Cyanogen bromide cleavage of proteins in sodium dodecyl sulphate/polyacrylamide gels

Diagonal peptide mapping of proteins from epidermis

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A two-dimensional electrophoretic procedure employing CNBr has been devised for the analysis of proteins in sodium dodecyl sulphate/polyacrylamide gels. The technique allows the detection of an unusual class of epidermal proteins that lack methionine. The proteins have been identified by this method in newborn mouse, rat, and rabbit, because they are stable in the presence of CNBr and consequently lie on a diagonal. Adult human epidermis also contains CNBr-stable proteins, but in lesser amounts than in the newborn rabbit or newborn rodents. The methionine-containing proteins (i.e., the keratins) are degraded by CNBr into a series of unique and characteristic peptides which lie below the diagonal. Inter- and intra-species similarities and differences exist between the individual keratins, depending on the number and distribution of their methionine residues. The peptide-map patterns for the rodent and lagomorph proteins are more similar to each other than to that for the human proteins. The maps for rat and rabbit skin proteins are the most similar. We conclude that the epidermal keratins are a closely related, yet individually distinct, group of proteins that are found in conjunction with a class of proteins that lack methionine. The latter proteins are related to the histidine-rich basic protein, an epidermal structural protein that aggregates with keratin filaments.

In the present paper we describe a technique for detecting, in SDS/polyacrylamide gels, proteins that lack methionine. The technique originated as a response to a need for a rapid and simple method for detecting an unusual class of proteins found in the epidermis of many animals. These proteins are rich in the amino acids glutamic acid, serine, glycine, arginine and histidine, but are deficient in hydrophobic residues, including methionine. They are found in the cornified and granular layers of the epidermis and have been called 'stratum corneum basic protein' (Dale, 1977) or 'histidine-rich proteins' (Tezuka & Freedberg, 1974; Balmain *et al.*, 1977; Ball *et al.*, 1978; Scott & Harding, 1980). Newborn-rat stratum-corneum basic protein and histidine-rich protein II have recently been shown to be identical and are now called 'histidine-rich basic protein' (Dale *et al.*, 1981). HRB protein has an apparent relative molecular mass ($\equiv$ molecular weight) (M_r) of 49 000. An equivalent protein with a

lower M_r (27 000) has been isolated from mouse epidermis (Steinert *et al.*, 1981). A similar bovine protein has been detected that has an even lower M_r (14 400) (Ugel *et al.*, 1971), and adult guinea pig may have analogous proteins of higher M_r (250 000 and 200 000) (Scott & Harding, 1980).

To date, detection of the histidine-rich proteins has only been by the pulse-chase methods or by immunological procedures. Since the histidine-rich proteins are unusual in that they lack methionine and are not broken down by CNBr, the present technique can be used to supplement the previously used procedures. The histidine-rich proteins lie on the diagonal resulting from electrophoresis in the second dimension, whereas the keratins and other proteins that contain methionine are broken down and lie below the diagonal. In addition, the method allows the simultaneous mapping of the peptides of the keratin filament proteins that are co-extracted with the HRB proteins. This method also complements the enzymic one-dimensional mapping techniques that have been used to compare many proteins (Cleveland *et al.*, 1977; Lee *et al.*, 1977; Fuchs &

Abbreviations used: SDS, sodium dodecyl sulphate; HRB protein, histidine-rich basic protein.

Green, 1979). Finally, we expect that the method will also be useful in protein-sequence studies. For example, its use would allow the rapid determination of methionine-containing peptides in a proteolytic digest of a protein without requiring the isolation of each of the different peptides.

Materials and methods

Isolation of epidermal proteins

Newborn Sprague-Dawley rats were killed by cervical dislocation. The dorsal skins were excised and defatted by rubbing with a Kimwipes tissue; the dermis and epidermis were separated by a modification of the method of Harris *et al.* (1980), by treating the skin with 5 mM-EDTA in phosphate-buffered saline (0.075 M-sodium phosphate/0.075 M-NaCl, pH 7.3) at 50°C for 3 min and then plunging the tissue into ice-cold phosphate-buffered saline. The dermis was readily teased from the epidermis after this treatment. The epidermis was stored frozen at -20°C overnight, after which it was extracted with 3 ml of 6 M-urea/0.05 M-Tris (pH 7.6)/2 mM-dithiothreitol/100 mM- β -mercaptoethanol. The supernatants were stored at -20°C.

