CHAPTER 5

# PATTERNS OF KERATIN EXPRESSION DEFINE DISTINCT PATHWAYS OF EPITHELIAL DEVELOPMENT AND DIFFERENTIATION

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## I. Introduction

Keratins are of particular interest to developmental biologists because they provide convenient markers of differentiation for all vertebrate and potentially certain invertebrate epithelia and their derivatives (Bartnik *et al.*, 1985; Franke *et al.*, 1981a; Lazarides, 1982; Osborn *et al.*, 1981; Steinert *et al.*, 1984; Sun *et al.*, 1984). During the past decade or so, keratin polypeptides have been subjected to an intense scrutiny which has resulted in the elucidation of a wealth of information on the physical, biochemical, immunological, and molecular properties of the products of this multigene family. These studies indicate that there are a number of characteristics unique to keratin polypeptides which make them especially valuable for studies dealing with epithelial determination and differentiation. Keratins are produced by virtually all epithelia and their expression is specifically restricted to epithelia and epithelial derivatives (Franke *et al.*, 1978, 1979; Sun and Green, 1978b; Sun *et al.*, 1979). They are expressed very early in development, concomitantly with the primary delineation of an epithelium (e.g., mammalian trophectoderm) (Jackson *et al.*, 1980; Lehtonen *et al.*, 1983). They demonstrate specific, coordinately regulated patterns of expression throughout embryogenesis which eventually reflect the stabilization of adult programs of epithelial differentiation (Banks-Schlegel, 1982; Dale *et al.*, 1976, 1985; Moll *et al.*, 1984). Individual keratin polypeptides are expressed in a differentiation-specific manner (Franke *et al.*, 1981b; Sun *et al.*, 1984) and also exhibit specific patterns of distribution in different layers of a given (stratified) epi-



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thelium (Fuchs and Green, 1980; Woodcock-Mitchell *et al.*, 1982). This tissue distribution of particular keratins is a function of the degree of differentiation or maturation of the individual cells as they express the phenotypic characteristics of their particular program of differentiation. Also, the polypeptide composition of keratin varies with disease (Moll *et al.*, 1982, 1984; Tseng *et al.*, 1984; Weiss *et al.*, 1984), transformation (Hronis *et al.*, 1984), and growth environment of epithelial cells (Eichner *et al.*, 1984).

Studies from this laboratory using monoclonal antibodies raised against human keratins have helped to establish definitive patterns of expression of keratin polypeptides within many epithelia of human origin (for review see Cooper *et al.*, 1985; Sun *et al.*, 1985; also see Moll *et al.*, 1982). That is, these mammalian keratins exist as two distinct subclasses of polypeptides which are distinguished by their reactivity with the AE-1 and AE-3 monoclonal antibodies and their charge characteristics as determined by two-dimensional polyacrylamide gels (Eichner *et al.*, 1984). These classes are composed of the basic (AE-3 positive) subfamily and the generally smaller, acidic (AE-1 positive) subfamily. This differential classification was consistent with information derived at the gene level by cDNA clones which distinguished subclasses of mRNA corresponding to the acidic and basic polypeptide subfamilies (Fuchs *et al.*, 1981). This resulted in the designation of type I and type II keratin genes which code for the acidic and basic subfamilies of polypeptides, respectively. Further, it has been determined by using one- and two-dimensional polyacrylamide gel electrophoresis in conjunction with monoclonal antibodies that frequently one member of the acidic subfamily and one member of the basic subfamily are coordinately regulated and expressed as a coupled "pair" of polypeptides in a differentiation-specific manner (Sun *et al.*, 1984). The realization of this "pair concept" of keratin polypeptides, as defined by coexpression, has allowed for the establishment of direct correlations between particular programs of epithelial differentiation and the synthesis of specific keratin pairs (Cooper *et al.*, 1985; Tseng *et al.*, 1982). These associations indicate that the expression of particular keratin polypeptides in an individual cell or tissue provides useful information on its program of epithelial differentiation, its relative degree of differentiation, and its potential to express other keratin polypeptides under various morphological and environmental conditions.

It has become increasingly obvious from these studies that valuable information concerning the mechanism(s) controlling epithelial determination and differentiation may be obtained through the analy-

sis of keratin expression during the developmental divergence and maturation of the various epithelial cell lineages, as well as through the examination of pathological and experimentally manipulated conditions. We will summarize the current information regarding the expression of differentiation-specific keratins under a variety of conditions and will discuss the implications of their existence toward our understanding of epithelial differentiation in general. However, the great majority of these studies have involved human tissues and cell types. Therefore, it is not entirely clear that these properties will be common to all mammals, much less whether they are ubiquitous among all higher vertebrates. Since it is not practical to perform extensive developmental studies designed to determine the nature of factors involved in regulating epithelial differentiation using tissue derived from human sources, it is essential to establish an equivalent set of standards for keratin expression in experimental systems which more readily lend themselves to convenient developmental analysis. Therefore, using the well-established concepts associated with human keratins as a model, we will also present data which demonstrate that the pair concept of keratin expression and the theory of differentiation-specific keratins are equally applicable to a range of mammalian as well as to at least one nonmammalian (e.g., avian) species which are more amenable to developmental analysis.

