We have characterized the keratin proteins of various bovine epithelial tissues by one- and two-dimensional gel electrophoresis, coupled with the immunoblot technique using AE1, AE2, AE3, AE5, CA20, BE14, and 6.11 monoclonal antikeratin antibodies. The results indicate that all known bovine keratins can be divided into two subfamilies. The "acidic" (Type I) subfamily consists of 41-, 43-, 45-, 46-, 50-, 54-, 56-, and 56.5-kDa keratins, all of which have a pI of <5.6, and most of them are recognized by our AE1 antibody, whereas the "neutral-to-basic" (Type II) subfamily consists of 55-, 57-, 58-, 62-65-, 66-, and 67-kDa keratins, all of which have a pI of >6.0 and are recognized by our AE3 antibody. Tissue distribution data and cell culture studies show that, within the two subfamilies, keratins with similar "size ranks" form a "pair" as defined by frequent co-expression. Furthermore, within most "keratin pairs," the basic keratin is larger than the acidic one by 8-10 kDa. These results provide further support for the concepts of "keratin subfamilies" and "keratin pairs" and are consistent with the possibility that the acidic and basic members of at least some keratin pairs may interact specifically during in vivo tonofilament assembly and/or function. Immunoblotting data derived from the use of several monoclonal antibodies show that although the size, charge, and pattern of expression of most bovine keratins are similar to those of the human counterparts, there are important exceptions to this rule.

Keratins are a family of water-insoluble proteins forming 10-nm tonofilaments that are present not only in the epidermis and its appendages, but also in a wide variety of other epithelial tissues (1-5). Two-dimensional gel analysis has established that in every mammalian species that has been studied there exist approximately 20 different keratin molecules (6-9). Detailed analyses of human keratins have firmly established that all of these keratins can be divided into two subfamilies (the so-called "acidic or Type I" and "basic or Type II" subfamilies) according to their immunoreactivities with our AE1 and AE3 monoclonal antibodies (10-12), their relative charges (6, 13, 14), their peptide mapping patterns (6, 7, 15), and their sequence homologies with the previously described Type I or Type II wool keratins (16-20). Tissue surveys and cell culture studies have shown that specific acidic and basic human keratins frequently co-express, forming "keratin pairs." Within each "pair," the basic member is usually larger than the acidic one by approximately 5-10 kDa (12). Moreover, the expression of many of these human keratin pairs can be correlated with different types of epithelial differentiation (10, 12, 21). To see if this concept of keratin "subfamilies" and "pairs" may also apply to animal species other than the human, we have characterized in detail the keratins of various bovine epithelia.

Using a panel of monoclonal antikeratin antibodies including several that have not previously been described, we show in this paper that: (i) the concept of keratin pairs is applicable to bovine keratins; (ii) the 56.5/67-, 56/66-, 54/62-65-, and 43/58'-kDa keratin pairs may be regarded, respectively, as markers of "snout," "corneal," "skin," and "esophageal" types of stratified epithelial differentiation; (iii) the snout epithelium is unusually complex in that it expresses a mixture of several "types" of differentiation markers; (iv) the 58-kDa keratin of normal esophagus can be distinguished immunologically from the 58-kDa keratin of most other keratinocytes since only the latter is BE14 positive; and (v) the acidic esophageal keratin of the cow, as defined immunologically by our new CA20 antibody, is unusually small (43 kDa as compared with 51 kDa in human). Taked together, our results indicate that the corresponding keratin species of cow and human are, by and large, highly conserved in their charge, size, and immunoreactivities; however, there are some notable exceptions to this rule.

Materials and Methods

Monoclonal Antibodies—Mouse monoclonal antibodies AE1, AE2, and AE5 were raised against SDS-denatured human epidermal (cal) keratins (22), CA20 against human esophageal keratins, BE14 against human hair keratins, and AE5 against rabbit corneal epithelial keratins. Mouse monoclonal anti-intermediate filament (aIF) antibody and 6.11 anti-keratin antibody were kindly provided by R. Pruss of the National Institutes of Health (23, 24) and R. Cardiff of the University of California, Davis (25), respectively.

