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Keratin cytoskeletons in epithelial cells of internal organs

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ABSTRACT An antiserum against human epidermal keratins was used to detect keratins in frozen sections of various rabbit and human tissues by indirect immunofluorescence. Strong staining was observed in all stratified squamous epithelia (epidermis, cornea, conjunctiva, tongue, esophagus, vagina, and anus), in epidermal appendages (hair follicle, sebaceous gland, ductal and myoepithelial cells of sweat glands), as well as in Hassall's corpuscles of the thymus, indicating that all contain abundant keratins. No staining by the antiserum was observed in fibroblasts, muscle of any type, cartilage, blood vessel, nerve tissue, iris or lens epithelium, or the glomerular or tubular cells of the kidney. In contrast, the antiserum stained the cells of most epithelia of the intestinal tract, urinary tract (urethra, bladder, ureter, collecting ducts of kidney), female genital tract (cervix, cervical glands, uterus, and oviduct), and respiratory tract (trachea and bronchi). Epithelial cells of the fine ductal system in the pancreas and submaxillary gland also stained well. When primary cultures of epithelial cells derived from bladder, intestine, kidney, and trachea were grown on glass coverslips and stained with anti-keratin, fiber networks similar to those of cultured keratinocytes were observed. These results show that keratins constitute a cytoskeleton in epithelial cells of diverse morphology and embryological origin. The stability of keratin filaments probably confers the structural strength necessary for cells covering a free surface. Keratin staining can be used to obtain information about the origin of cell lines.

The stratified squamous epithelium is the most common covering or lining surface of the animal body. It may be of ectodermal origin (the epidermis) or of endodermal origin (the esophageal epithelium). The dominant cell type of these epithelia (the keratinocyte) contains abundant 80-Å filaments composed of keratin proteins. Cells of this type cultivated from different stratified squamous epithelia are rather similar (1), keratins accounting for about 30% of the cellular protein.

Most other epithelia, such as those of the intestinal, respiratory, genital, and urinary tracts, seem quite different. Although the cells of these epithelia frequently contain 80- to 100-Å filaments, they are not as abundant as those of the keratinocyte and may not show their typical lateral aggregation. The cells of epithelia other than stratified squamous are often secretory and have morphology and specialization very different from those of the keratinocytes.

The keratins of human stratum corneum have been purified as a group and used to make a rabbit antiserum (2). This antiserum produces specific precipitin lines when tested against extracts of keratinocytes, and it stains by indirect immunofluorescence a network of fibers within the cytoplasm of keratinocytes (3). Using this serum, we show here that keratins are present not only in cells of all stratified squamous epithelia (of human and rabbit) but also in many other epithelial cell types of diverse morphology and function.

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MATERIALS AND METHODS

Preparation of Keratins and Antiserum. The purification and characterization of keratins from stratum corneum of human epidermis and the preparation of antiserum specific for keratins have been described in detail elsewhere (2). Briefly, human epidermal callus clipped from fingers and toes was minced, vigorously homogenized, and serially extracted with 20 mM Tris·HCl (pH 7.4) and the same buffer containing 8 M urea to remove the nonkeratin proteins. The keratin filaments (containing subunits stabilized by intermolecular disulfide bonds) were then dissolved in 8 M urea and 2-mercaptoethanol. The dissolved keratins produced four major bands and three minor bands in sodium dodecyl sulfate gel electrophoresis, all with molecular weight between 40,000 and 63,000, and could be assembled *in vitro* into typical 80-Å filaments (2).

Cell Culture. Epidermal cells (4) and corneal epithelial cells (1) were grown in the presence of lethally irradiated mouse 3T3 cells in fortified Eagle's medium supplemented with 20% fetal bovine serum and hydrocortisone (0.4 μg/ml) as described previously. The same medium was used for all other cells (Table 1) except the fibroblasts, which were grown in medium containing 10% bovine serum without hydrocortisone, and XB-2 cells (5), which were grown in medium containing 20% fetal bovine serum without hydrocortisone.