## II. Differentiation-Specific Keratin Pairs

### A. Human Keratin Pairs Defined by Coexpression

It has by now become well established that human keratins are composed of roughly 20 distinct polypeptides, the majority of which have been shown to be distinct translational products. All of the known human keratins have been compiled into a catalog which assigns an empirical numerical value (1–19) to each polypeptide in order to provide a standard system of nomenclature (Moll *et al.*, 1982). Further, by examining a large number of normal and abnormal tissues and cell types, it was described that specific acidic and basic polypeptides form "pairs" which came to be associated with particular programs of epithelial differentiation (Sun *et al.*, 1984; Tseng *et al.*, 1982). The 56.5K acidic and 65–67K basic keratins (keratins number 10 and 1–2, respectively, in the Moll *et al.* nomenclature) are usually found in association with keratinized stratified squamous epithelia of all types (Moll *et al.*, 1982; Tseng *et al.*, 1982, 1984). The 55K acidic and 64K basic keratins (keratins 12, 3) are found mainly in association with corneal epithelium in humans, and the 51K acidic and 59K basic ker-

atins (keratins 13, 4) are found in most other nonkeratinized stratified squamous epithelia (Moll *et al.*, 1982; Sun *et al.*, 1984). In addition, the 50/58K (keratins 14, 5) pair of keratins is found to be present in variable quantities in all keratinocytes (which are defined as being the major cell type in all stratified epithelia) but is not expressed in simple epithelial cells, whereas the 48/56K (keratins 15, 6) pair is found only in "hyperproliferative keratinocytes" (Moll *et al.*, 1982; Sun *et al.*, 1984; Weiss *et al.*, 1984). There are also a number of additional keratins which are mainly restricted to simple epithelia or pseudostratified epithelia. These pairs, which were defined by coexpression, are probably the result of a combination of specialized functional characteristics of the polpeptides which dictate their differential expression and the fact that keratin filament formation requires an equimolar ratio of acidic to basic components (Hatzfeld and Franke, 1985).

### B. Keratin Pairs Defined by Coexpression in Nonhuman Epithelia

As in the case of human keratins, the keratins from cow, rabbit, and chicken can also be subclassified into acidic and basic subfamilies based on both their isoelectric points and their reactivity with AE-1 and AE-3 (Figs. 1–11). Also, Pruss *et al.* (1981) have produced a broadly reactive monoclonal antibody (aIF) which recognizes most intermediate filament polypeptides. This antibody, used in conjunction with AE1 and AE3 on two-dimensional immunoblots, allows for the identification of most keratin polypeptides (Cooper *et al.*, 1984).

In bovine epithelia, Schiller *et al.* (1982) and Cooper and Sun (1986) have shown that the basic subfamily consists of about eight polypeptides ranging in molecular weight from 55K to 67K. The acidic subfamily also consists of eight polypeptides which range in molecular weight from 41K to 56.5K (Cooper and Sun, 1986). Cooper and Sun (1986) have also shown that bovine acidic and basic polypeptides show

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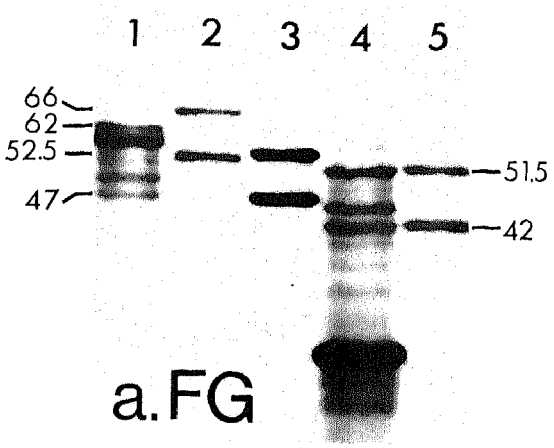
Fig. 1. Keratin extracted from representative chicken tissues and subsequently separated by SDS–PAGE and blotted to nitrocellulose. (a) Fast Green-stained (FG) blot of protein from (1) middorsal epidermis, (2) corneal epithelium, (3) esophageal epithelium, (4) ventricular (gizzard) epithelium, and (5) intestinal epithelium. (b and c) Blots similar to a except that they have been immunostained by the peroxidase–antiperoxidase (PAP) procedure with AE3 (b) and AE1 (c) monoclonal keratin antibodies. The pattern seen in middorsal epidermis (lane 1) is identical to that found in epidermis taken from comb, scutate scale, and reticulate scale skin. The epithelium from the tongue and crop produces the same keratin profile as that seen in esophagus (lane 3) and the proventriculus (fore stomach) produces the same keratins as the ventriculus.