Cell Culture— Cultures of human epidermal cells and bovine snout epithelial cells were grown in the presence of lethally irradiated 3T3 feeder cells in Dubecco's modified Eagle's medium containing 20% fetal calf serum, epidermal growth factor (10 ng/ml), and hydrocortisone (0.4 μg/ml (26, 27)). Cultures of bovine esophageal epithelia were grown as above and were kindly provided by Dr. Joseph McGuire of Yale University (28). MDBK cells (a bovine kidney-derived simple epithelial cell line (29)) were obtained from the American Type Culture Collection (ATCC CCL 22) and were grown in Dubecco's

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§ Recipient of a Monique Weill-Caulier Career Scientist Award.

1 The abbreviations used are: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; aIF, anti-intermediate filament; EGTA, ethyleneglycoldinitril; tetraacetic acid.

2 A. Schmerer, C. Hardy, and T.-T. Sun, unpublished data.
modified Eagle’s medium containing 10% fetal calf serum.

Tissues—All bovine tissues came from freshly slaughtered animals. Bovine "epidermis" was obtained from several regions including 1) the snout (so-called "snout epidermis" or, perhaps more correctly, snout epithelium; see below), 2) an area slightly above the posterior hoof (so-called "hoof epidermis"), 3) skin epidermis from an area caudal to the snout. Epithelia were also isolated from the middle portion of the esophagus and from the cornea.

Tissue Extraction—Cultured cells were rinsed twice with phosphate-buffered saline, scraped from culture dishes with a rubber policeman, and homogenized in a solution containing 25 mM Tris-HCl (pH 7.4), 1% Triton, and 0.6 M KCl to remove water-soluble proteins (cf. Ref. 13). To minimize proteolytic degradation, antipain (5 µg/ml), pepstatin (5 µg/ml), 1 mM EDTA, 1 mM EGTA, and 1 mM phenylmethanesulfonyl fluoride were added to all buffers (22). After centrifugation at 10,000 × g for 20 min at 4°C, the supernatant was discarded, and the water-insoluble fraction was solubilized in 25 mM Tris-HCl (pH 7.4) and 2% SDS, followed by heating for 5 min at 95°C. Water-insoluble protein was cleared of SDS-insoluble material and other debris by centrifugation for 5 min in a Fisher microcentrifuge. Alternatively, the water-insoluble fraction was homogenized in 25 mM Tris-HCl (pH 7.4) and 9.5 M urea followed by centrifugation for 20 min at 10,000 × g to remove water-insoluble debris. 9 M urea or 2% SDS (in 25 mM Tris-HCl, pH 7.4, without a reducing agent) was used for the selective extraction of keratins from the living layers ("prekeratin" equivalence (30)) of fresh (unfrozen) epidermis (22).

Gel Electrophoresis and Immunoblot Analysis—Proteins were separated either by SDS-PAGE (12.5% acrylamide (31)) or by two-dimensional gel electrophoresis in which the first-dimensional separation was nonequilibrium pH-gradient electrophoresis and the second-dimensional separation was SDS-PAGE (32). For immunoblotting, separated proteins were electrophoretically transferred to a sheet of nitrocellulose paper (Millipore) using an E-C blotting apparatus (30 min at 4°C with a full power setting (33)). These blots were then stained with Fast Green to visualize all proteins, followed by staining the same blot with monoclonal antibodies. Also note in c that AE3 recognizes the 55-, 57-, 58-, and 62-67-kDa keratins, whereas in d that AE1 recognizes 41-, 50-, 54-, and the 56.5-kDa keratins, whereas in d that AE1 recognizes 41-, 50-, 54-, and the 56.5-kDa keratins, whereas in d that AE1 recognizes 41-, 50-, 54-, and the 56.5-kDa keratins, whereas in d that AE1 recognizes 41-, 50-, 54-, and the 56.5-kDa keratins.