The following cell lines were obtained from American Type Culture Collection: A2, NBL-11, D562, SW-13, M3, and Pt K2 (See Table 1).

Indirect Immunofluorescent Staining. Various human tissues were obtained through the courtesy of Richard Schlegel of Harvard Medical School from autopsies performed within the previous 16 hr. Rabbit tissues were obtained from freshly killed New Zealand White rabbits. Small pieces of tissues were snap frozen in isopentane at −160°C and embedded in Tec II medium. Sections (5 μm) were air dried on glass slides with a fan for 60 min. Nerve tissues were subsequently delipidized by immersion in chloroform/methanol (2:1 vol/vol) (6). Sections of the digestive tract were air dried only briefly (5–10 min) and then fixed in 3.7% (wt/vol) formaldehyde in isotonic phosphate buffer (NaCl/P_i) for 30 min.

The air-dried tissue sections were hydrated in NaCl/P_i, covered with 20 μl of anti-keratin serum previously diluted 1:48 with NaCl/P_i, and filtered through a Millipore filter (0.22 μm). The treated sections were incubated in a humidified chamber at 37°C for 60 min. They were then washed in three changes of NaCl/P_i for a total of 30 min and overlaid with fluorescein-conjugated goat anti-rabbit IgG (Miles) at a dilution of 1:16 and incubated at 37°C for 60 min. After rinsing again, samples were mounted in Gelvatol (7) and viewed in a Zeiss universal microscope using transmitted illumination. Photographs were taken with Kodak Tri-X pan film.

Cultured cells to be stained were grown on 12-mm glass

Abbreviation: NaCl/P_i, isotonic phosphate buffer.