1 2 3 4 5
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62
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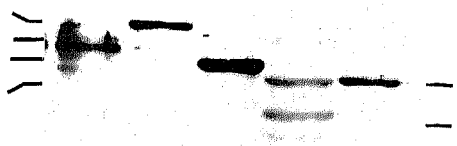
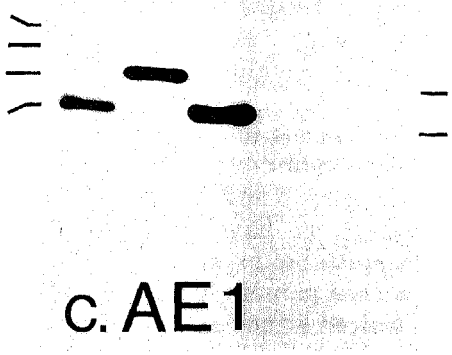
a. FG


b. AE3


c. AE1

specific patterns of coexpression similar to human keratin. In cows, the 56.5 acidic keratin and the 62–65K keratins form a pair which is restricted to keratinized stratified squamous epithelia such as skin and hoof and are therefore functionally equivalent to the human 56.5/65–67 pair. The bovine 56K acidic and 66K basic keratins form a pair which is mainly found in corneal epithelium, making them equivalent to the human 55/64K cornea pair. The 43K acidic and 58'K basic keratin polypeptides of cow are found in all nonkeratinized stratified squamous epithelia (other than cornea) such as esophagus and are therefore equivalent to the human 51/59K pair. The 50/58K keratinocyte marker in humans is represented by the 50/58K cow keratins and is found in most stratified epithelia as well as cultured epithelia derived from stratified epithelia. Hyperproliferative bovine keratinocytes produce a 46/57K pair which is equivalent to the human 48/56K pair. The remaining cow keratins are found in varying amounts among simple and pseudostratified epithelia (Cooper and Sun, 1986; Franke *et al.*, 1981a; Schiller *et al.*, 1982).

The analysis of chicken keratin has so far yielded two subfamilies of keratin polypeptides composed of six major basic and six acidic keratins (Figs. 1 and 2). Of these keratins, the 59K and 62K keratins are found only in keratinized stratified squamous epithelia including epidermis from skin, comb, scutate, and reticulate scales and are therefore equivalent to the human 56.5/65–67K pair (Fig. 3). The chick 52.5/66K pair is found in corneal epithelium (Figs. 1 and 4) and the 47/55.5K pair is restricted to all other nonkeratinized stratified squamous epithelia including esophagus, crop, and tongue (Figs. 1 and 5). The chick 47/60K pair appears to be the keratinocyte-specific marker although the 60K polypeptide is more easily detected. Due to the lack of availability of cultured chicken epithelia or hyperproliferative diseased epithelia, a marker for hyperproliferative keratinocytes from chick has not been determined. The remaining keratins are found in various combinations in simple and pseudostratified epithelia such as intestine, trachea, proventriculus (fore stomach), and ventriculus (gizzard) (Figs. 1, 6, and 7).

A very similar situation is found in rabbits. The rabbit keratins may be divided into acidic and basic subfamilies consisting of about eight polypepties each (Figs. 8 and 9). Based on their distribution and patterns of coexpression we have determined that all keratinized stratified squamous epithilia express the 56.5/63.5K keratin pair (Figs. 8 and 9). The cornea pair is represented by the 55/64K pair, and most other nonkeratinized stratified squamous epithelia express the 46/59K pair (Figs. 8 and 9). All rabbit keratinocytes express the 50/58K