RESULTS

Analyses of Bovine Epidermal Keratins Using the Subfamily-specific AE1 and AE3 Antibodies—Fig. 1a shows the SDS-gel patterns of various bovine epidermal-related tissues including the epidermis (lane 2), snout (muzzle) epithelium (lanes 3 and 9), "hoof" epidermis (lanes 4 and 8), and cultured snout epithelial cells (lane 10 (35)). These samples were compared with several hoof keratin components isolated by Peter M. Steinert of the National Institutes of Health (lanes 5-7 (36, 37)), as well as the previously characterized human
epidermal keratins (in vivo normal epidermis, lane 1; cultured
epidermal keratinocytes, lane 11 (11, 38, 39)). Almost all of
these major water-insoluble cytoskeletal proteins represent
intermediate filament proteins, as is shown by immunoblot-
ting using a monoclonal antibody known to cross-react with
all classes of intermediate filaments (Fig. 1b (23, 24)). More-
over, all of these keratins can be divided into 2 groups accord-
ing to their immunoreactivities with our AE3 and AE1 mono-
clonal antibodies. Thus, our AE3 antibody reacts with the
55-, 57-, 58-, and 62-67-kDa proteins3 (Fig. 1c), all of which
are relatively basic (Fig. 2, a’-d’), whereas our AE1 antibody
recognizes the 41-, 50-, and 54-kDa keratins and a 56.5-kDa
protein (which is present in the epidermis only in minute
quantities (Fig. 1d)), all of which are relatively acidic (Fig. 2,
a’-d’).

Two-dimensional gel electrophoresis coupled with immu-
noblotting showed that the keratins of skin and “hoof” are
virtually identical (Fig. 2, a and b), both possessing mainly
50-, 54-, 58-, and 62-65-kDa keratins. Hoof epidermis, how-
ever, expresses a small amount of an additional 57-kDa kerat-
in, as well as a major 65-kDa acidic component (arrowhead
in Fig. 2b), which may correspond to the No. 9 human keratin
previously described by Moll et al. (6). The snout epithelium
is unique in that it expresses a 67-kDa component instead of
the 62-65-kDa keratin characteristic of the skin and hoof
epidermis (Fig. 2c (40-42)). When snout cells are placed in
culture, they lose their 54- and 67-kDa proteins and gain a
41- and a 46-kDa protein (Fig. 2, c and d). The three hoof
keratin components purified and kindly provided by P. M.
Steinert (“bands 3, 4, and 5” of Refs. 36 and 37; Fig. 1a, lanes
5-7) correspond to proteins of 57, 54, and 50 kDa in our hoof
keratin preparations (Fig. 1a, lanes 4 and 8).

Analyses of Other Bovine Epithelial Keratins Using the
Subfamily-specific Antibodies—Fig. 3a shows the SDS-gel pat-
tterns of the water-insoluble proteins from bovine corneal
epithelium (lane 2), esophageal epithelium (lane 3), cultured
esophageal epithelial cells (lane 4), and MDBK cells (a bovine
kidney-derived simple epithelial cell line, lane 6). Normal
esophageal epithelium expresses 43- (major), 57- (minor), and
58-kDa (major) keratins (lane 3; this 58-kDa component is
BE14 negative and is, therefore, distinguishable from the
BE14-positive 58-kDa keratin of other keratinocytes, see be-
low). In addition to the above “in vivo” components, cultured
esophageal cells express the 41-, 46-, 50-, and 55-kDa keratins
(lane 4). This pattern is, therefore, quite similar to that of
cultured snout epithelial cells (lane 5), the only significant
difference being a 43-kDa band that is synthesized by esoph-
ageal but not by snout cells. In vivo corneal epithelium ex-
presses 56- (major), 58- (minor), and 66-kDa (major) proteins
(lane 2), while MDBK cells express two proteins of 45 and 55
kDa in addition to a 57-kDa vimentin (lane 6 (9, 43)).