The epidermis of newborn mouse was obtained as described for the rat. The epidermal layer of newborn rabbit was separated from the dermis by the NH_4Cl technique described by Sibrack *et al.* (1974), because EDTA did not give a good separation. A 2 cm $\times$ 2 cm section of 69-year-old human adult epidermis was obtained from the abdomen of a female cadaver during autopsy. This work was authorized by the Human Subjects Committee of the University of Washington, and informed consent was obtained from the next of kin. The epidermis was separated from the dermis by the procedure described for the rat. Each piece of epidermis was extracted with 6 M-urea/0.05 M-Tris (pH 7.6)/2 mM-dithiothreitol/100 mM- β -mercaptoethanol.

Determination of suitable CNBr (see below) reaction conditions

The proteins, in 4 μ l portions, from newborn-rat epidermal extracts were separated by electrophoresis on discontinuous SDS/polyacrylamide slab gels (Laemmli, 1970) until the Bromophenol Blue front had passed through the stacking gel and 0.5–1.0 cm into the resolving gel. The gel was stained with Coomassie Brilliant Blue R [0.05% (w/v) in propan-2-ol/acetic acid/water (5:2:13, by vol.)] and then destained with 10% (v/v) acetic acid and stored in this solution until required. The lanes containing protein were cut out and the leading edge of the gel (the low- M_r end) was cut into a point for identification later.

Note: CNBr is a hazardous material that should be used only in a well-ventilated fume hood. Before disposal, the CNBr (in excess NaOH) should be de-activated by an equimolar concentration of hypochlorite for 24 h. CNBr is oxidized by the hypochlorite. If the oxidation is incomplete, the addition of ferrous ions will turn the mixture a Prussian Blue colour.

Immediately before CNBr treatment, the gels were equilibrated with 70% (v/v) formic acid (2 $\times$ 4.5 ml, 10 min each). Each gel was then allowed to react separately in a 7 ml scintillation vial with 4.5 ml of 70% formic acid containing CNBr at various concentrations under N_2 , in the dark and at room temperature. After 45 min the CNBr-containing solutions were decanted and the gels washed with several changes of 10% acetic acid (10 min per wash). Efficient stirring of the reactions was accomplished on a rocker platform or by placing the vials with their lids tightly closed in a 2 litre beaker of water and by stirring vigorously. A time course was obtained by the above technique with a 5 mg/ml solution of CNBr in 70% formic acid and by withdrawing samples at the requisite times. Control runs were performed in 70% formic acid without CNBr.

After the acetic acid washes, the CNBr-treated gel pieces and their controls were re-equilibrated with 4 $\times$ 20 min washes of four-times-concentrated upper Tris buffer (0.5 M, pH 6.8, 0.04% SDS), then finally with undiluted buffer containing 2% β -mercaptoethanol. Each gel piece was then placed into the well of an SDS/polyacrylamide slab gel, with its point uppermost so that the slower-moving high- M_r proteins had less distance to migrate before entering the stacking gel than the lower- M_r proteins. Electrophoresis was then performed as usual and terminated when the Bromophenol Blue marker reached the end of the running gel.

Two-dimensional analysis of epidermal proteins

Approx. 100 μ g portions of the epidermal extracts were separated by electrophoresis on SDS/8.75%-polyacrylamide slab gels (Laemmli, 1970). Electrophoresis was continued until the Bromophenol Blue reached the bottom of the resolving gel. The gels were stained overnight with Coomassie Brilliant Blue R [0.05% (w/v) in propan-2-ol/acetic acid/water (5:2:13, by vol.)] and destained in 10% acetic acid. The lanes containing the proteins from each animal were excised, equilibrated with 70% formic acid (2 $\times$ 10 min), and then incubated with 5% CNBr in 70% formic acid for 1 h under N_2 . On completion of the reaction the gels were washed with several changes of 10% acetic acid to remove CNBr. The gel can be re-stained at this time, because the above procedure partly destains the proteins.

Electrophoresis in the second dimension was

performed after the gel had been equilibrated in the upper Tris buffer described above. The pH of the buffer was checked to make sure that equilibration had been achieved. The gel was placed horizontally on the top of a discontinuous SDS/polyacrylamide slab gel whose stacking gel was prepared without loading wells in the centre. Wells were constructed at either end to provide lanes for standards. The resolving gel was usually a gradient gel of 10–15% polyacrylamide. The proteins were stained with Coomassie Brilliant Blue R and the gels were de-stained with 10% acetic acid.

