The EMBO Journal Vol.1 No.6 pp.761–769, 1982

A subfamily of relatively large and basic cytokeratin polypeptides as defined by peptide mapping is represented by one or several polypeptides in epithelial cells

Dorothea L.Schiller, Werner W.Franke*, and Benjamin Geiger[1]

Division of Membrane Biology and Biochemistry, Institute of Cell and Tumor Biology, German Cancer Research Center, D-6900 Heidelberg, FRG, and [1]Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel

Communicated by Werner W.Franke
Received on 25 June 1982

Epithelial cells contain a class of intermediate-sized filaments formed by proteins related to epidermal α-keratins ('cytokeratins'). Different epithelia can express different combinations of cytokeratin polypeptides widely varying in apparent mol. wt. (40 000 – 68 000) and isoelectric pH (5.0 – 8.5). We have separated, by two-dimensional gel electrophoresis, cytokeratin polypeptides from various tissues and cultured cells of man, cow, and rodents and examined their relatedness by tryptic peptide mapping. By this method, a subfamily of closely related cytokeratin polypeptides has been identified which comprises the relatively large (≥ mol. wt. 52 500 in human cells) and basic (pH ≥ 6.0) polypeptides but not the smaller and acidic cytokeratins. In all species examined, the smallest polypeptide of this subfamily is cytokeratin A, which is widespread in many simple epithelia and is the first cytokeratin expressed during embryogenesis. This cytokeratin polypeptide subfamily is represented by at least one member in all epithelial and carcinoma cells examined, indicating that polypeptides of this subfamily serve an important role as tonofilament constitutents. Diverse stratified epithelia and tumours derived therefrom contain two or more polypeptides of this subfamily, and the patterns of expression in different cell types suggest that some polypeptides of this subfamily are specific for certain routes of epithelial differentiation.

Key words: keratins/cytoskeleton/intermediate filaments/ protein families/epithelial differentiation

Introduction

Unlike the other classes of intermediate-sized filaments (for reviews, see Lazarides, 1980; Anderton, 1981; Franke *et al.*, 1982b), the cytokeratin filaments are not identical in their polypeptide composition in different cells. Filaments of this category can be formed, in different epithelial cell types, by different polypeptides of a complex family of proteins (Drochmans *et al.*, 1978; Doran *et al.*, 1980; Fuchs and Green, 1980, 1981; Winter *et al.*, 1980; Franke *et al.*, 1981a – d; Milstone and McGuire, 1981; Wu and Rheinwald, 1981). In particular, patterns of cytokeratin polypeptides from various cell types of simple epithelia of internal organs and of early embryos have been shown to differ markedly from those of epidermal α-keratins (Jackson *et al.*, 1980, 1981; Winter *et al.*, 1980; Franke *et al.*, 1981a – d, 1982a). However, remarkably different cytokeratin polypeptide patterns can also be found when different cell types are compared within the same organ. For example, in human skin different cytokeratins are synthesized not only in different epidermal strata (Fuchs and Green, 1980; Bowden and Cunliffe, 1981) but also in different lateral domains of the epidermis and its appendages (Drochmans *et al.*, 1978; Kubilus *et al.*, 1979; Steinert *et al.*, 1980a; Moll *et al.*, 1982). An increasing number of cytokeratin polypeptides has been described which have been prepared and identified as components of filaments insoluble in high and low salt buffers and by their high tendency to reassemble into such filaments from denatured solubilized monomers (Lee and Baden, 1976; Steinert *et al.*, 1976; Sun and Green, 1978; Milstone, 1981; Renner *et al.*, 1981). Figures 1A – C present schematic summaries of the cytokeratin polypeptides so far described in bovine (22 components Figure 1A), murine (22 components, Figure 1B) and human (19 components, Figure 1C) cells arranged according to their appearance on two-dimensional gel electrophoresis. The diverse cytokeratin polypeptides differ greatly in apparent mol. wts. (from 40 000 to 68 000) and isoelectric pH (denatured molecules show isoelectric point (IEP) values from ~5.0 to 8.5).

Although polypeptide patterns of cytokeratin filaments from the same tissue are similar in different species, the putatively corresponding polypeptides from different species are not identical in size and charge but often show interspecies differences (Figure 1A – C; Franke *et al.*, 1981a 1981c; Denk *et al.*, 1982). The relatedness of the different cytokeratin polypeptides has so far only been examined by their cross-reactivity using antisera raised against epidermal α-keratins (Lee *et al.*, 1975; Franke *et al.*, 1978a, 1978b, 1981a, 1981c, 1981d; Fuchs and Green, 1978, 1981; Sun *et al.*, 1979; Wu and Rheinwald, 1981) and by one-dimensional electrophoresis of products of partial proteolytic digestion or cyanogen bromide cleavage from polypeptide bands separated by SDS-polyacrylamide gel electrophoresis (Lee *et al.*, 1975, 1979; Fuchs and Green, 1978, 1979, 1981; Steinert *et al.*, 1980a, 1980b; Milstone and McGuire, 1981). Such studies have revealed that certain antigenic determinants are present in some cytokeratin polypeptides, but not in others, and that certain peptide fragments from some of the polypeptides have similar electrophoretic mobilities. On the other hand, differences of one-dimensional peptide patterns between certain cytokeratin polypeptides have also been noted. Clearly, such limited information, mostly based on epidermal keratins, does not allow conclusions on the relatedness of the various cytokeratin polypeptides found in the diverse tissues of different species. Therefore, we have separated the individual cytokeratin and polypeptides from various tissues by two-dimensional gel electrophoresis and compared their maps of tryptically obtained peptides using the sensitive radioiodination method. In this report, we define a subfamily of closely related cytokeratin polypeptides which comprises the relatively large and basic components and is represented by at least one member in all tissues and cells studied.

Results

Separation of cytokeratin polypeptides

Because of the broad range of IEPs of denatured cytokeratin polypeptides it is advisable to use both isoelectric

*To whom reprint requests should be sent.

© IRL Press Limited, Oxford, England. 0261-4189/82/0106-0761$2.00/0.