One- and two-dimensional immunoblot analyses indicate
that almost all major proteins shown in Fig. 3a react with the
aIF antibody (Fig. 3b). AE3 monoclonal antibody recognizes
a subset of larger keratins (55-, 57-, 58-, and 66-67-kDa
proteins, Fig. 3c), all of which are relatively basic in charge

3 To facilitate the comparison of keratins of the human and bovine,
we have determined the SDS-gel molecular masses of individual
bovine keratins using masses of known human epithelial keratins as
standards (6, 12).
a 66-kDa band derived from human epithelium "(10-12. 21, 22, 44). In the cow, this antibody stains which are markers for keratinization or "skin-type differ-

Fast Green. AE3 recognizes 55-, 57-, 58-, and 66-67-kDa keratins, and cultured esophageal epithelial cells (lane 5), but fails to react with the major 58-kDa component of in vivo esophageal epithelium (lane 4). This observation supports an earlier suggestion by Schiller et al. (7) that the major 58-kDa keratin of esophagus (designated by them as component "No. 6") is closely related to, but distinguishable by peptide mapping from, the 58-kDa component ("No. 6") of other stratified tissues.

We have recently produced another monoclonal antibody, tentatively designated CA20, that is highly specific for the 51-kDa (No. 13) acidic human keratin. This antibody is monospecific for a 43-kDa bovine esophageal component (Fig. 5d, lanes 4 and 5) which is, therefore, immunologically equivalent to the 51-kDa human keratin.

Fig. 5f shows the immunoblotting data using AE5 antibody which recognizes a 66-kDa band in both snout (lane 2) and corneal epithelia (lane 3). This band is, therefore, homologous to the 64-kDa (No. 3) human keratin that was once thought to be corneal specific (12, 13, 47).

Immunoblotting of bovine keratins with a 6.11 monoclonal antibody recently described by Brabon et al. (25) showed that in both human and cow, this antibody is monospecific for a 45-kDa keratin (Fig. 5e, lane 6).

DISCUSSION

Using monoclonal antibodies as a tool, we have characterized a number of bovine epithelial keratins with respect to their immunoreactivities, electrophoretic properties, and their tissue distribution pattern. The results have allowed us to divide all known bovine epithelial keratins into two subfamilies (Fig. 6 and Table I), to provide additional support for our recently proposed concept of specific "keratin pairs" as defined by co-expression (Table I (12)), and to demonstrate several unique features of bovine keratins.

Nomenclature of Bovine Keratins—The existing nomenclatures of bovine keratins, which have been studied by a number of investigators over the past decade, are extremely confusing. Therefore, in order to facilitate a comparison of our data with those obtained by others we have: 1) listed in Table I bovine keratins that we have studied so far with regard to their apparent masses, immunoreactivities with various monoclonal antibodies, and their bovine keratin catalogue numbers according to Schiller et al. (7); 2) assigned their human keratin counterparts (their masses and catalogue numbers (6, 12) are shown in parentheses in Table I); 3) summarized in Table II some of the previous nomenclatures. The basis for some of these assignments will be discussed below.

Bovine and Human Keratins Are Similar, but There Are Some Notable Exceptions—Our results confirm and extend the earlier finding by Franke et al. (7, 13) that bovine and human keratins are highly conserved, with the corresponding members showing similar size, charge, immunoreactivities, and patterns of expression (Table I). However, there are several important exceptions.

<sup>4</sup> A. Schermer, S. Galvin, and T.-T. Sun, unpublished observations.
The human 51-kDa (Moll's human catalogue number 13) and 59-kDa (Moll No. 4) keratins are characteristic of esophageal, tongue, cervical, and some other internal nonkeratinized tissues (6, 12). The bovine keratin that corresponds to the 51-kDa human keratin, as identified by its CA20 reactivity (Fig. 5d), is unusually small (43 kDa; see Refs. 13, 48, and 49). The size difference between the major basic (58 kDa) and acidic (43 kDa) bovine esophageal keratins is, therefore, approximately 15 kDa, which is significantly larger than the 8–10-kDa difference observed in most other bovine and human keratin "pairs" (Table I).

