 hr indicates the likelihood of a considerable amount of lipid being extracted in the early stages of the digestion.

The graphs in Figure 1 cross over, which shows that further digestion of formic acid-pretreated wool is faster than for virgin wool. This probably results simply from an increase in the rates of diffusion of enzyme and products of reaction through the fibre which is brought about by the opening up of the structure due to the formic acid pretreatment (Bradbury and Chapman 1963; Bradbury, Leeder, and Watt 1971). If this is the case the formic acid pretreatment should not increase the limiting amount of material obtained by enzyme treatment. Unfortunately we were unable to check this point because of experimental difficulties in keeping the pH-stat stable for long periods of time. An alternative explanation is that formic acid treatment for 2 hr at room temperature has an appreciable degradative chemical effect. We believe this to be unlikely since it has been shown that only 7% of wool is dissolved after immersion in formic acid for 20 weeks (Bradbury and King 1967). Furthermore, treatment of the low-sulphur and high-sulphur proteins from wool with formic acid for 4 hr at room temperature causes no change in their gel-filtration chromatograms, thus showing the absence of an appreciable amount of peptide bond fission (Bradbury and O'Shea 1972).

### (*b*) *Limiting Amount of Material Digested from Wool*

The total amount of material which can be dissolved by enzyme treatments on wool has been variously estimated as 14% with pancreatin (Elöd and Zahn 1946),

TABLE 2

AMINO ACID ANALYSES (MOLE %) OF TRYPSIN DIGESTS OF WOOL AND CORTICAL CELLS*

| Amino Acid | Trypsin digestion for 1·67 hr of | | Trypsin digestion for 20–84 hr of | | | Trypsin digestion for 36 hr of cortical cells | Endocuticle‡ |
|---|---|---|---|---|---|---|---|
| | Wool | Formic-acid-treated wool | Wool† | Formic-acid-treated wool† | Mean | | |
| Alanine | 7·16 | 7·33 | 7·16 | 7·57 | 7·36 | 7·46 | 6·65 |
| Arginine | 2·59 | 2·86 | 3·58 | 6·38 | 4·98 | 6·19 | 4·98 |
| Aspartic acid | 10·88 | 11·01 | 10·85 | 9·66 | 10·26 | 9·89 | 7·41 |
| Cysteic acid | 0·64 | 0·18 | 0·23 | 0·15 | 0·19 | 0·81 | 0·90 |
| ½Cystine | 4·14 | 3·92 | 2·51 | 0·56 | 1·54 | 2·25 | 2·20 |
| Cysteic acid + ½cystine | 4·78 | 4·10 | 2·74 | 0·71 | 1·73 | 3·06 | 3·10 |
| Glutamic acid | 8·84 | 8·76 | 9·46 | 11·56 | 10·51 | 11·23 | 10·31 |
| Glycine | 13·37 | 12·54 | 12·50 | 9·05 | 10·78 | 9·42 | 8·15 |
| Histidine | 1·31 | 1·67 | 1·48 | 1·87 | 1·68 | 1·67 | 1·07 |
| Isoleucine | 6·27 | 6·16 | 6·10 | 5·04 | 5·57 | 5·60 | 3·94 |
| Leucine | 6·78 | 7·67 | 7·60 | 9·37 | 8·48 | 8·69 | 9·31 |
| Lysine | 5·87 | 6·84 | 6·61 | 6·89 | 6·75 | 6·51 | 4·21 |
| Methionine | 1·07 | 1·01 | 1·44 | 1·81 | 1·62 | 1·43 | 0·81 |
| Phenylalanine | 1·87 | 1·79 | 2·16 | 3·38 | 2·77 | 2·98 | 3·85 |
| Proline | 5·09 | 5·64 | 5·11 | 5·14 | 5·13 | 4·75 | 8·91 |
| Serine | 9·91 | 7·87 | 8·49 | 6·81 | 7·65 | 7·12 | 10·70 |
| Threonine | 3·85 | 3·82 | 4·33 | 5·24 | 4·78 | 4·34 | 5·54 |
| Tyrosine | 3·68 | 3·99 | 3·54 | 3·18 | 3·36 | 3·11 | 3·58 |
| Valine | 6·68 | 6·94 | 6·85 | 6·34 | 6·59 | 6·55 | 7·47 |
| % recovery of anhydroamino acids | 59 | 50 | 72 | 83 | 78 | 94 | — |

* All analyses corrected for trypsin or pronase present in digests.