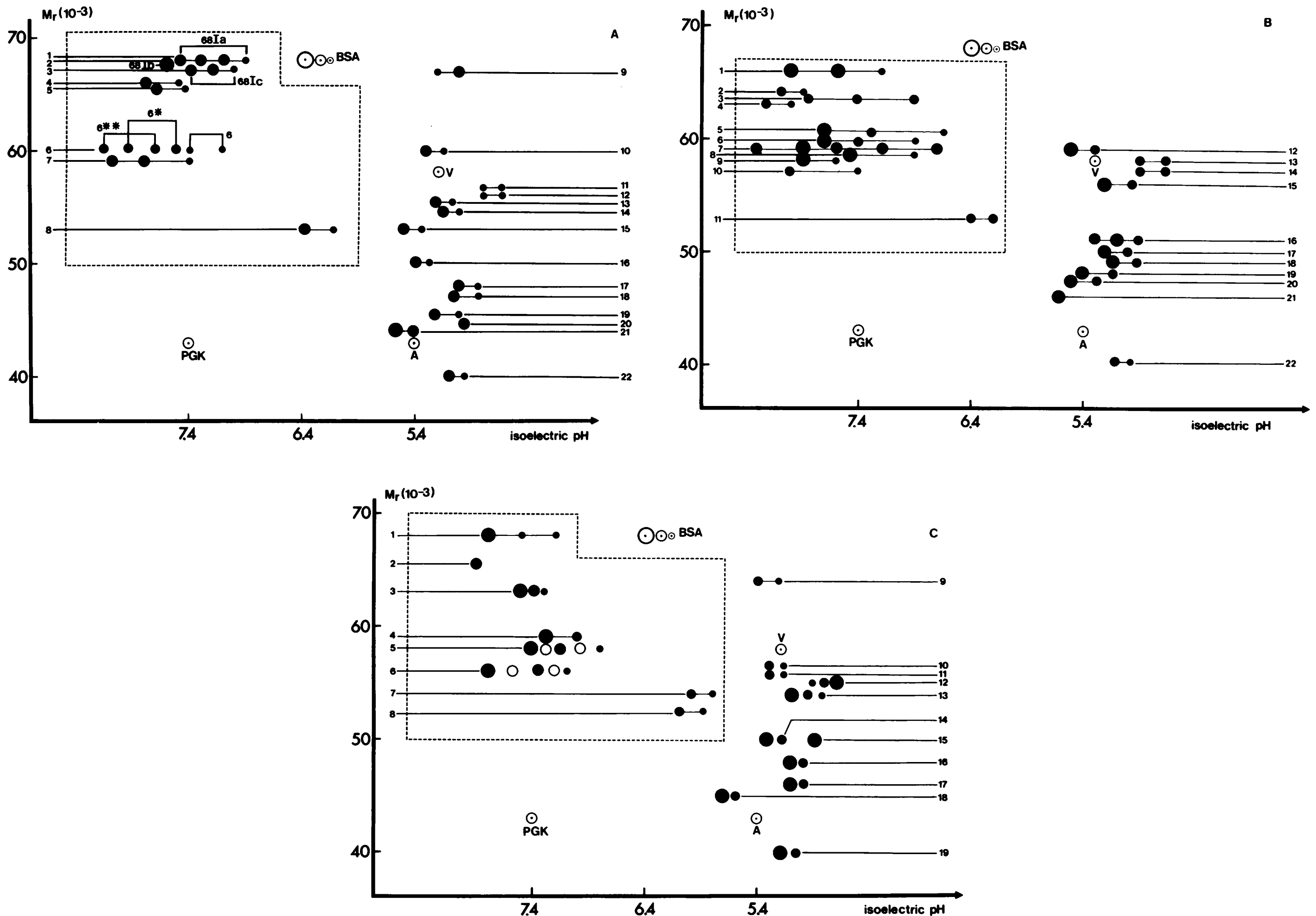

Fig. 1. Schematic and simplified presentation of cytokeratin polypeptides from various cell types as separated by two-dimensional gel electrophoresis (for original data see Franke *et al.*, 1981a–d, 1982a, 1982b; Moll *et al.*, 1982). Major isoelectric variants are shown (diameters of circles indicate relative staining intensities with Coomassie blue). Distinct polypeptides are numbered in the specific system (Roman numerals in **A** relate to the designation of bovine epidermal keratins used previously; Franke *et al.*, 1978a, 1980a, 1981a, 1981c). Reference proteins used in co-electrophoresis are: bovine serum albumin (BSA, mol. wt. 68 000), vimentin (V, mol. wt. 57 000), actin (A, mol. wt. 42 000), and 3-phosphoglyceric kinase (PGK, mol. wt. 42 000). **(A)** Bovine cytokeratins, combined from muzzle and hoof epidermis, hair follicle sheath, hepatocytes, small intestine, lactating udder, cornea, esophagus and tongue mucosa, transitional epithelium of bladder, MDBK cells and BMGE cells. 6* and 6** denote two polypeptides of mol. wt. 59 000 of esophagus and BMGE cells which, on two-dimensional gel electrophoresis, partly overlap with each other and with epidermal component 6 (III); these polypeptides, however, are not identical as judged from peptide mapping. **(B)** Murine (rat and mouse) cytokeratins, combined from body epidermis of neonatal animals, lip epidermis, esophagus and tongue mucosa, cornea, hepatocytes, small intestine, mouse embryos, HEL cells and MH_1C_1 cells. **(C)** Human cytokeratins, combined from epidermis of various locations, hair follicle sheaths, sebaceous, sweat glands and mammary glands, esophagus and tongue mucosa, cornea, exocervix, bladder epithelium, tracheal epithelium, hepatocytes, small intestine and colon, and various cell culture lines (HeLa, MCF-7, A-431). Open circles denote minor components that are not recognized in all tissues. For comparison see also Sun and Green (1978) and Fuchs and Green (1978, 1979, 1980, 1981).

focusing and non-equilibrium pH gradient electrophoresis to separate cytokeratins (Franke *et al.*, 1981c). As an example of the complexity of cytokeratin composition in some tissues, notably in stratified squamous epithelia, we describe the cytokeratin polypeptides of bovine muzzle epidermis in some detail in Figure 2 (for immunological identification by immuno-replica and blotting techniques see Franke *et al.*, 1980a, 1981a, 1981c). Tonofilaments from this tissue contain a number of major and minor polypeptides differing in electrical charge as demonstrated by non-equilibrium pH gradient electrophoresis (Figure 2a). The basic character of the major polypeptides nos. 1–7 as listed in Figure 1A (previously introduced as components Ia–c, III, and IV by Franke *et al.*, 1981c) can also be shown by isoelectric focusing, using an ampholine combination allowing high resolution in the range of pH 7–10 (Figure 2b; isoelectric focusing in the pH range 4.5–6.5 is shown in Figure 2c). All the components described in Figure 2a are also recovered in filaments reconstituted from monomers denatured in solutions of 8 or 9 M urea (Figure 2d; Renner *et al.*, 1981). Translation *in vitro*, using mRNA from bovine muzzle epidermis, shows that these different polypeptides can also be identified as translation products and therefore represent genuine polypeptides (Figure 2e, and f), including the minor polypeptides nos. 4 and 5.

Characteristically, cytokeratin polypeptides appear in series of isoelectric variants. These series of spots have been shown for several cytokeratins of human epidermis, murine hepatocytes, and HeLa cells to represent different degrees of phosphorylation, the most basic spot usually being the non-phosphorylated polypeptide (Sun and Green, 1978; Gilmartin *et al.*, 1980; Franke *et al.*, 1981c; Steinert *et al.*, 1982). Figure 2g shows the pattern of labelled cytokeratin polypeptides ob-