Another interesting feature of keratin expression in the cow relates to the so-called keratinization markers. Four bovine keratins, two acidic (54 and 56.5 kDa) and two basic (62–65 and 67 kDa), appear to be related to some form of keratinization. In most cases, the 54-kDa bovine component was found to be the predominant acidic keratin, with only traces of the 56.5-kDa acidic keratin (which corresponds to the 56.5-kDa No. 10 human keratin, Fig. 1d). Immunolocalization data suggest that the 54-kDa keratin is present in all layers of the snout epithelium (50). This is in sharp contrast with the suprabasal location of similar "keratinization markers" in human and rodent epidermis (22, 51–53). Moreover, the presence of the 54-kDa keratin in snout basal cells indicates this keratin cannot be regarded as a marker for a "terminally differentiated" compartment (21, 54).

The basic keratins that co-express with the 54-kDa acidic keratin appear to vary depending on the tissue; the 62–65 and 67-kDa keratins are found in the normal epidermis and snout, respectively (Figs. 1 and 2). The structural and functional significance of the 54-kDa keratin pairing in vivo with multiple basic "partners" in a tissue-dependent fashion is not clear.

The presence of the 66-kDa corneal keratin in bovine snout epithelium attests that this keratin is not corneal specific (cf. Refs. 12, 13, 15, 47, 57). This finding reinforces our recent suggestion that keratins are in general not cell specific; rather, the expression of individual keratins is mainly related to various pathways of epithelial differentiation (21). Our results also emphasize that a given keratinocyte can sometimes express more than one type of differentiation marker (21).
FIG. 5. Immunoblot analysis of bovine keratins using monoclonal antibodies that react with only one or a few keratins. Samples are: 1, skin; 2, snout epithelium in vivo; 3, corneal epithelium in vivo; 4, esophageal epithelium in vivo; 5, cultured esophageal epithelial cells; and 6, MDBK cells. The proteins are transferred to nitrocellulose and (a) stained with Fast Green (FG) or immunoblotted using (b) AE2, (c) BE14, (d) CA20, (e) 6.11, or (f) AE5 monoclonal anti-keratin antibody.

FIG. 6. Classification of bovine epithelial keratins into an acidic and a basic subfamily. Keratins are identified by their molecular masses and by the catalogue numbers of their corresponding human keratins (in parentheses). For a comparison, see Franke et al. (9) and Schiller et al. (7). Filled-in circles denote AE1-positive keratins, hatched circles denote AE3-positive ones, and empty circles denote keratins that react with neither antibody.

well established that keratin expression in stratified squamous epithelia can be modulated significantly and reversibly according to the state of cellular growth and differentiation (see Refs. 11, 38, 39, 47, 52, 55, and 56). Consistent with this concept, we have found that the expression of the bovine 54/62-67-kDa skin-type keratins and the 43/58-kDa esophageal-type keratins is dependent upon normal (in vivo) differentiation, because they are diminished in cultured bovine snout and esophageal epithelial cells, respectively (Figs. 1-4). Although we have not yet analyzed keratins of cultured bovine corneal epithelial cells, data from human and rabbit cells indicate that the 56/66-kDa bovine keratins most likely will also be expressed only during normal differentiation (47, 57). The function of these differentiation-dependent keratins (13) remains unknown. However, the fact that many of them are expressed preferentially in suprabasal layers strongly suggests that they may play a role during advanced stages of stratified epithelial differentiation (22, 51–53, 56; but for an exception see Ref. 50).

Table III summarizes the keratin composition of several cultured bovine epithelial cells including snout epithelial cells, esophageal epithelial cells, BMGE+H and BMGE−H cells (bovine mammary epithelial cells established in the presence and absence of hormones, respectively, by Schmid et al. (58, 59, 77)), and MDBK cells. The fact that the 50/58-kDa keratins are present in large quantities in cultured snout, esophageal, and BMGE+H cells, but not in BMGE−H or MDBK cells, is consistent with the notion that the former three are keratinocytes or keratinocyte-related, while the latter two are simple epithelial related (also see Ref. 50).