Results

Reaction conditions

The incubation (1h) of newborn rat epidermal proteins in SDS/polyacrylamide gels with increasing concentrations of CNBr results in the complete cleavage of the keratins by CNBr concentration in excess of 1% (w/v). The proteins are stable in the absence of the CNBr. Not all the keratins are cleaved equally readily by the CNBr. The smaller keratin (M_r 57000) was less easily broken down than was the larger (M_r 68000). This is clearly demonstrated in Fig. 1, which shows the time course of cleavage by CNBr (10mg/ml). The larger keratin was completely degraded within 25–30min and the smaller at about 1h. Consequently, to ensure complete cleavage of the proteins in subsequent reactions, the concentration of the reagent was increased to 50mg/ml (i.e., 5%), with a reaction time of 1.5h. Subjective assessment of the amount of stained material in the first- and second-dimension gels (Fig. 2) suggests that minimal loss of material occurred by diffusion out of the gel under these reaction conditions. However, the results cannot be assumed to be quantitative, particularly for low-molecular-weight peptides, which diffused rapidly from the gel at higher reaction temperatures (50°C) (J. D. Lonsdale-Eccles, unpublished work). Longer reaction times were also avoided to minimize the potential loss of proteins and peptides by diffusion, a problem encountered by Lam & Kasper (1979) with 40h incubation periods. Cleavage of aspartic acid–proline bonds might also be encountered with extended periods of incubation under the acidic conditions used in the CNBr reactions. In all the systems tested, the present reaction conditions were found to be adequate for the complete breakdown of the keratin filaments, but for an unknown system it is probably wise to test a variety of conditions in order to find the optimal one.

On several occasions during the electrophoresis in the second dimension, some of the proteins (less than 5% of the total protein) did not move into the second gel, but appeared to be permanently bound to the first-dimension gel. Lacks & Springhorn (1980)

Fig. 1. Time course of the cleavage of epidermal proteins by 1% CNBr in 70% formic acid

Samples were treated with CNBr as described in the text for 0, 0.2, 0.5 and 1.5 h and analysed by electrophoresis on SDS/8.75%-polyacrylamide gels. The control sample (C) was incubated for 1.5 h in 70% formic acid without CNBr. The keratins (arrows) are completely degraded between 0.5h and 1.5h; HRB protein is stable.

made similar observations in experiments on the renaturation of proteins from SDS/polyacrylamide gels. They suggested that this could be the result of folding of the protein around the gel matrix or perhaps the result of covalent linkage by transamidation to the polyacrylamide gel. In the present study the effect seems to be more pronounced with certain higher- M_r proteins and with prolonged storage in the first-dimension gel (up to several weeks). However, no retention of peptides was observed in the first-dimension gel when the CNBr cleavage and re-electrophoresis was performed within 1–2 days of the first-dimension electrophoresis.

CNBr-stable proteins

Fig. 2(a) shows the diagonal CNBr cleavage of a 6M-urea extract of newborn-rat epidermis and it

Fig. 2. Two-dimensional electrophoresis patterns for epidermal proteins after CNBr treatment (a), newborn rat; (b), newborn rabbit; (c), newborn mouse; and (d), adult human. CNBr treatment was performed as described in the Materials and methods section. Arrows show some apparently identical peptides derived from different keratin molecules; asterisks show some CNBr-stable proteins on the diagonals. The keratin peptide patterns are divided into three groups: A, M_r 8000–15 000; B, M_r 25 000–35 000; C, M_r 18 000–22 000. The various standard proteins which were employed were: 1, β -galactosidase, M_r = 135 000; 2, bovine serum albumin, M_r = 68 000; 3, pyruvate kinase, M_r = 57 000; 4, ovalbumin, M_r = 43 000; 5, deoxyribonuclease I, M_r = 31 000; 6, cytochrome c, M_r = 12 300.

clearly demonstrates the potential of this technique for identifying methionine-deficient proteins. We used newborn-rat epidermis as a model because it histidine-rich proteins have been the most extensively studied. These proteins (HRB protein, HRB protein precursor, and HRB protein degradation products), are all aligned on the diagonal and have no degradation products directly beneath them, whereas the methionine-containing keratins are all degraded and lie below the line. HRB protein (M_r 49 000) is the most clearly identified CNBr-stable protein. The high- M_r polymeric precursor of HRB protein reported by a number of laboratories (Ball *et al.*, 1978; Ugel & Idler, 1972; Lonsdale-Eccles *et al.*, 1980a; Dale *et al.*, 1980) can be seen in the upper left of the picture. A number of proteins with a lower M_r than that of HRB protein are also present and

are probably the degradation products of HRB protein reported in the literature (Dale, 1977; Dale & Ling, 1979a). These are seen on the diagonal to the right and below HRB protein. The CNBr-stable proteins identified by asterisks in Fig. 2(a), and that are presumed to be HRB protein and HRB-protein precursors, have been shown to cross-react with antibody to HRB protein (A. M. Lynley, unpublished work).