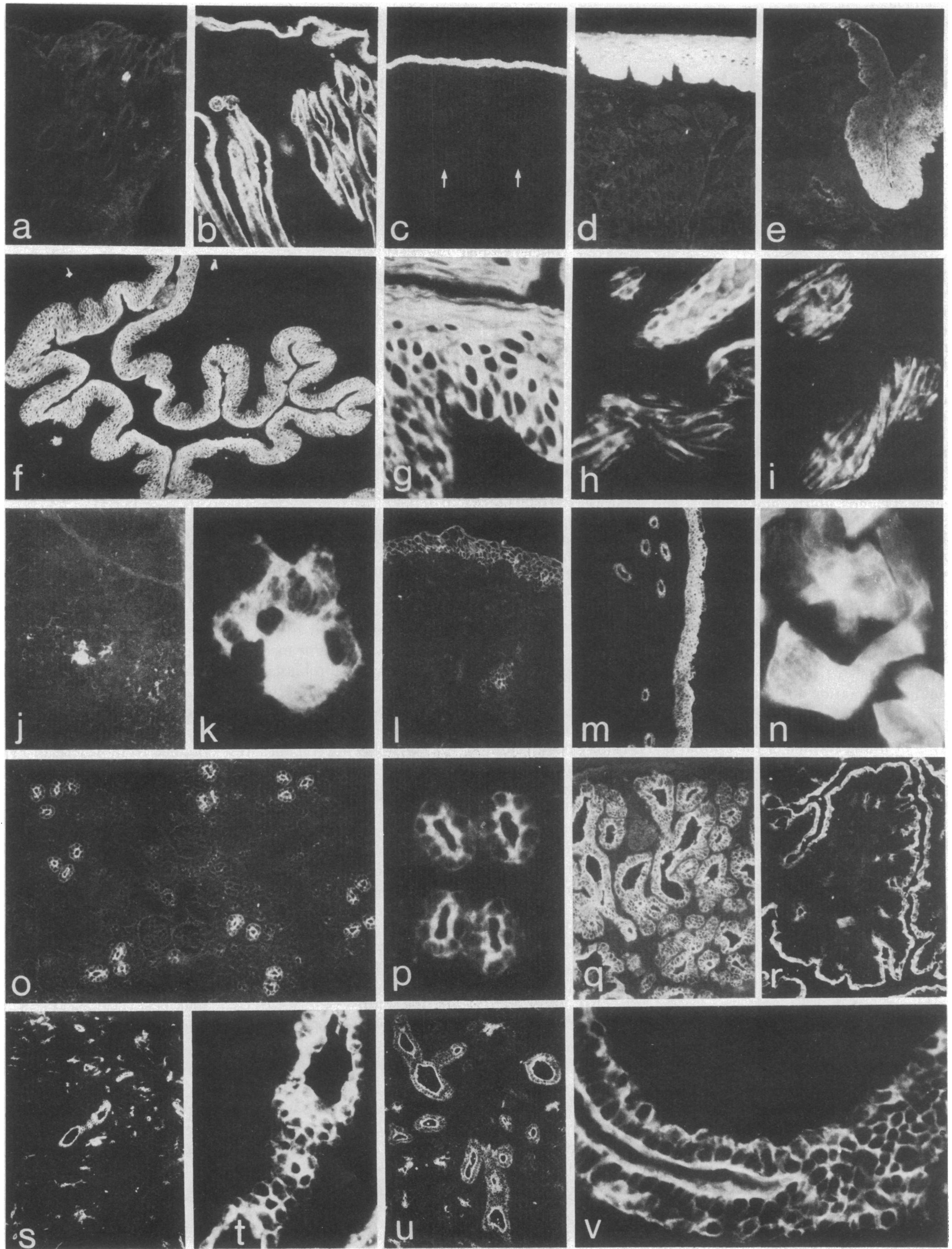

FIG. 1. (*Legend appears at the bottom of the next page.*)

coverslips, rinsed with NaCl/P_i, fixed at room temperature for 30 min in 3.7% formaldehyde in NaCl/P_i, made permeable by immersion in methanol chilled to −20°C for 5 min, stained, and viewed under epifluorescent illumination (3). Photographs were taken with Kodak Plus-X film and developed in Diafine (Acufine, Inc., Chicago, IL).

RESULTS

Immunofluorescent Staining of Tissue Sections. Frozen sections of different tissues were tested with our antiserum to human epidermal keratins. Some of the tissues were obtained from human autopsy specimens, but others could be obtained in a better state of preservation from the rabbit. Because the antiserum reacts well against rabbit keratins (3), the results were similar for the two species. The immunological nature of the fluorescent staining by our anti-keratin serum was established by controls in which cells were unstained after the application of preimmune serum (e.g., Fig. 1*a*) or antiserum preabsorbed with isolated keratins. The antiserum has been shown to be specific for keratins by immunodiffusion, immunoelectrophoresis, and immunofluorescent staining techniques (1–3).

All stratified squamous epithelia were stained very brightly, including those of epidermis (Fig. 1*b*), cornea (Fig. 1*c*), tongue (Fig. 1*d*), anus (Fig. 1*e*), esophagus (Fig. 1 *f*, *g*), and vagina. Appendages of the epidermis also stained brightly, including hair follicles (Fig. 1*b*), sebaceous glands, and the ducts of eccrine sweat glands (Fig. 1*h*). Surprisingly, groups of eccrine myoepithelial cells, identifiable by their large size and characteristic shape (8), stained well and showed fibrous distribution of the stain (Fig. 1 *h*, *i*). Hassall's corpuscles of the thymus, believed to be of endodermal origin but whose histological appearance is epidermoid, stained strongly, whereas most of the thymus remained unstained (Fig. 1 *j*, *k*).

All connective tissues tested failed to stain. This category included fibroblasts, muscle (cardiac, striated, and smooth), cartilage, and blood vessels. Some tissues of ectodermal origin also failed to stain (brain and peripheral nerve, iris epithelium, and lens). Finally, the parenchymal cells of organs of endodermal origin such as liver and pancreas also did not stain.