† Result for wool is the mean of three duplicate analyses on digests obtained after treatment for 21·5, 42·5, and 44·5 hr; that for formic-acid-treated wool is mean of three duplicate analyses on digests obtained after treatment for 24, 30, and 84 hr.

‡ Obtained from pronase digestion of cuticle (see Bradbury and Ley 1972).

10–17% with trypsin (Crewther 1956), 10–20% (Springell 1963), and 12% (Leeder 1969) with pronase. The general semiquantitative agreement between these results indicates that these three enzymes, with markedly different specificities, probably extract essentially the same material from the fibre.

Another estimate can be made of the total amount of the enzymically digestible (non-keratinous) material from wool. We assume that it originates from the endocuticle, the nuclear remnants, and intermacrofibrillar material of the cortex and the cell membrane complex. The endocuticle accounts for $36 \times 10/100 = 3 \cdot 6\%$ of the fibre (Bradbury and Ley 1972), the nuclear remnants and intermacrofibrillar cement $14 \cdot 6 \times 86 \cdot 5/100 = 12 \cdot 6\%$ of the fibre (Table 1), and the non-keratinous material of the cell membrane complex $1 \cdot 8\%$ of the fibre (Bradbury, Leeder, and Watt 1971). A total value of 18% is obtained, which is slightly greater than most of the direct measurements, but shows that the whole scheme appears to be reasonable.

### (c) *Site of Non-keratinous Material*

By comparison with electron micrographs of stained cross-sections of untreated wool (Birbeck and Mercer 1957; Rogers 1959*a*, 1959*b*) the electron micrograph in Figure 2 shows removal of material from the endocuticle, the cell membrane complex, and the nuclear remnants of the cortex. Not all material has been extracted from these components in this treatment since the extraction amounts to $6 \cdot 9\%$. Virtually complete extraction has been achieved with cortical cells after digestion for 36 hr with trypsin, since no further extraction occurs with prolonged treatment (Table 1) and also there is no evidence of retention of nuclear remnants or intermacrofibrillar material in Figures 3 and 4. Kulkarni, Robson, and Robson (1971) have previously obtained similar results on prolonged treatment of wool with trypsin, which they used in the separation of orthocortical and paracortical cells.* Similarly prolonged pronase extraction of separated cuticle cells removes the endocuticle cleanly (Bradbury and Ley 1972).

### (d) *Amino Acid Analyses*

The amino acid analyses of the material removed by trypsin digestion for $1 \cdot 67$ hr of virgin wool and formic acid pretreated wool shown in Table 2 are very similar. In each case there is about 50% of non-protein material obtained in this early, rapid stage of the digestion of about $1–1 \cdot 4\%$ of material from the fibre. As digestion is continued the protein content of the digest increases to about 80% and there are some changes in the composition of the protein particularly a decrease in the content of cysteic acid + ½cystine and increases in arginine, methionine, phenylalanine, and possibly threonine. Differences also appear between the amino acid analyses of the digests from virgin wool and formic acid pretreated wool, particularly in ½cystine + cysteic acid.