Keratin Subfamilies—Our results indicate that all of the bovine keratins that we have studied can be divided into two mutually exclusive subfamilies according to their charge properties and their immunoreactivities with the AE3 and AE1 monoclonal antibodies (Fig. 6). The 67- (Nos. 1–3 according to Schiller et al. (7)), 66- (Nos. 1–3), 62-65- (Nos. 4–5), 58- (No. 6), 58’- (No. 6’), 57- (No. 7) and 55-kDa (No. 8) keratins are all relatively basic and share an AE3 epitope and form the neutral-to-basic (Type I) subfamily. These results are consistent with the earlier data from Schiller and co-workers who have demonstrated by peptide mapping that all of these proteins are structurally related (“subfamily of large and basic cytokeratins” (7)).

In contrast, the 56.5- (No. 10), 56- (Nos. 11 and 12), 54- (Nos. 13 and 14), 50- (No. 16), 46- (No. 19), 45- (No. 21), 43- (No. 18), and 41-kDa (No. 22) keratins are all relatively acidic, and many of them (56.5, 54, 50, 43, and 41 kDa) share an AE1 epitope. These acidic proteins are apparently less conserved structurally than the basic subfamily proteins since Schiller...
## Table I

Classification and rules for expression of bovine epithelial keratins

The keratin catalogue numbers (No.) are according to Schiller et al. (7). The "Ab" columns show the immunoreactivities with monoclonal antibodies (− denotes negative reaction with AE1 antibody). The (B − A) column denotes the molecular weight difference between the basic and acidic members within the keratin "pair." The masses and catalogue numbers (according to Moll et al. (6)) of the corresponding human keratins are shown in parentheses (cf. Ref. 72). Transitional, pseudostratified, and some glandular epithelia express a combination of "simple" and "stratified" types of keratins (6, 10, 12, 72). Most of these bovine keratins have been shown to represent products of different genes (50, 60, 61, 73).

<table>
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<th>Acidic (type I)</th>
<th>Basic (type II)</th>
<th>dM&lt;sub&gt;i&lt;/sub&gt; × 10&lt;sup&gt;-3&lt;/sup&gt; (B − A)</th>
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## Table II

Correlation of the nomenclature of bovine epidermal keratins as described by previous authors

The masses in the left-hand column were determined using known human keratins as standards (2, 6, 38, 39).

<table>
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<th>Backen&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Steinert&lt;sup&gt;c&lt;/sup&gt;</th>
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</table>

<sup>a</sup> Skerrow et al. (74).
<sup>b</sup> Matoltsy et al. (75).
<sup>c</sup> Lee et al. (76).
<sup>d</sup> Lee et al. (41).
<sup>e</sup> Steinert and Idler (36).
<sup>f</sup> Steinert et al. (42).
<sup>g</sup> Franke et al. (3).
<sup>h</sup> Franke et al. (13).
<sup>i</sup> Schiller et al. (7).
<sup>j</sup> Not applicable.
hydrocortisone, and prolactin. These two keratins may, therefore, be regarded as markers for "corneal-type" cells which, thus, are related to a hyperproliferative state but are not found in any simple epithelia. These two keratins and, thus, may be regarded as markers for keratinocytes and related myoepithelial origin; this line was also established in media containing hormones; its keratin composition suggests a simple epithelial (ductal) differentiation (57, 58).