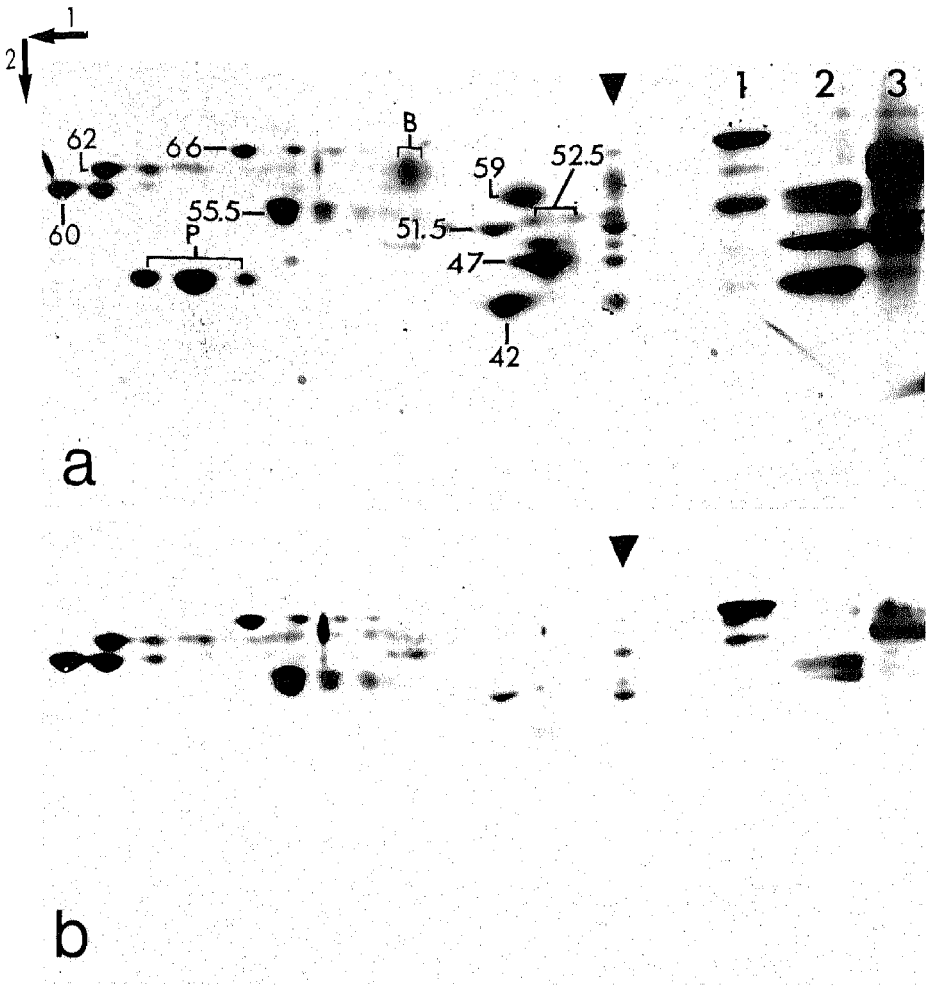



FIG. 2. Two-dimensional analysis of a mixture of keratins from chicken epidermis, cornea, esophagus, and intestine. The first dimension (1) separation was achieved by nonequilibrium pH gradient gel electrophoresis (NepHGE) and the second dimension (2) was SDS–PAGE. This figure allows direct evaluation of the relative position of the majority of chicken keratin polypeptides which are indicated by approximate molecular weight ($\times 10^{-3}$). Arrowhead indicates aggregated polypeptides not entering the first dimensional gel. Side lanes are (1) cornea keratins, (2) a mixture of esophageal and intestinal keratins, and (3) epidermal keratins. a is stained with Fast Green and b is the same blot after staining with aIF. Note that all of the basic keratins are stained but only a subset of the acidic keratins are stained. This pattern is frequently observed when using aIF (Cooper *et al.*, 1984). Bovine serum albumin (B) and 3-phosphoglycerate kinase (P) are included as standards.