In contrast, epithelial lining cells of the urinary, female genital, respiratory, and intestinal tracts were stained by the antiserum, but less strongly than the cells of stratified epithelia. This was true for the entire length of the urinary tract, including urethra, bladder (Fig. 1*l*), ureters, and the collecting ducts of the kidney (Fig. 1 *o*, *p*). Positive staining was also observed in the covering epithelial cells of the trachea (Fig. 1*m*), bronchus, small intestine (Fig. 1*n*) and large intestine, oviduct, cervix and cervical glands (Fig. 1 *q*), and uterus (Fig. 1*r*). The ducts of the pancreas (Fig. 1 *s*, *t*) and submaxillary gland (Fig. 1 *u*, *v*) also stained well, though the parenchymal cells did not. The bronchioles stained quite definitely, but the lung parenchyma stained very weakly.

Immunofluorescent Staining of Primary Cultures and Cell Strains. As shown earlier (3), the anti-keratin serum stained cultured keratinocytes originating from skin and cornea, revealing keratin fibers. To these cell types may now be added cultured keratinocytes originating from normal human nasopharyngeal epithelium and briefly cultured epithelial cells derived from rabbit bladder, small intestine, kidney, and trachea. Primary cultures of cells from various rabbit tissues were grown on glass coverslips. Because no precautions were taken to prevent the growth of fibroblasts, most of the cultures consisted of a mixture of fibroblasts and epithelial cells. Consistent with previous observations (3, 9), none of the fibroblastic cells derived from any tissue of human or rabbit were stained by the antiserum (e.g., bladder fibroblast as shown in Fig. 2*a*); but epithelial colonies derived from bladder (Fig. 2*a*), intestine (Fig. 2*b*), and kidney (Fig. 2 *c*, *d*) were stained by the antiserum and revealed a fibrous distribution of the stain. Stained fibers were also observed in epithelial colonies derived from rabbit trachea. On the other hand, cultured epithelial cells derived from rabbit ocular lens were not stained.

Immunofluorescent Staining of Established Cell Lines. The study of cultured cell lines gave results consistent with those of the tissues and in one case gave information about the origin of a line. The established line D562, derived from a human nasopharyngeal carcinoma (10), was found to stain brightly. As recently reported (11), HeLa cells, known to have originated from a cervical tumor (12) and diagnosed as adenocarcinoma (13), stained moderately brightly. This result is consistent with an origin of HeLa cells from cervical epithelium or cervical glands. As might be expected from an earlier study (14), Pt K2, a line derived from kidney of the rat kangaroo, was also stained by our anti-keratin serum. This suggests that the line arose from a collecting duct rather than from other cell types in the kidney. In all these cultured cell types, flattened cells revealed a fibrous distribution of the stain.

Established fibroblast lines of the hamster (BHK), mouse (3T3), and rabbit (NBL-1) were not stained by the antiserum; also not stained were cell lines derived from a human adenocarcinoma of the adrenal cortex (SW-13) (15), a mouse melanoma (M-3) (16), a mouse neuroblastoma (2A) (17), and a rat hepatoma (H4). These results are summarized in Table 1.

DISCUSSION

Tissue Distribution of Keratin-Containing Cells. Stratified squamous epithelia of all types were stained very strongly by antiserum to keratin, and the staining was evident in all cell layers of the epithelia (Fig. 1 *b*–*g*). Cells of epidermal appendages, such as hair follicles and ducts of the sweat glands, were also stained. This is consistent with the electron microscopic finding that these cells contain abundant tonofilaments (18–20). Hassall's corpuscles of the thymus are similar histologically to epidermis; they contain abundant tonofilaments and desmosomes (21, 22) as well as crossreacting antigens (23, 24). The present finding that the antiserum to keratin reacts with Hassall's corpuscles shows that they contain keratins immunologically related to those of stratum corneum.