In order to test the present method in less-well-documented systems, we have also analysed mouse, rabbit and human epidermal extracts. Fig. 2(b) demonstrates the presence of several CNBr-stable proteins in the epidermis of newborn rabbit. The major stable protein, which has not been previously identified, has an M_r of about 61 000. Two proteins, about 2000 and 4000 daltons smaller, are

present in lesser amounts. A stable protein of about M_r 25 000 also appears on the diagonal.

The diagonal produced by newborn-mouse proteins showed two discrete CNBr-stable spots of 27 000 and 60 000 M_r (Fig. 2c). The 27 000- M_r protein has already been isolated and shown to be a functional equivalent of HRB protein (Steinert *et al.*, 1981). The diagonal for mouse proteins also shows a number of additional high- M_r proteins that are stable in the presence of CNBr. These probably are precursors of the functional mouse HRB protein.

Unlike the rat and rabbit, the human epidermis produced only faint diffuse spots on the diagonal line (Fig. 2d). The major CNBr-stable protein had an M_r of about 40 000, whereas a minor protein had an M_r of approx. 30 000. Since there is no evidence of the cleavage of these proteins by CNBr (i.e. no peptides lie immediately below these faint spots), the amount of HRB protein in human epidermis must be much lower than that found in the newborn-rat epidermis. Furthermore, the human epidermis showed no very-high- M_r species and so presumably has very little precursor HRB protein.

Keratin peptide patterns

The majority of the proteins synthesized by epidermal cells are keratins. They are extracted from these cells by the procedure employed in the present study. The keratins of the different species are all degraded by the CNBr treatment and the resultant peptides lie below the diagonals (Fig. 2). A broad similarity can be seen in the protein-cleavage patterns between the species. Each pattern can be divided into three distinct groups of peptides; A, M_r 8000–15 000; B, M_r 25 000–35 000; and C, M_r 18 000–22 000. Most of the peptides fall into groups A and B. Group C has only one peptide in the human, but three peptides in the rat, mouse and rabbit. These three peptides in group C are all derived from the ~57 000- M_r keratin. However, they do not lie in a straight line directly below this keratin, but are at a slightly oblique angle to the vertical, suggesting that the parent keratin is actually a heterogeneous population of proteins. The overall peptide patterns of the rat, mouse and rabbit are generally more similar to each other than to the pattern of the human keratin peptides. The rabbit and human have two lower- M_r proteins, whose peptide patterns have little similarity to their respective larger keratins. In the rabbit and rat, which have the most similar peptide maps, the highest- M_r keratin is a doublet in the first dimension; however, within each species, each member of this pair gives the same peptide map. There are several additional peptides that are common to more than one keratin in a species (arrows in Fig. 2). Despite such similarities, the inter- and intra-species degradation patterns are not identical. The peptide pattern from

each species is both reproducible and characteristic of that species.