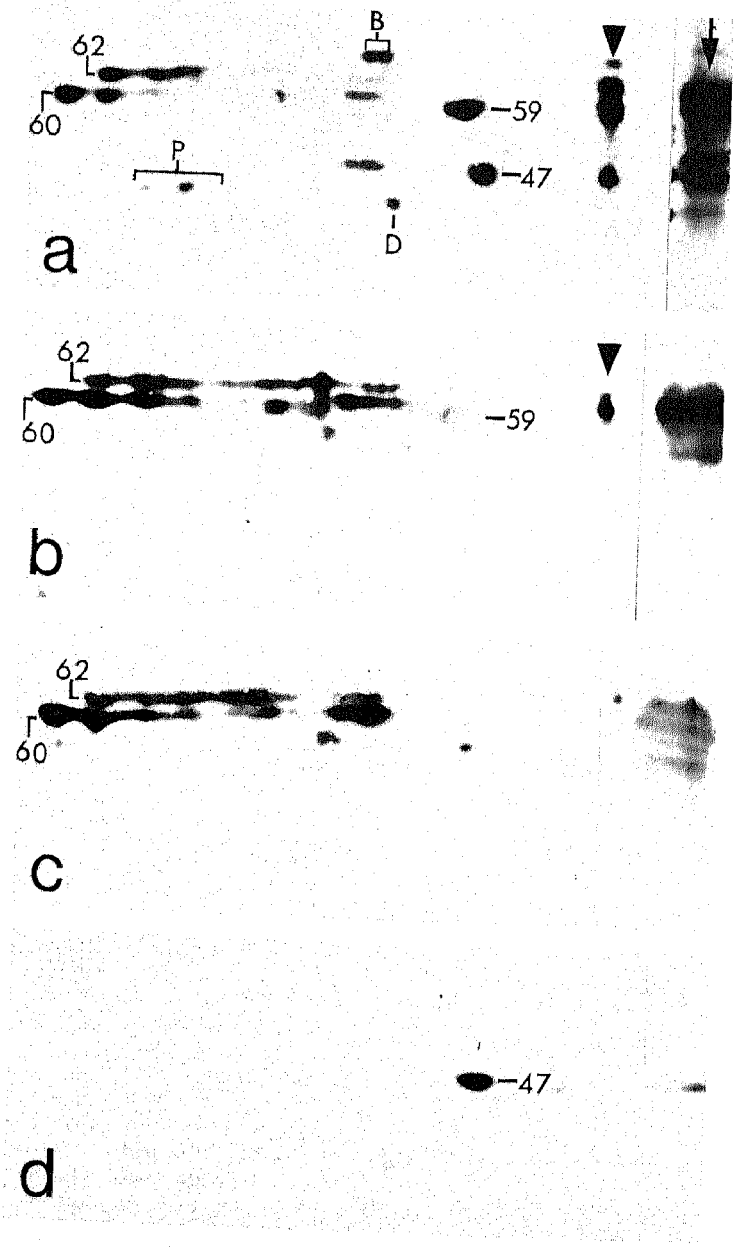



FIG. 3. Two-dimensional polyacrylamide gel electrophoresis as described in Fig. 2 of keratins extracted from chicken epidermis. Arrowheads indicate the location of aggregated material not entering the first dimensional gel and the arrow indicates a one-dimensional side lane provided for comparison. (a) Fast Green-stained blot with the major epidermal keratins indicated by molecular weight ($\times 10^{-3}$). Standards included on the gel were bovine serum albumin (B), 3-phosphoglycerate kinase (P), and deoxyribonuclease (D). (b–d) Immunoblots of gels similar to a stained with (b) aIF mono-