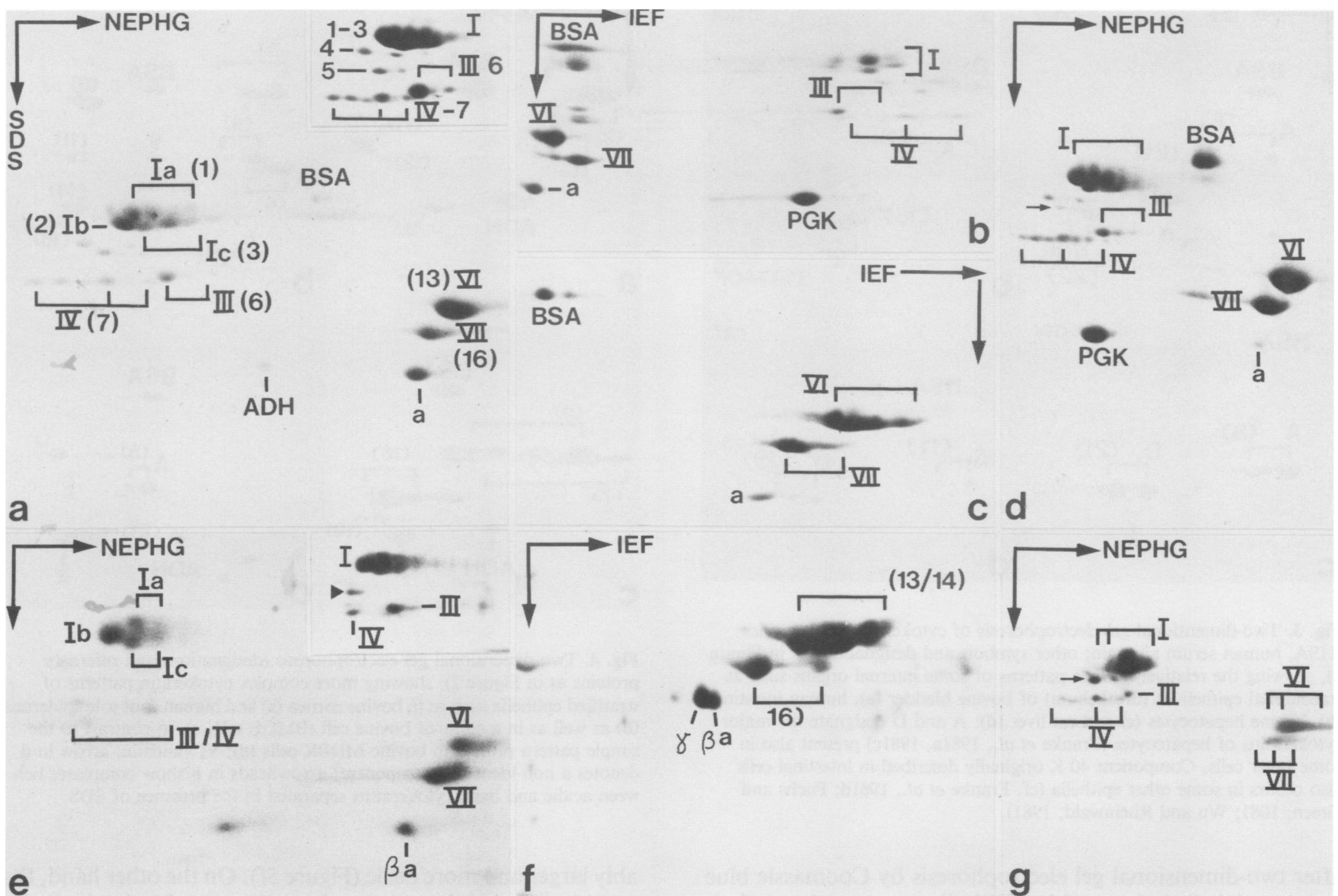

Fig. 2. Two dimensional gel electrophoresis of epidermal cytokeratin polypeptides from bovine muzzle, using isoelectric focusing (IEF) with ampholines ranging from pH 7 to 10 (**b**) or from 4 to 7 (**c,f**) or non-equilibrium pH gradient electrophoresis (**a,d,e,g**; NEPHG) in the first dimension; SDS, direction of electrophoresis in the presence of SDS. (**a – c**) Coomassie blue staining of cytokeratins from native tonofilaments (minor components are seen in the insert in **a**). Note in **b** that components nos. 1 – 7 (I – IV of Franke *et al.*, 1978a, 1981c) are more basic than PGK. (**d**) Coomassie blue staining of polypeptides from filaments reconstituted *in vitro*. Note presence of all cytokeratins found in native filaments, including component no. 5 of mol. wt. 64 000 (arrow). (**e**) and (**f**) Fluorographs showing *in vitro* translation products labelled with [^{35}S]methionine. Note appearance of all cytokeratins found in the tissue, including component no. 5 (K 64, arrowhead in insert of **e**). Some phosphorylated variants are also seen (for phosphorylation in the reticulocyte system see also O'Connor *et al.*, 1981). (**g**) Fluorograph showing cytokeratin polypeptides labelled with [^{32}P]phosphate in slices of bovine muzzle tissue. Note that all cytokeratins are labelled (the position of the specific non-phosphorylated polypeptides are denoted by the left vertical bars of the brackets). Roman numerals used are according to the literature (Franke *et al.*, 1978a, 1981a, and c). Arabic numerals as in Figure 1A. In addition to reference proteins shown in Figure 1, alcohol dehydrogenase (ADH, mol. wt. 43 500) and internal residual actin (β, γ-a) is shown.

tained after [^{32}P]phosphate incorporation in tissue slices of bovine muzzle epidermis: all detectable cytokeratin polypeptides occur with at least one phosphorylated modification, including the relatively large and basic polypeptides nos. 1 – 7. The latter finding is at variance with a recent report by Steinert *et al.* (1982) who did not observe any detectable phosphorylation of the largest bovine muzzle keratin polypeptides.

Complex patterns showing a similarly high degree of correspondence of cytokeratin polypeptides present in cytoskeletons, translational products *in vitro*, and phosphorylated variants have been found in various other tissues and several cultured cells (not shown; for *in vitro* translation of RNA from human, murine, or guinea pig epidermis see Fuchs and Green, 1979; Schweizer and Goerttler, 1980; Gibbs and Freedberg, 1982). In the present study, we have isolated polypeptide spots of cytokeratins from diverse epithelial tissues, including examples with relatively simple patterns such as transitional epithelium ('urothelium') of bovine bladder (Figure 3a), human small intestine (Figure 3b), and hepatocytes of bovine (Figure 3c) and rat (Figure 3d) liver as well as other tissues showing more complex patterns including non-cornified epithelia (corneal epithelium, Figure 4a; for esophagus see Franke *et al.*, 1981c; Milstone, 1981; Milstone and McGuire, 1981) and highly cornified epidermis (Figure 4b). Various degrees of cytokeratin complexity can also be found in different cultured cell lines: some cell lines express considerable amounts of basic cytokeratins such as the BMGE cell line derived from bovine mammary gland epithelia (Figure 4c) whereas others contain only simple patterns of cytokeratins characteristic of simple epithelia of internal organs, and lack components with IEP values higher than pH 6.5 (e.g., bovine MDBK cells: Figure 4d; HeLa cells: Franke *et al.*, 1981c; Bravo *et al.*, 1982; rat MH_1C_1 and Novikoff hepatoma cells: Franke *et al.*, 1981b; Schmidt *et al.*, 1982).