FIG. 1 (*on preceding page*). Immunofluorescent staining of frozen sections of tissues by antiserum to human epidermal keratins. Frozen sections (5 μm) of various tissues were stained with rabbit antiserum and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG. All tissues were identified in a neighboring section stained with hematoxylin and eosin. Except for the human eccrine sweat glands (*h*, *i*) and intestine (*n*), all tissues are from rabbit. (*a*) Skin. Preimmune serum control. (*b*) Skin. Note the staining of epidermis and hair follicles. (*c*) Cornea. Note staining of epithelium. Arrows mark the endothelial layer. (*d*) Tongue (inferior surface). (*e*) Anorectal junction. Rectal epithelium stained much more weakly than anal epithelium. (*f* and *g*) Esophagus. (*h*) Eccrine sweat gland. Ductal portion is seen at top right. Several groups of myoepithelial cells of the secretory portion can also be seen. (*i*) Groups of myoepithelial cells of eccrine sweat gland. (*j*) Thymus. Note the staining of a Hassall's corpuscle. (*k*) Hassall's corpuscle of the thymus. (*l*) Bladder. Note staining of transitional epithelium. (*m*) Trachea, tangential section. Note staining of the epithelium and ducts of the tracheal glands. (*n*) Small intestine. Desquamated epithelial cells obtained from human autopsy. Note the fibrous distribution of the stain in some cells. (*o* and *p*) Kidney. Note the staining of collecting ducts. (*q*) Cervix. Note staining of endocervical glands. (*r*) Uterus. Note staining of uterine epithelium. (*s* and *t*) Pancreas. Note staining of ducts. (*u* and *v*) Submaxillary gland. Note staining of ducts. (Magnification for *n* is ×1080; for *g*, *h*, *i*, *k*, *p*, *t*, and *v*, ×430; and for all others, ×70.)