Keratin Subfamilies and Pairs

**Table III**

<table>
<thead>
<tr>
<th>Keratin*</th>
<th>Cultured snout epi-</th>
<th>BMGE-H*</th>
<th>BMGE-H*</th>
<th>MDBK*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dermal cell</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>58 (6)</td>
<td>++</td>
<td>+</td>
<td>++/-</td>
<td>-</td>
</tr>
<tr>
<td>57 (7)</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>55 (8)</td>
<td>+</td>
<td>+</td>
<td>-/-</td>
<td>+</td>
</tr>
<tr>
<td>50 (16)</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>46 (19)</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>45 (21)</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>+</td>
</tr>
<tr>
<td>43 (18)</td>
<td>+</td>
<td>+</td>
<td>-/-</td>
<td>+</td>
</tr>
<tr>
<td>41 (22)</td>
<td>+</td>
<td>+</td>
<td>-/-</td>
<td>-</td>
</tr>
<tr>
<td>Vimentin</td>
<td>-</td>
<td>-</td>
<td>-/-</td>
<td>+</td>
</tr>
</tbody>
</table>

* Keratins are identified by masses and (in parentheses) Schiller's (7) catalogue numbers. The tentative correlation of the masses described here and those used by Schmid et al. (58) are as follows: 58 kDa = their 59 kDa; 57 kDa = 58.5 kDa; 55 kDa = 53 kDa; 50 kDa = 50 kDa; 46 kDa = 45.5 kDa; and 45 kDa = 44 kDa.

**REFERENCES**


e.t. (7) can demonstrate the relatedness of only a few of them according to peptide mapping (also Refs. 12 and 24). However, our immunoblotting results, in conjunction with some recent sequence data (50, 60, 61), clearly indicate that these keratins form another (acidic, Type I) subfamily immunologically and biochemically distinct from the basic subfamily (Fig. 6).

**Keratin Pairs as Defined by Co-expression**—Our data indicate that, similar to the human situation, most acidic bovine keratins have favorite in vivo "partners" in the basic subfamily, forming keratin pairs. Within most pairs, the two keratins have about the same "size ranks" in their respective subfamilies and follow similar rules of expression (Table I; also see Refs. 9 and 13). For example, the 45- and 55-kDa keratins of the acidic and basic subfamilies, respectively, are found mainly in simple epithelia (13, 62, 63). The next larger keratins, i.e. the 46- and 57-kDa keratins of the acidic and basic subfamilies, respectively, are present in cultured keratinocytes and in small amounts in snout epithelium and hoof epidermis (Figs. 1 and 2), both of which are unusually thick and are, therefore, probably hyperproliferative (64). These two keratins may, therefore, be related to a hyperproliferative state of keratinocytes (cf. Refs. 65-67). The acidic 50- and basic 58-kDa components are present in various quantities in stratified epithelia as well as in cells that have a potential to stratify but are not found in any simple epithelia. These two keratins may thus be regarded as markers for keratinocytes and related cells (10, 45, 46) also see Ref. 68). The acidic 43- and basic 58-kDa keratins are found in large quantities in esophageal epithelium, and thus may be considered markers for esophageal differentiation (13, 48, 49). The 56- acidic and 66-kDa basic proteins are found mainly in corneal epithelium and thus may be regarded as markers for "corneal-type" differentiation (13, 47). Finally, the 54- and 56.5-kDa keratins of the acidic subfamily and the 62-65- and 67-kDa keratins of the basic subfamily are found mainly in keratinized epithelia. These four proteins may, therefore, be related to the keratinization process or "skin-type differentiation" (reviewed in Ref. 21; see below).

**Possible Significance of Keratin Pairs**—In the present study, we have characterized many bovine epithelial keratins in terms of their mass, charge, and immunoreactivities with a panel of well characterized monoclonal anti-keratin antibodies. The results confirmed that all the known bovine keratins can be divided into 2 subfamilies. In addition, the data have allowed us to identify for the first time many bovine keratin pairs as defined by co-expression and by their immunological homologies to known human keratins. The recently proposed concept of keratin pair is, therefore, applicable not only to human but also to bovine and possibly other species. Although the structural significance of the keratin pair is not yet clear, the fact that under certain in vivo conditions members of some of the pairs show preferential binding (69, 70) suggests that keratins of these pairs may interact specifically during in vivo tonofilament assembly and/or function.