Discussion

The cleavage of proteins at methionine residues by CNBr is a routine technique used in peptide-mapping studies and for the generation of fragments useful for determining the sequence of the amino acids of proteins. Cleavage can be readily accomplished in SDS/polyacrylamide gels using high concentrations of CNBr. Two-dimensional patterns can then be obtained by re-electrophoresis at an angle of 90° to the original direction. We have used the technique to map different epidermal keratins and to identify a group of epidermal proteins called 'histidine-rich proteins', which have been shown to lack methionine (Ugel & Idler, 1972; Dale, 1977; Ball *et al.*, 1978). One of these proteins, HRB protein, appears to act as the interfibrillar matrix of keratin in the cornified layer (Dale *et al.*, 1978). Biochemical studies of rat skin have shown that HRB protein is derived from a phosphorylated precursor protein of higher M_r that is a component of the amorphous keratohyalin granules (Ball *et al.*, 1978; Dale & Ling, 1979a,b; Dale *et al.*, 1980; Lonsdale-Eccles *et al.*, 1980a,b). The precursor also lacks methionine. HRB protein and the precursor proteins can be identified by the present technique as spots that lie on the diagonal line (Fig. 2). Newborn-rat, rabbit, mouse, and adult-human epidermis all possess CNBr-stable proteins, although human epidermis appears to have less of these proteins. This quantitative difference may be a consequence of age, since the human sample was obtained from an elderly adult, whereas the others were all from newborn animals. The adult rat is known to have less HRB protein than the newborn (Dale & Thompson, 1978). The CNBr-stable proteins that we have identified all have different relative molecular masses (M_r); however, both the mouse (Holbrook *et al.*, 1981) and the human proteins (B. A. Dale, unpublished work) as identified by asterisks in Figs. 2(c) and 2(d) are immunologically related to rat HRB protein. Furthermore, the mouse 27 000- M_r protein has been isolated and shown to aggregate keratin filaments, as does rat HRB protein (Steinert *et al.*, 1981). Thus these proteins form a family of biochemically related, but species-specific, proteins.

In addition to identifying the CNBr-stable species, we are also able to compare the fragmentation patterns of the different keratin proteins. Each species possesses its own characteristic degradation pattern of similar and dissimilar peptides. Such intraspecies similarities have been demonstrated also in human keratins by means of enzymic degradation (Fuchs & Green, 1978). CNBr digests of isolated bovine hoof and snout keratins also showed

similarities, although Lee *et al.* (1977), who described them, emphasized the differences rather than the similarities. In the experiments by Fuchs & Green (1978), fragmentation, by enzymic cleavage, of human, mouse and rabbit keratins produced similar patterns, which fell into two large groups (A = 8000–13 000 M_r ; B = 20 000–40 000 M_r). Our observations with CNBr are in broad agreement, except that the subdivision we see is into three groups (A, 8000–15 000 M_r ; B, 25 000–35 000 M_r ; C, 18 000–22 000 M_r) rather than into two. A group of three peptides, which appear to be common among rat, mouse and rabbit and are derived from the ~57 000- M_r keratin, falls in between the two major ranges described by Fuchs & Green (1978). Cleavage into three major groups (A, B and C) is common to the keratins of all the different species investigated. The similar cleavage patterns produced by the CNBr and the enzymic procedures suggest that the sites at which cleavages occur may be relatively close to one another. Possibly the sites of cleavage in the proteins of each species are all located in similar domains in the keratins. These domains may be related to the two helical and three random portions of the secondary structure described by Steinert (1978). The helical portions of the keratins, and of other intermediate filaments, have been shown to be genetically well conserved, whereas the non-helical portions show considerable variations (Steinert *et al.*, 1980b). The close similarities of the peptide patterns suggest that the genetically conserved helical portions may have remained intact and that the cleavages may have occurred mainly in the non-helical segments.

Summing the relative molecular masses of the fragments from any one single keratin gives a value greater than that of the parent molecule. Thus the cleavage by CNBr is incomplete under the present conditions; that is, some methionine residues are more susceptible to cleavage than others. It is also possible that each protein band, which is presumed to be a single keratin, may actually consist of a heterogeneous population of keratins of the same size. Evidence for this latter interpretation has been shown by a number of studies in which the individual keratins have been further resolved into different polypeptides (Fuchs & Green, 1978; Steinert *et al.*, 1980a,b). However, some of this apparent heterogeneity may be due to post-translational modifications, such as phosphorylation (Sun & Green, 1976, 1978; Gilmartin *et al.*, 1980).

It is clear from the present and from previous studies (Lee *et al.*, 1977; Fuchs & Green, 1978; Steinert, 1978) that the keratins are a closely related, yet individually distinct, group of proteins. The present study, which combines analytical SDS/polyacrylamide-gel electrophoresis with CNBr cleavage to create two-dimensional diagonal peptide

maps, provides not only a comparison of the keratin polypeptides within and among species, but also demonstrates that the epidermis of each of the species investigated possesses an unusual group of proteins that lack methionine. The use of similar CNBr procedures for mapping the peptides of collagen (Barsh & Byers, 1981) and the polypeptides of the nuclear envelope (Lam & Kasper, 1979, 1980) became known to us during the preparation of the present paper. The results obtained confirm that this technique should have wide application in the analysis of proteins that contain methionine. Furthermore, the procedure affords a simple method for identifying proteins that lack this amino acid and may be useful for the identification of other methionine-deficient proteins; e.g., certain high-mobility-group-like non-histone chromosomal proteins (Walker *et al.*, 1980).