Tryptic peptide maps

We have excised individual polypeptide spots identified

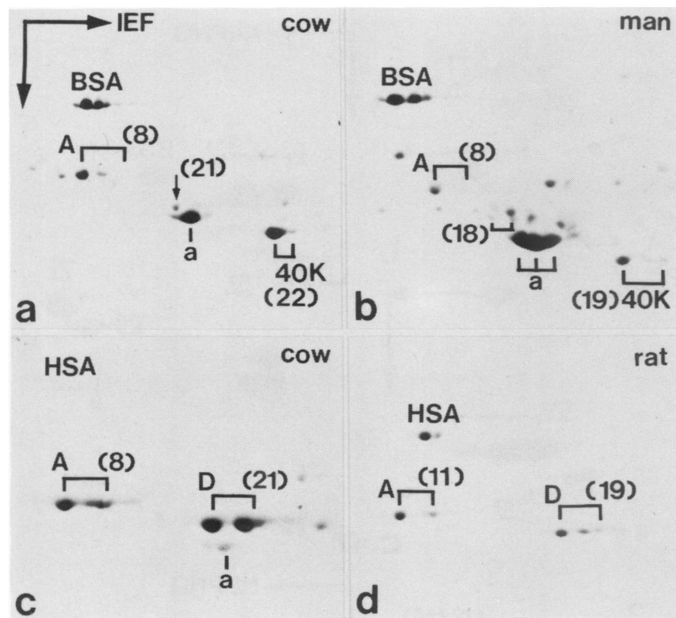

Fig. 3. Two-dimensional gel electrophoresis of cytokeratin polypeptides (HSA, human serum albumin; other symbols and designations as in Figure 2), showing the relatively simple patterns of some internal organs such as transitional epithelium (urothelium) of bovine bladder (**a**), human intestine (**b**), bovine hepatocytes (**c**) and rat liver (**d**). A and D designate the major cytokeratins of hepatocytes (Franke *et al.*, 1981a, 1981c) present also in some other cells. Component 40 K originally described in intestinal cells also occurs in some other epithelia (cf. Franke *et al.*, 1981d; Fuchs and Green, 1081; Wu and Rheinwald, 1981).

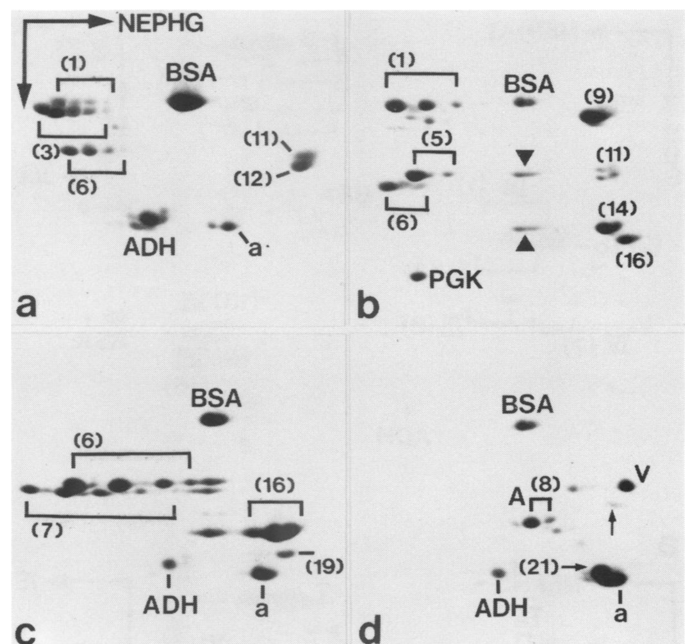

Fig. 4. Two-dimensional gel electrophoresis (designations and reference proteins as in Figure 2), showing more complex cytokeratin patterns of stratified epithelia such as in bovine cornea (**a**) and human foot sole epidermis (**b**) as well as in a cultured bovine cell (BMGE + H, **c**), in contrast to the simple pattern present in bovine MDBK cells (**d**); V, vimentin; arrow in **d** denotes a non-identified component; arrowheads in **b** show complexes between acidic and basic cytokeratins separated in the presence of SDS.

after two-dimensional gel electrophoresis by Coomassie blue staining, labelled them with [^{125}I]iodine and analysed their tryptic peptides according to Elder *et al.* (1977). In Figure 5 the peptide maps of several polypeptide spots from different bovine tissues and cells are compared. Practically identical maps are obtained for the various isoelectric variant spots of a distinct polypeptide, in agreement with the interpretation that these are modifications of the same polypeptide (Figure 5a and b presents an example of cytokeratin A, no. 8, and its phosphorylated satellite spot A′; Franke *et al.*, 1981a – d). Identical peptide maps are also obtained when polypeptides with identical co-ordinates from different cell types are compared. For example, Figure 5a – e and g demonstrate the identity of bovine cytokeratin no. 8 in hepatocytes of liver, urothelium of bladder, cultured mammary gland epithelial cells (BMGE – H), and kidney epithelial cells (MDBK). Very similar tryptic peptide maps have also been obtained in several other cases of cytokeratin polypeptides which have the same electrophoretic co-ordinates in different tissues. Therefore, we conclude that most of the cytokeratin polypeptides from different cell types which co-migrate on two-dimensional gel electrophoresis are very similar, probably identical. Should more subtle cell type-specific microheterogeneity occur in some of the polypeptide spots denoted by Arabic numerals in Figure 1A – C (for examples see actins; Vandekerckhove and Weber, 1979), it could only be of a minor degree not detectable by the method used.

Comparison of tryptic peptide maps of different cytokeratin polypeptides has revealed similarities as well as dissimilarities. For example, the tryptic map of bovine component no. 8 shows several peptides in common with the map of cytokeratin no. 7 from bovine esophagus, which is considerably larger and more basic (Figure 5f). On the other hand, the peptide maps of these two polypeptides can be clearly distinguished by some peptide fragments specific for one or the other. Such peptide map analysis indicates a close relationship of bovine cytokeratin no. 8 to the whole group of the larger and more basic bovine polypeptides nos. 1 – 7 (see below). In contrast, when cytokeratins nos. 8 and 1 – 7 are compared with the more acidic cytokeratins from the same species completely different maps are noted (compare Figures 5a – g with Figures 5h and i).

We have also examined possible species differences of the seemingly corresponding cytokeratin polypeptides from the same tissue, as these can differ in their electrophoretic co-ordinates (for demonstration of such differences in the case of hepatic cytokeratins A and D in mouse, rat and man see Franke *et al.*, 1981a; Denk *et al.*, 1982). Figure 6 shows, again for cytokeratin A, that the tryptic peptides from rat (Figure 6a), mouse (Figure 6b), cow (Figure 6c), and human (not shown) liver are very similar, and this also holds when this cytokeratin is isolated from other organs (Figure 6d and e). This similarity indicates that certain cytokeratin polypeptides characteristic of specific routes of epithelial differentiation have been highly conserved during mammalian evolution.

Comparison of the peptide map of cytokeratin A (no. 8 in bovine and human cells, no. 11 in the rodent system; cf. Figure 1A – C) with other cytokeratin polypeptides of the specific species shows various degrees of similarities amongst polypeptides that are larger and relatively basic (nos. 1 – 7 in human and bovine material). Figure 6a – f presents, as an example, a comparison of cytokeratin A with one of the major keratin polypeptides of human foot sole epidermis, namely the basic component no. 6 (cf. Fuchs and Green, 1978, 1980).