In general, the close similarity between these values and those for the trypsin digest of cortical cells and the pronase digest of cuticle (i.e. endocuticle) encourage us to average the results as shown in Table 2 and obtain a mean value for the trypsin extract from whole wool. This should lie intermediate between the values obtained for the enzymatic digestion from cortical cells and cuticle and this occurs for most of the amino acids. Those amino acids for which this is not the case are particularly

* Their published amino acid analyses are therefore those for the keratin of these cells, and do not include the non-keratinous material, as was obtained by Chapman and Bradbury (1968).

cysteic acid+½cystine, methionine, and to a much lesser extent aspartic acid, glycine, lysine, and phenylalanine. Furthermore the analysis of the trypsin digest from wool is very similar to that of the trypsin digest from cortical cells (important deviations occurring only with arginine and cysteic acid+½cystine) since the latter makes up $12 \cdot 6 \times 100/18 = 70\%$ [see Section IV(*b*)] of the former. However, the analysis of endocuticle shows much greater deviations from that of the trypsin extract of wool for aspartic acid, glycine, histidine, isoleucine, lysine, methionine, proline, serine, and threonine. There is a close parallel between this situation and the amino acid analyses of wool and cortical cells which are virtually the same and cuticle which is appreciably different (Bradbury, Chapman, and King 1965*b*; Bradbury *et al.* 1970).

### (*e*) *Degradative Effect of Formic Acid and of Trypsin*

As already discussed in Section IV(*a*) it is unlikely that the formic acid treatment of keratin, which is required to produce separated cortical and cuticle cells (Bradbury and Ley 1972) for enzymatic digestion, causes main chain fission or other chemical reactions other than formylation, which is probably reversible (Bradbury, Chapman, and King 1965*b*). This conclusion also stems from the general consistency of the whole picture with regard to both the total amount of material extracted by enzymes from wool and its various components and the amino acid analyses of these digests.

However, a more difficult question to answer is whether the enzymic treatment removes *only* the endocuticle, the non-keratinous part of the cell membrane complex, and the nuclear remnants and intermacrofibrillar material of the cortex or whether it is also capable of digesting part of the keratin. It is clear that the enzymic attack of the keratin must be small or negligible because (1) there is no visual evidence of digestion of keratin in electron micrographs (see Figs. 2 4); (2) there is a limiting amount of material digested by enzymes from wool (Leeder 1969), cortical cells (Table 1), and cuticle (Bradbury and Ley 1972); and (3) Kulkarni, Robson, and Robson (1971) find no increase in *N*-terminal residues after prolonged trypsin treatment of wool to produce orthocortical and paracortical cells.

Attack of the keratin by the enzyme is probably limited to amorphous regions, since ordered regions are much more resistant to digestion (Harrington, von Hippel, and Milhalyi 1959; Crewther and Harrap 1967). Dissolution of peptides will be limited by the disulphide cross-links which occur on the average every tenth residue over the whole fibre and about every fifth residue in the amorphous region, if we assume that the latter consists of the high sulphur protein from wool (Crewther *et al.* 1965). However, there is still the likelihood of some peptide-bond fission within the three dimensional network (Gillespie 1970), even though dissolution of peptides from the keratin is probably slight.

### (*f*) *Analysis of Nuclear Remnants and Intermacrofibrillar Material*

All available evidence points to the likelihood of, at the most, only a small amount of contamination of the trypsin digest with material dissolved from the keratin itself. Thus, the analysis of the trypsin digest from cortical cells in Table 2 is to a first approximation that of the nuclear remnants and intermacrofibrillar material of the cortex. Compared with wool the nuclear remnants contain more aspartic acid, histidine, isoleucine, valine, twice as much lysine, almost three times as much methionine, only

one-third as much cysteic acid+½cystine, and less proline, serine, threonine, and tyrosine.

It is clear that enzymic digestion is a very useful procedure for separating the non-keratinous part of the fibre from the keratin itself, to allow amino acid analysis of the former. However, the method would be useless for studies on soluble proteins from the non-keratinous region, because of the extensive peptide bond hydrolysis which occurs.

## V. Acknowledgments

We wish to thank Dr. W. L. Nicholas for access to transmission electron microscope facilities in the Zoology Department and the Australian Wool Board for a research scholarship to Dr. D. E. Peters.