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References

- Ball, R. D., Walker, G. K. & Bernstein, I. A. (1978) *J. Biol. Chem.* **253**, 5861–5868
- Balmain, A., Loehren, D., Fischer, J. & Alonso, A. (1977) *Dev. Biol.* **60**, 442–452
- Barsh, G. S. & Byers, P. H. (1981) *Proc. Natl. Acad. Sci. U.S.A.* in the press
- Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102–1106
- Dale, B. A. (1977) *Biochim. Biophys. Acta* **491**, 193–204
- Dale, B. A. & Ling, S. Y. (1979a) *Biochemistry* **18**, 3539–3546
- Dale, B. A. & Ling, S. Y. (1979b) *J. Invest. Dermatol.* **72**, 257–261
- Dale, B. A. & Thompson, W. B. (1978) *J. Dent. Res.* **57**, 222
- Dale, B. A., Holbrook, K. A. & Steinert, P. M. (1978) *Nature (London)* **226**, 729–731
- Dale, B. A., Lonsdale-Eccles, J. D. & Holbrook, K. A. (1980) in *Biochemistry of Normal and Abnormal Epidermal Differentiation* (Bernstein, I. A., & Seiji, M., eds.), Tokyo University Press, pp. 311–325
- Dale, B. A., Vadlamudi, B., DeLapp, L. W. & Bernstein, I. A. (1981) *Biochim. Biophys. Acta* **668**, 98–106
- Fuchs, E. & Green, H. (1978) *Cell* **15**, 887–897
- Fuchs, E. & Green, H. (1979) *Cell* **17**, 573–582
- Gilmartin, M. E., Culbertson, V. B. & Freedberg, I. M. (1980) *J. Invest. Dermatol.* **75**, 211–216
- Harris, R. R., Mackenzie, I. C. & Williams, R. A. D. (1980) *J. Invest. Dermatol.* **74**, 402–406
- Holbrook, K. A., Dale, B. A. & Brown, K. S. (1981) *J. Invest. Dermatol.* **76**, 331–332

- Lacks, S. A. & Springhorn, S. S. (1980) *J. Biol. Chem.* **255**, 7467–7473
- Laemmli, U. K. (1970) *Nature (London)*, **227**, 680–685
- Lam, K. S. & Kasper, C. B. (1979) *J. Biol. Chem.* **254**, 11713–11720
- Lam, K. S. & Kasper, C. B. (1980) *Anal. Biochem.* **108**, 220–226
- Lee, L. D., Kubilus, J. K. & Baden, H. P. (1977) *Biochem. J.* **187**–196
- Lonsdale-Eccles, J. D., Dale, B. A., & Haugen, J. A. (1980a) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 1866
- Lonsdale-Eccles, J. D., Haugen, J. A. & Dale, B. A. (1980b) *J. Biol. Chem.* **255**, 2235–2238
- Scott, I. R. & Harding, C. R. (1980) *J. Invest. Dermatol.* **74**, 456
- Sibrack, L. A., Gray, R. H. & Bernstein, I. A. (1974) *J. Invest. Dermatol.* **62**, 394–405
- Steinert, P. M. (1978) *J. Mol. Biol.* **123**, 49–70
- Steinert, P. M., Idler, W. W. & Wantz, M. L. (1980a) *Biochem. J.* **187**, 913–916
- Steinert, P. M., Idler, W. W. & Goldman, R. D. (1980b) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4534–4538
- Steinert, P. M., Cantieri, J. S. Teller, D. C., Lonsdale-Eccles, J. D. & Dale, B. A. (1981) *Proc. Natl. Acad. Sci. U.S.A.* in the press
- Sun, T.-T. & Green, H. (1976) *Cell* **9**, 511–521
- Sun, T.-T. & Green, H. (1978) *J. Biol. Chem.* **253**, 2053–2060
- Tezuka, T. & Freedberg, I. M. (1974) *J. Invest. Dermatol.* **63**, 402–406
- Ugel, A. R. & Idler, W. (1972) *J. Cell Biol.* **52**, 453–464
- Ugel, A. R., Chrambach, A. R. & Rodbard, D. (1971) *Anal. Biochem.* **43**, 410–426
- Walker, J. M., Brown, E., Goodwin, G. H., Stearn, C. & Johns, E. W. (1980) *FEBS Lett.* **113**, 253–257