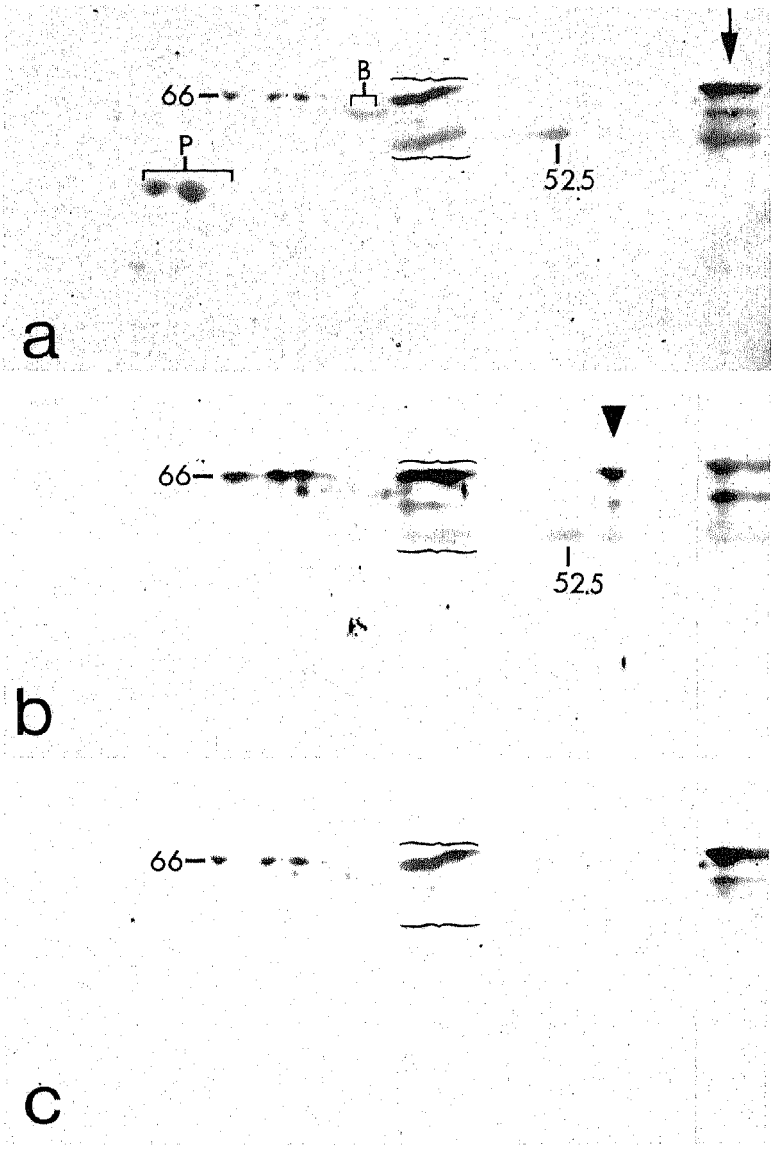



FIG. 4. Two-dimensional gel analysis, as described for Fig. 2, of chicken corneal epithelial keratin. (a) Fast Green-stained blot, with the major keratin polypeptides indicated by molecular weight ($\times 10^{-3}$). The brackets indicate the presence of a strong complex of corneal 66K and 52.5K keratin not dissociated in the first dimension. (b) Immunoblot of corneal keratin using the aIF antibody; (c) immunoblot with AE3.

clonal antibody which recognized most intermediate filament proteins, (c) AE3 monoclonal antibody which recognizes all basic keratin polypeptides, and (d) AE1 monclonal antibody which recognizes most acidic keratin polypeptides.

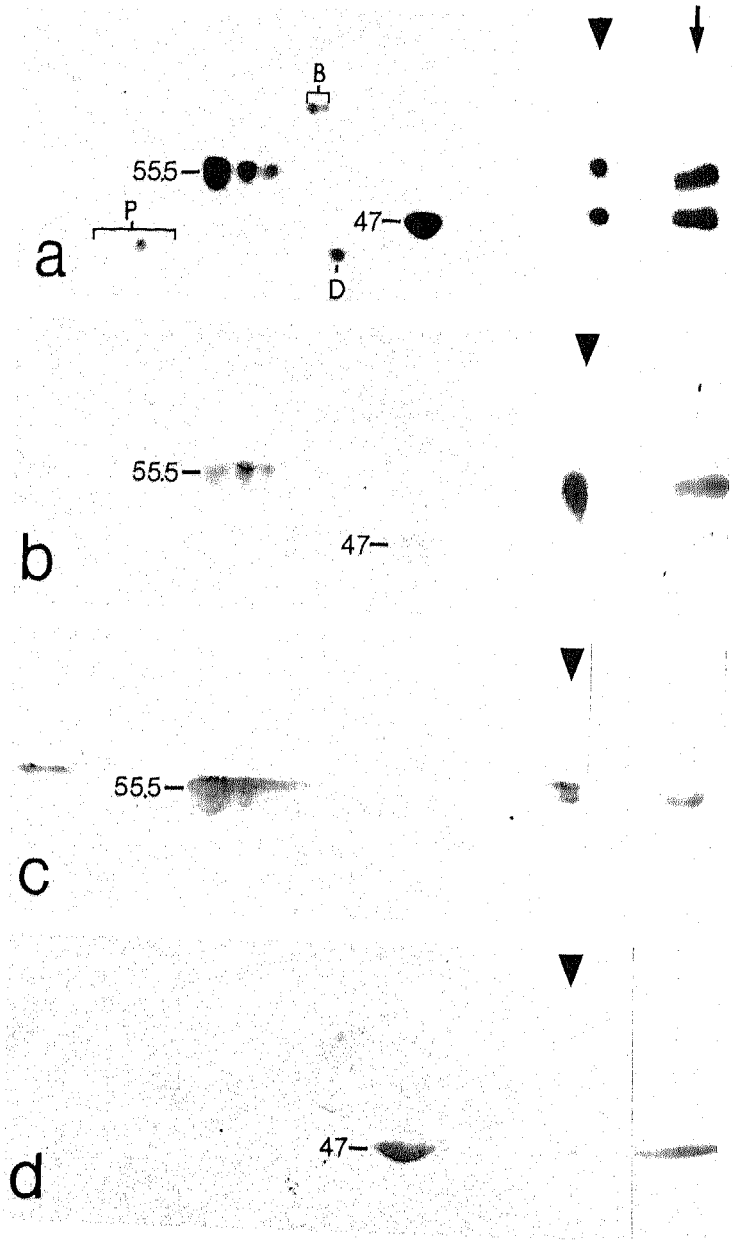



FIG. 5. Two-dimensional analysis of chicken esophageal keratins as described in Fig. 2. (a) Fast Green staining pattern; (b–d) immunoblots. (b) aIF staining pattern of esophageal keratin. Note the weak staining of the 47K polypeptide. This is frequently seen in aIF stains of various acidic keratins (see Cooper *et al.*, 1984). The acidic (47K) and basic (55.5K) polypeptides are well distinguished by AE3 (c) and AE1 (d).