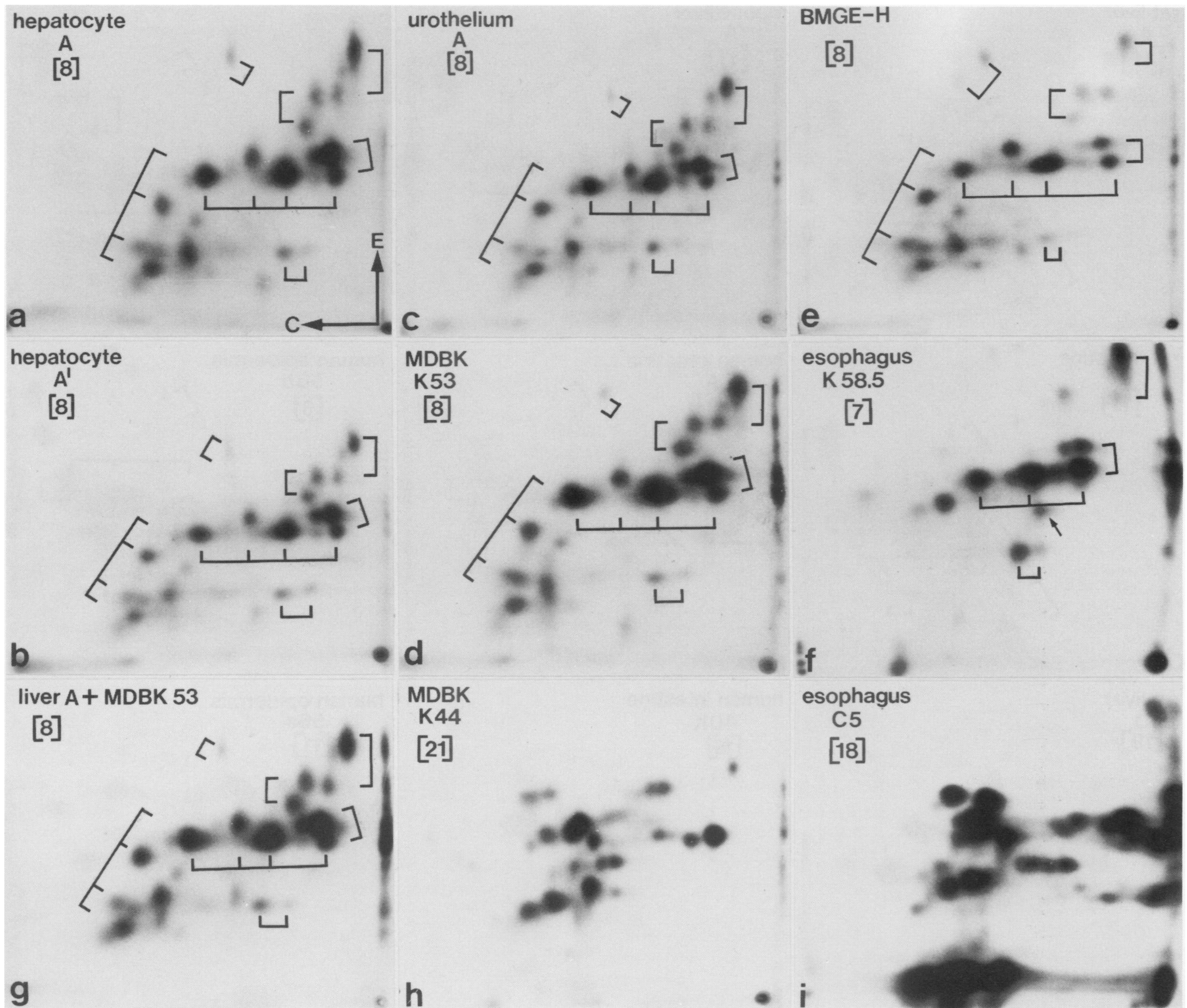

Fig. 5. Tryptic peptide map comparison of bovine cytokeratins (E, electrophoresis; C, chromatography) of radio-iodinated polypeptide spots excised after two-dimensional gel electrophoresis (Arabic numerals according to Figure 1A; only major isoelectric variants are shown). **a – e**: Identity of component no. 8 isolated from various cell types. **(a)** Component A (no. 8) from hepatocytes, major isoelectric variant; **(b)** Component A', i.e., a minor, phosphorylated variant of A; **(c)** Component A from urothelium of bladder; **(d)** Cytokeratin K 53 (A) from MDBK cells; **(e)** cytokeratin K 53 (A) from BMGE – H cells. For comparison **(f)**, cytokeratin K 58.5 (no. 7) from esophagus is shown. Note relationship, but also differences, to cytokeratin no. 8 (a specific additional spot is denoted by an arrow; corresponding spots are denoted by bars and brackets). **(g)** Co-migration of nearly equal amounts of labelled peptides from component A (no. 8) of liver and MDBK cells. **h** and **i**: Different maps of tryptic peptides obtained from smaller and acidic cytokeratins are shown for comparison. **(h)** Cytokeratin K 44 (no. 21) from MDBK cells; **(i)** Cytokeratin C 5 (no. 18) from esophagus (see also Franke *et al.*, 1981c).

In contrast, none of the smaller acidic cytokeratins of the diverse species (Figure 6g – i presents some examples) has yielded peptide maps related to cytokeratin A. Remarkable relatedness has also been observed between the peptide maps of the various cytokeratin polypeptides that are relatively large and basic and cytokeratin A. Figure 7 presents a small selection of examples of several bovine cytokeratins, including the largest cytokeratins (no. 1, mol. wt. 68 000) obtained from muzzle epidermis and cornea. This similarity among all cytokeratin polypeptides of the nos. 1 – 8 group, and their dissimilarity when compared with the more acidic cytokeratin polypeptides defines a subfamily of bovine cytokeratin polypeptides. Corresponding data have been obtained for cytokeratins nos. 1 – 8 from human tissues and cells and for cytokeratins nos. 1 – 11 from mouse or rat (not shown).

Discussion

From our peptide map analysis we conclude that certain cytokeratin polypeptides, i.e., the relatively large and basic ones (demarcated by hatched lines in Figure 1A – C), represent a subfamily of distinct polypeptides that are closely related to each other in primary structure, irrespective of the still considerable differences in mol. wt. and IEP. Since most of these polypeptides are also identified as translational products we further conclude that they are coded by different genes of a multigene family that is differentially expressed in