## VI. References

Anderson, C. A., and Lipson, M. (1970).—*Text. Res. J.* **40**, 88.
Birbeck, M. S. C., and Mercer, E. H. (1957).—*J. Biophys. Biochem. Cytol.* **3**, 203, 215.
Bradbury, J. H., and Chapman, G. V. (1963).—*Text. Res. J.* **33**, 666.
Bradbury, J. H., and Chapman, G. V. (1964).—*Aust. J. biol. Sci.* **17**, 960.
Bradbury, J. H., Chapman, G. V., Hambly, A. N., and King, N. L. R. (1966).—*Nature, Lond.* **210**, 1333.
Bradbury, J. H., Chapman, G. V., and King, N. L. R. (1965*a*).—Proc. Third Int. Wool Text. Res. Conf., Paris. Vol. 1. p. 359.
Bradbury, J. H., Chapman, G. V., and King, N. L. R. (1965*b*).—*Aust. J. biol. Sci.* **18**, 353.
Bradbury, J. H., Chapman, G. V., King, N. L. R., and O'Shea, J. M. (1970).—*Aust. J. biol. Sci.* **23**, 637.
Bradbury, J. H., and King, N. L. R. (1967).—*Aust. J. Chem.* **20**, 2803.
Bradbury, J. H., Leeder, J. D., and Watt, I. C. (1971).—Appl. Polymer Symp. No. 18. p. 227.
Bradbury, J. H., and Ley, K. F. (1972).—*Aust. J. biol. Sci.* **25**, 1235.
Bradbury, J. H., and O'Shea, J. M. (1972).—*Aust. J. biol. Sci.* **25**, 963.
Bradbury, J. H., and Peters, D. E. (1972).—*Text. Res. J.* **42**, 471.
Burgess, R. (1934).—*J. Text. Inst.* **25**, T289.
Chapman, G. V., and Bradbury, J. H. (1968).—*Archs Biochem. Biophys.* **127**, 157.
Crewther, W. G. (1956).—Proc. Int. Wool Text. Res. Conf., Aust., 1955. Vol. C. p. 227.
Crewther, W. G., Fraser, R. D. B., Lennox, F. G., and Lindley, H. (1965).—*Adv. Protein Chem.* **20**, 191.
Crewther, W. G., and Harrap, B. S. (1967).—*J. biol. Chem.* **242**, 4310.
Elöd, E., and Zahn, H. (1943).—*Melliand Textilber.* **24**, 245.
Elöd, E., and Zahn, H. (1946).—*Melliand Textilber.* **27**, 68.
Gillespie, J. M. (1970).—*Science, N.Y.* **170**, 1100.
Harrington, W. H., Hippel, P. H. von, and Milhalyi, E. (1959).—*Biochim. Biophys. Acta* **32**, 303.
Kulkarni, V. G., Robson, R. M., and Robson, A. (1971).—Appl. Polymer Symp. No. 18. p. 127.
Leeder, J. D. (1969).—M.Sc. Thesis, Australian National University.
Mercer, E. H., and Rees, A. L. G. (1946).—*Aust. J. exp. Biol. Med. Sci.* **24**, 147, 175.
Mercer, E. H., Farrant, J. L., and Rees, A. L. G. (1956).—Proc. Int. Wool Text. Res. Conf. Aust., 1955. Vol. F. p. 120.
Peters, D. E. (1971).—Ph.D. Thesis, Australian National University.
Reynolds, E. S. (1963).—*J. Cell Biol.* **17**, 208.
Rogers, G. E. (1959*a*).—*Ann. N.Y. Acad. Sci.* **83**, 378.
Rogers, G. E. (1959*b*).—*J. Ultrastruct. Res.* **2**, 309.
Springell, P. H. (1963).—*Aust. J. biol. Sci.* **16**, 727.