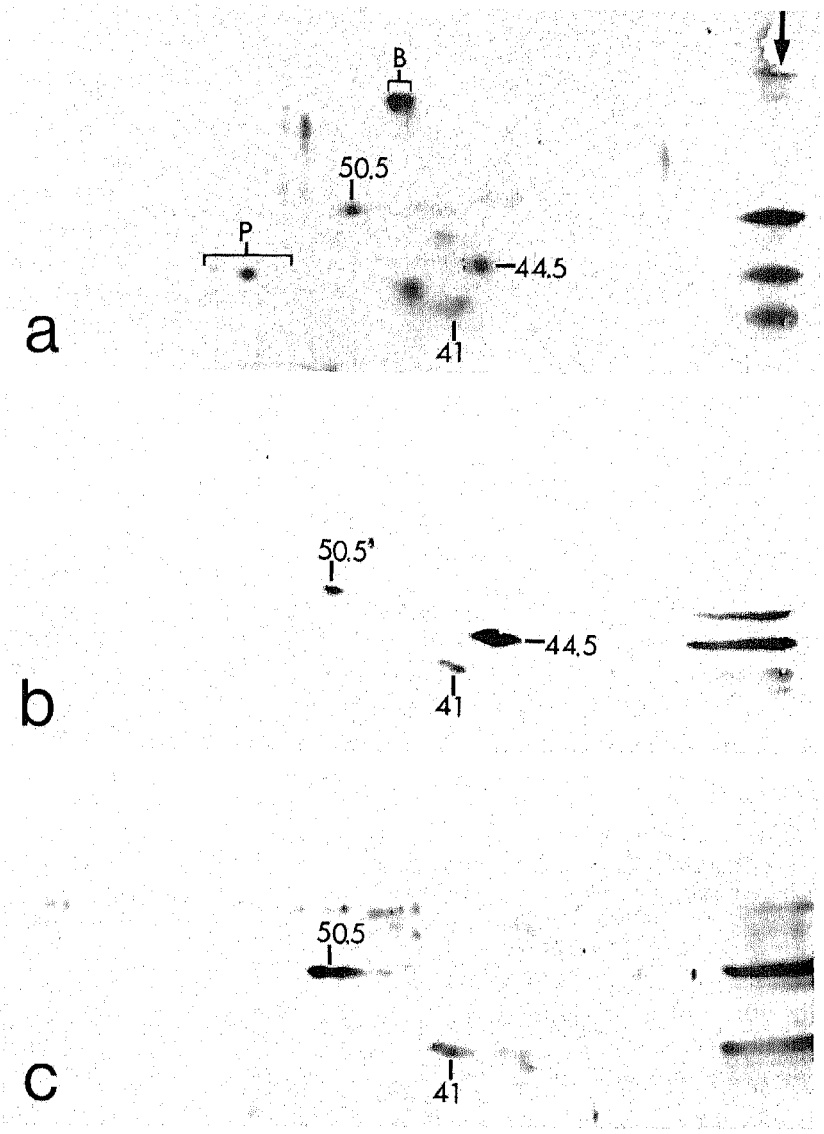



FIG. 6. Two-dimensional immunoblot analysis of chicken ventriculus (gizzard) keratins. (a) Fast Green-stained proteins blotted onto nitrocellulose as described in Fig. 2; (b) shows that all of the keratins are recognized by aIF; (c) shows the reactivity of AE3 with these keratins. AE1 does not recognize any keratin from the ventriculus.