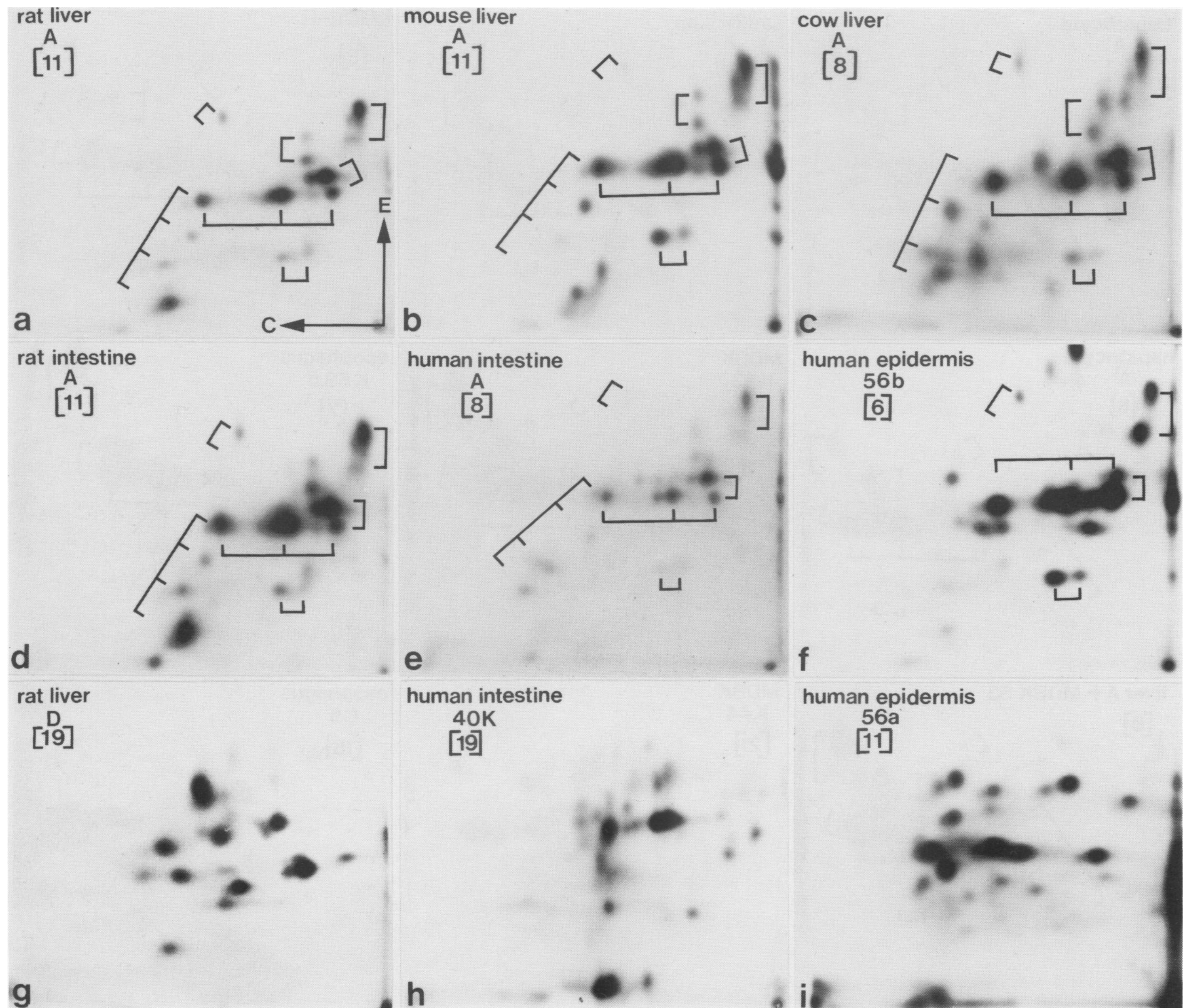

Fig. 6. Comparison of radio-iodinated tryptic fragments of components A from different species (**a**–**d**: designation as in Figure 5). (**a**) Rat liver; (**b**) mouse liver; (**c**) cow liver; (**d**) rat intestine; (**e**) human intestine. For comparison we show peptide maps of a related component from foot sole epidermis (no. 6; **f**) as well as the unrelated patterns of the acidic and smaller cytokeratins: (**g**) component D (no. 19) from rat liver; (**h**) component K 40 (no. 19) from human intestine; (**i**) component K 56a (no. 11) from human epidermis.

different epithelia. Whether the similar, in some cases identical, peptide fragments are derived from homologous regions located in a common α-helical core portion (Steinert, 1978; Steinert *et al.*, 1980b; Anderton, 1981) remains to be seen.

Our results are in line with several observations of other authors. (1) Milstone and McGuire (1981) have emphasized that one-dimensional peptide maps obtained by limited proteolysis are different for the two major bovine esophageal cytokeratin bands designated E_1 (which includes cytokeratins nos. 6* and 7; cf. Figure 1A) and E_2 (mostly cytokeratin no. 18 of Figure 1A; see also Franke *et al.*, 1981). Likewise, several differences and similarities in their one-dimensional peptide maps of bovine hoof keratin bands can be explained by our findings of peptide map similarities among the members of the subfamily of large and basic polypeptides. (2) Although Fuchs and Green (1978) have emphasized similarities of band patterns in their one-dimensional analysis of protease-digested keratin polypeptides from human, murine, and rabbit epidermis, they also have noted that keratins of similar size are more closely related than keratins of different size. The one-dimensional peptide map data of Fuchs and Green (1981) also indicate a close relationship of the small and acidic cytokeratin of mol. wt. 40 000 (no. 19 of Figure 1C) to some other keratins of the small and acidic class (nos. 14 and 16 of Figure 1C) but apparently not to the larger (and more basic) polypeptides (see also Wu and Rheinwald, 1981). In general, however, it has to be said that one-dimensional analysis of peptides according to Cleveland *et al.* (1977) is not sensitive enough to reveal the kind of subfamily relationship described in the present study. (3) Recently, Fuchs *et al.* (1981) have reported that cloned cDNA sequences to mRNA coding for large (and basic) human epidermal keratins (nos. 5

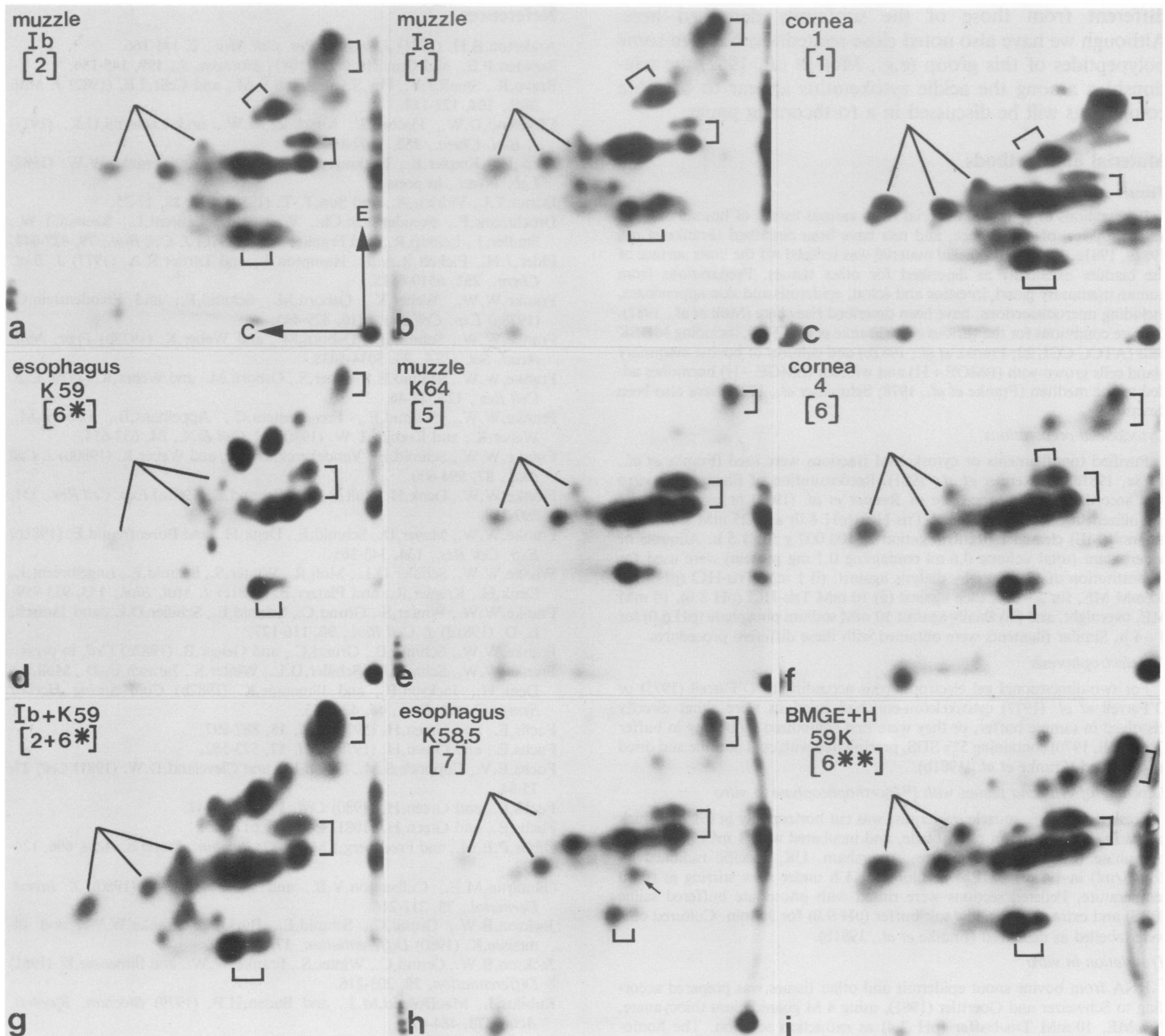

Fig. 7. Peptide maps (designation as Figure 5) of relatively large and basic cytokeratins from bovine stratified epithelia and BMGE cells; **(a)** component Ib (no. 2) from muzzle epidermis; **(b)** component Ia (no. 1) of muzzle epidermis; **(c)** component 1 (no. 1) from cornea; **(d)** component K 59 (no. 6*), from esophagus; **(e)** component K 64 (no. 5) from muzzle epidermis; **(f)** component 4 (no. 6) from cornea; **(g)** co-migration of tryptic peptides from epidermal prekeratin Ib (no. 2) and component K 59 (no. 6*) from esophagus (note similarities of these cytokeratins as well as some distinct differences); **(h)** component K 58.5 (no. 7) from esophagus; **(i)** component K 59 (no. 6**) from BMGE + H cells.

and 6 of Figure 1C) do not hybridize with sequences coding for small (and acidic) keratins (nos. 14 and 16 of Figure 1C), which also indicates the existence of different subfamilies of cytokeratin genes. (4) Several monoclonal antibodies that react with cytokeratin polypeptides of relatively high mol. wt. and IEP do not react with most of the cytokeratins of the small and more acidic group (Lane, 1982; Gigi *et al.*, in preparation).

The close relationship of the relatively large and basic cytokeratins demarcated in Figures 1A–C, and the difference between these cytokeratin polypeptides and the smaller and acidic ones, is also interesting in relation to our findings that all epithelial and carcinoma cell types examined contain at least one representative of this polypeptide subfamily. In certain simple epithelia such as hepatocytes and intestinal cells as well as in early embryonal cells, only one polypeptide of this category is found, namely cytokeratin A (no. 8 in cow and man, no. 11 in murine systems). Characteristically, stratified squamous epithelia contain more than one member of this polypeptide subfamily, and epidermis shows a special wealth and complexity of expression of a number of keratins of this type. The widespread, probably ubiquitous occurrence of this cytokeratin subfamily suggests that polypeptides of this subfamily are necessary as constitutive elements for the construction of tonofilaments. Using the same procedure of peptide mapping we have also examined the cytokeratin polypeptides

different from those of the subfamily described here. Although we have also noted close relatedness between some polypeptides of this group (e.g., Moll *et al.*, 1982) the relationships among the acidic cytokeratins appear to be more complex as will be discussed in a forthcoming paper.

Material and methods

Tissues and cells

Preparations of epithelial material from various tissues of human biopsies and autopsies, of cows, mice, and rats have been described (Franke *et al.*, 1981a, 1981c, 1981d). Urothelial material was scraped off the inner surface of the bladder essentially as described for other tissues. Preparations from human mammary gland, intestine and colon, epidermis and skin appendages, including microdissections, have been described elsewhere (Moll *et al.*, 1982). Culture conditions for the various cells (Franke *et al.*, 1979), including MDBK cells (ATCC CCL 22; Franke *et al.*, 1982b) and cultures of bovine mammary gland cells grown with (BMGE + H) and without (BMGE – H) hormones added to the medium (Franke *et al.*, 1978; Schmid *et al.*, 1982) have also been described.

Cytoskeletal preparations

Purified tonofilaments or cytoskeletal fractions were used (Franke *et al.*, 1978a, 1981a – d; Renner *et al.*, 1981). Reconstitution of filaments *in vitro* was according to the procedure of Renner *et al.* (1981) or using material solubilized in 8 M urea, 10 mM Tris-HCl (pH 8.0) and 25 mM mercaptoethanol (ME) cleared by centrifugation at 100 000 *g* for 1.5 h. Aliquots of supernatant (total volume 0.6 ml containing 0.1 mg protein) were used for reconstitution of filaments by dialysis against: (i) 1 mM Tris-HCl (pH 8.0), 10 mM ME, for 2 – 3 h, then against (ii) 10 mM Tris-HCl (pH 8.0), 10 mM ME, overnight, and (iii) finally against 50 mM sodium phosphate (pH 6.0) for 3 – 4 h. Similar filaments were obtained with these different procedures.

Gel electrophoresis

For two-dimensional gel electrophoresis according to O'Farrell (1975) or O'Farrell *et al.* (1977) cytoskeleton-enriched fractions were either directly dissolved in sample buffer, or they were first solubilized by boiling in buffer (Laemmli, 1970) containing 5% SDS, precipitated with cold acetone and dried as described (Franke *et al.*, 1981b).

Labelling of cells and tissues with [^{32}P]orthophosphate in vitro

Fresh tissue, e.g., muzzle epidermis, was cut horizontally in 0.1 mm thick pieces of 1 mm^2 using a razor-blade, and incubated with 1 mCi [^{32}P]orthophosphate (Radiochemical Centre, Amersham, UK, specific radioactivity 2 mCi/ml) in 1.5 ml BMEM medium for 1 h under slow stirring at room temperature. Pelleted sections were rinsed with phosphate buffered saline (PBS) and extracted with low salt buffer (pH 9.0) for 30 min. Cultured cells were labelled as described (Franke *et al.*, 1981b).

Translation in vitro

RNA from bovine snout epidermis and other tissues was prepared according to Schweizer and Goerttler (1981), using 4 M guanidinium thiocyanate, 7% ME, 10 mM Tris-buffer (pH 7.4) as extraction solution. The homogenized material was made 2% in N-lauroyl sarcosine, heated to 65°C for 10 min, immediately cooled to room temperature, filtered through a fine mesh gauze, loaded on a CsCl cushion (6.5 M CsCl, 10 mM Na-EDTA, pH 7.5), and centrifuged at 44 000 r.p.m. for 2 h using Beckman VTi50 rotor. The nucleic acid pellet was solubilized in 0.3 M sodium acetate (pH 5.5), extracted with phenol, and precipitated with cold ethanol. RNA from cultured cells was extracted and analysed as described (Franke *et al.*, 1980b). Total or purified poly(A)$^+$-RNA was translated using a rabbit reticulocyte system and L-[^{35}S]methionine as label (500 Ci/mMol; New England Nuclear, Dreieich, FRG).

Peptide mapping

The procedure of Elder *et al.* (1977) was used. Approximately 0.2 mCi of ^{125}I (Radiochemical Centre) was added to each gel slice. For digestion (24 h, 37°C) TPCK-treated trypsin (50 µg, i.e. 217 units; Millipore, Molsheim, France) was applied per gel slice.

Acknowledgements

We thank Drs. R.Moll, R.Sommer, and J.Jorcano (Heidelberg) as well as Drs. Denk and R.Krepler (University of Vienna, Austria) for their help and valuable discussions. The work has been supported by the Deutsche Forschungsgemeinschaft (Fr. 308/15).

References

Anderton,B.H. (1981) *J. Muscle Res. Cell Mot.*, **2**, 141-166.