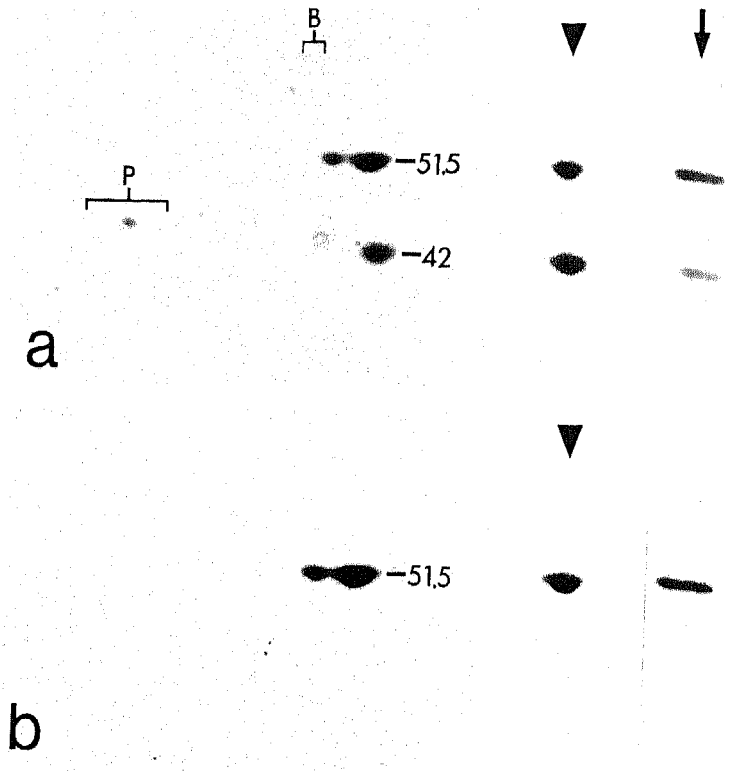



Fig. 7. Two-dimensional immunoblot analysis of a cytoskeletal preparation from chicken intestinal epithelium which has been separated by two-dimensional gel electrophoresis. (a) Fast Green staining pattern. (b) The staining pattern of AE3 on intestinal keratin shows strong reactivity with the 51.5K polypeptide; the same pattern was also seen with aIF (not shown). AE1 does not stain any intestinal polypeptide.

pair while those keratinocytes undergoing hyperproliferation additionally express a 48/56K pair (Figs. 8 and 9). The remaining polypeptides are found in a variety of nonstratified epithelia (Fig. 10).

### C. Species Differences in Keratin Pairs

It becomes obvious from the above comparisons that both the patterns of keratin expression and their reactivity with AE-1, AE-3, and aIF are highly conserved over a wide range of species. There are a few notable differences in the characteristics of the human keratin pairs and those from other species (Table I). One difference is that the molecular weight difference of 8–11K between the acidic and basic members of most human keratin pairs is much more variable among the

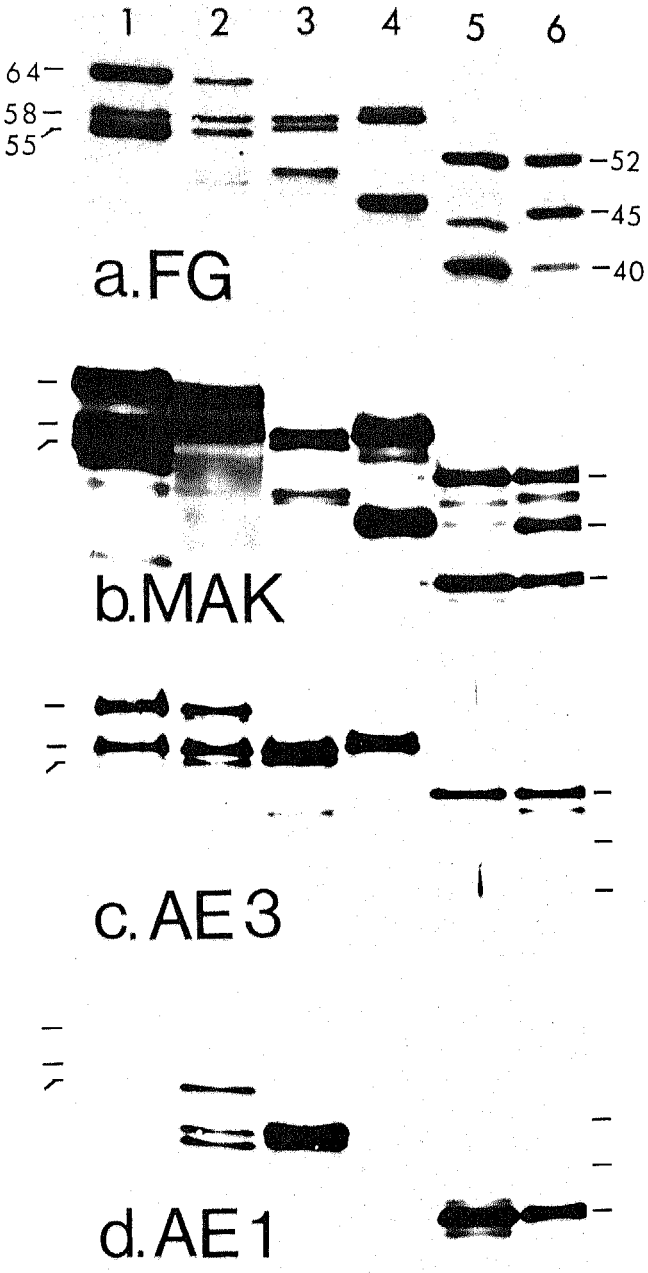



FIG. 8. Keratins extracted from representative rabbit epithelial tissues and separated by SDS–PAGE. (a) Fast Green blot of proteins from (1) cornea, (2) epidermis, (3) epidermal cells cultured *in vitro,* (4) esophagus, (5) intestine, and (6) bladder. (b–d) Immunoblots of the same samples in a using (b) a mixture of monoclonal antibodies (AE1, AE3, aIF, and CA-20) which recognize almost all keratins or (c) AE3 alone and (d) AE1 alone.