Bowden,P.E., and Cunliffe,W.J. (1981) *Biochem. J.*, **199**, 145-154.

Bravo,R., Small,J.V., Fey,S.J., Larsen,P.M., and Celis,J.E. (1982) *J. Mol. Biol.*, **154**, 121-143.

Cleveland,D.W., Fischer,S., Kirschner,M.W., and Laemmli,U.K. (1977) *J. Biol. Chem.*, **252**, 1102-1106.

Denk,H., Krepler,R., Lackinger,E., Artlieb,U., and Franke,W.W. (1982) *Lab. Invest.*, in press.

Doran,T.I., Vidrich,A., and Sun,T.-T. (1980) *Cell*, **22**, 17-25.

Drochmans,P., Freudenstein,Ch., Wanson,J., Laurent,L., Keenan,T.W., Stadler,J., Leloup,R., and Franke,W.W. (1978) *J. Cell Biol.*, **79**, 427-443.

Elder,J.H., Pickett,R.A.,II, Hampton,J., and Lerner,R.A. (1977) *J. Biol. Chem.*, **252**, 6510-6515.

Franke,W.W., Weber,K., Osborn,M., Schmid,E., and Freudenstein,C. (1978a) *Exp. Cell Res.*, **116**, 429-445.

Franke,W.W., Schmid,E., Osborn,M., and Weber,K. (1978b) *Proc. Natl. Acad. Sci. USA*, **75**, 5034-5038.

Franke,W.W., Schmid,E., Winter,S., Osborn,M., and Weber,K. (1979) *Exp. Cell Res.*, **123**, 25-46.

Franke,W.W., Schmid,E., Freudenstein,C., Appelhans,B., Osborn,M., Weber,K., and Keenan,T.W. (1980a) *J. Cell Biol.*, **84**, 633-654.

Franke,W.W., Schmid,E., Vandekerckhove,J., and Weber,K. (1980b) *J. Cell Biol.*, **87**, 594-600.

Franke,W.W., Denk,H., Kalt,R., and Schmid,E. (1981a) *Exp. Cell Res.*, **131**, 299-318.

Franke,W.W., Mayer,D., Schmid,E., Denk,H., and Borenfreund,E. (1981b) *Exp. Cell Res.*, **134**, 345-365.

Franke,W.W., Schiller,D.L., Moll,R., Winter,S., Schmid,E., Engelbrecht,I., Denk,H., Krepler,R., and Platzer,B. (1981c) *J. Mol. Biol.*, **153**, 933-959.

Franke,W.W., Winter,S., Grund,C., Schmid,E., Schiller,D.L., and Jarasch, E.-D. (1981d) *J. Cell Biol.*, **90**, 116-127.

Franke,W.W., Schmid,E., Grund,C., and Geiger,B. (1982a) *Cell*, in press.

Franke,W.W., Schmid,E., Schiller,D.L., Winter,S., Jarasch,E.-D., Moll,R., Denk,H., Jackson,B., and Illmensee,K. (1982b) *Cold Spring Harbor Symp. Quant. Biol.*, **46**, 431-453.

Fuchs,E., and Green,H. (1978) *Cell*, **15**, 887-897.

Fuchs,E., and Green,H. (1979) *Cell*, **17**, 573-582.

Fuchs,E.V., Coppock,S.M., Green,H., and Cleveland,D.W. (1981) *Cell*, **27**, 75-84.

Fuchs,E., and Green,H. (1980) *Cell*, **19**, 1033-1042.

Fuchs,E., and Green,H. (1981) *Cell*, **25**, 617-625.

Gibbs,P.E.M., and Freedberg,I.M. (1982) *Biochim. Biophys. Acta*, **696**, 124-133.

Gilmartin,M.E., Culbertson,V.B., and Freedberg,I.M. (1980) *J. Invest. Dermatol.*, **75**, 211-216.

Jackson,B.W., Grund,C., Schmid,E., Bürki,K., Franke,W.W., and Illmensee,K. (1980) *Differentiation*, **17**, 161-179.

Jackson,B.W., Grund,C., Winter,S., Franke,W.W., and Illmensee,K. (1981) *Differentiation*, **20**, 203-216.

Kubilus,J., MacDonald,M.J., and Baden,H.P. (1979) *Biochim. Biophys. Acta*, **578**, 484-492.

Laemmli,U. (1970) *Nature*, **227**, 680-685.

Lane,E.B. (1982) *J. Cell Biol.*, **92**, 665-673.

Lazarides,E. (1980) *Nature*, **283**, 249-256.

Lee,L.D., and Baden,H.P. (1976) *Nature*, **264**, 377-378.

Lee,L.D., Kubilus,J., and Baden,H.P. (1979) *Biochem. J.*, **177**, 187-196.

Lee,L.D., Fleming,B.C., Waitkus,R.F., and Baden,H.P. (1975) *Biochim. Biophys. Acta*, **412**, 82-90.

Milstone,L. (1981) *J. Cell Biol.*, **88**, 317-322.

Milstone,L.M., and McGuire,J. (1981) *J. Cell Biol.*, **88**, 312-316.

Moll,R., Franke,W.W., Volc-Platzer,B., and Krepler,R. (1982) *J. Cell Biol.*, in press.

O'Connor,C.M., Asai,D.J., Flytzanis,C.N., and Lazarides,E. (1981) *Mol. Cell Biol.*, **1**, 303-309.

O'Farrell,P.H. (1975) *J. Biol. Chem.*, **250**, 4007-4021.

O'Farrell,P.Z., Goodman,H.M., and O'Farrell,P.H. (1977) *Cell*, **12**, 1133-1142.

Renner,W., Franke,W.W., Schmid,E., Geisler,N., Weber,K., and Mandelkow,E. (1981) *J. Mol. Biol.*, **149**, 285-306.

Schmid,E., Schiller,D.L., Grund,C., Stadler,J., and Franke,W.W. (1982) *J. Cell Biol.*, in press.

Schmidt,W.N., Pardue,R.L., Tutt,M.C., Briggs,R.C., Brinkley,B.R., and Hnilica,L.S. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 3138-3142.

Schweizer,J., and Goerttler,K. (1980) *Eur. J. Biochem.*, **112**, 243-249.
Steinert,P.M. (1978) *J. Mol. Biol.*, **123**, 49-70.
Steinert,P.M., Idler,W.W., and Zimmermann,S.B. (1976) *J. Mol. Biol.*, **108**, 547-567.
Steinert,P.M., Idler,W.W. and Wantz,M.L. (1980a) *Biochem. J.*, **187**, 913-916.
Steinert,P.M., Idler,W.W., and Goldman,R.D. (1980b) *Proc. Natl. Acad. Sci. USA*, **77**, 4534-4538.
Steinert,P.M., Wantz,M.L., and Idler,W.W. (1982) *Biochemistry (Wash.)*, **21**, 177-183.
Sun,T.-T., and Green,H. (1978) *J. Biol. Chem.*, **253**, 2053-2060.
Sun,T.-T., Shih,C., and Green,H. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 2813-2817.
Vandekerckhove,J., and Weber,K. (1979) *Differentiation*, **14**, 123-133.
Winter,S., Jarasch,E.-D., Schmid,E., Franke,W.W., and Denk,H. (1980) *Eur. J. Cell Biol.*, **22**, 371 Abstract.
Wu,Y.-J., and Rheinwald,J.G. (1981) *Cell*, **25**, 627-635.