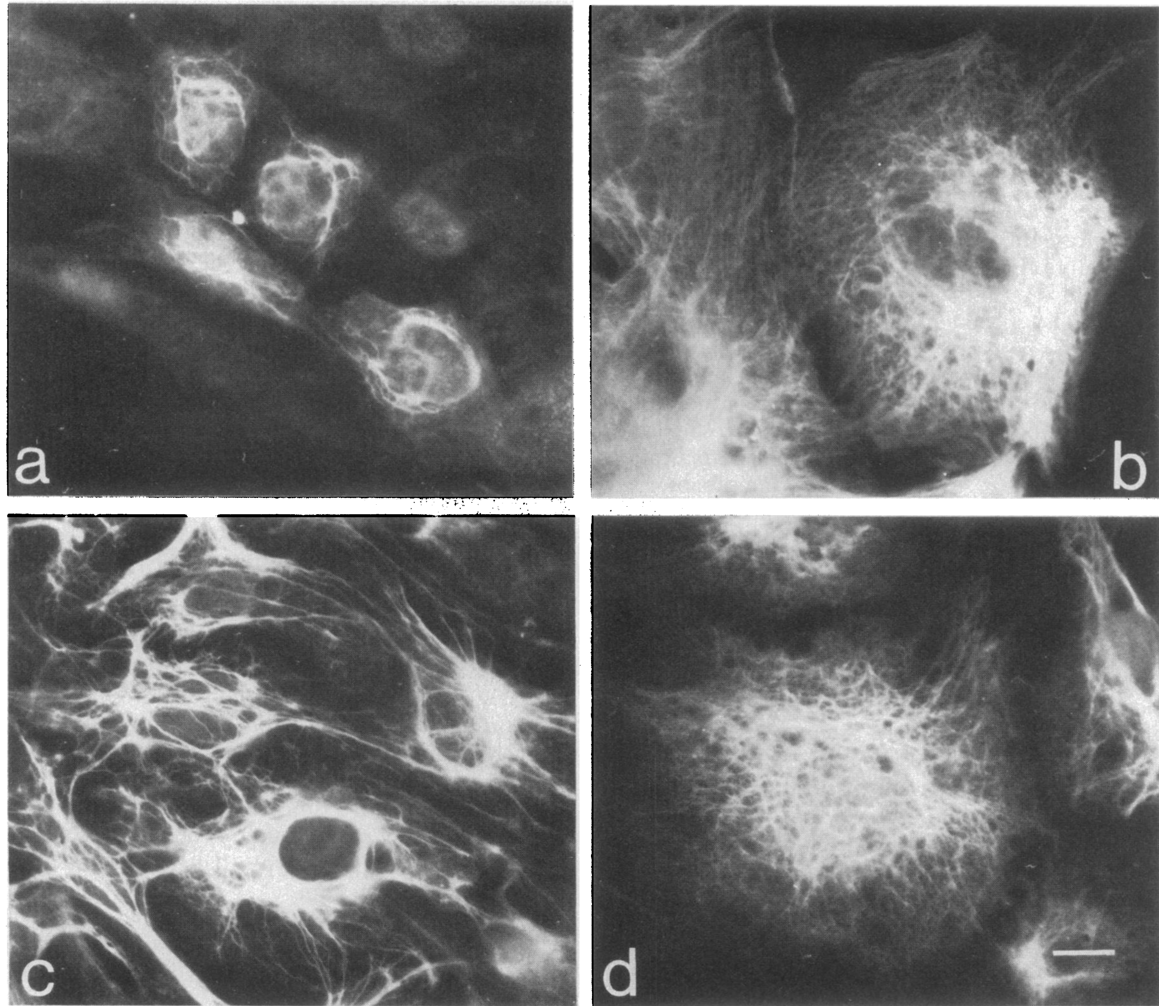

FIG. 2. Immunofluorescent staining of primary cultures of rabbit epithelial cells by antiserum to keratin. Tissues from a freshly killed rabbit (New Zealand White, 2-week-old female) were isolated, minced, and digested with 0.25% trypsin and 0.001% EDTA at 37°C for 45 min. The released cells were plated on glass coverslips. Five days later the cells were fixed for 30 min in 3.7% formaldehyde, treated with methanol, and stained with anti-keratin. (*a*) Bladder. Note the fibrous staining of the small epithelial colony; the fibroblasts surrounding the colony were not stained. (*b*) Small intestine. This is an outgrowth from a tissue fragment that lies to the left. (*c* and *d*) Kidney. The most common cell type stained is shown in *c*, but occasionally there are cells with elaborate fibers such as the one shown in *d*. These cells are probably derived from collecting ducts, as shown in Fig. 1 *o* and *p*. (All pictures ×820. Bar = 10 μm.)

More surprising was the presence of keratins in the epithelia of the intestinal, genitourinary, and respiratory tracts. Epithelia of many different kinds have been known to contain filaments approximately 80 Å in diameter (25–30). These are especially prominent in relation to desmosomes (31) and have often been referred to as tonofilaments; but, in comparison with the filaments of the keratinocytes, they are much less abundant and their biochemical nature was not known. The reaction by immunofluorescence of such cell types with the anti-keratin serum suggests that the filaments detected by electron microscopy in those cell types are actually composed of keratins. The epithelial cells of ducts stain strongly, particularly in the region close to their luminal surface (Fig. 1 *p*, *u*, and *v*), where desmosomes are known to be concentrated (31, 32). Parenchymal tissue of glands were not stained, indicating that if keratins are present, their concentration is too low to be detected.

Another unexpected finding was that myoepithelial cells of the eccrine sweat gland stained strongly and characteristically (Fig. 1 *h*, *i*). Bundles of filaments described as myofibrils are present in these cells (33), and it has been suggested that they have a contractile role (8). Even if myofibrils are present (34), keratin fibrils must be present as well, in accord with the view that another function of these cells may be supportive and protective (35).

Significance of Keratins as Cytoskeletal Proteins. The principal conclusion of these experiments is that, in addition to the keratinocytes of stratified squamous epithelia, many other but *not all* epithelial cell types contain keratin fibers. This form of cytoskeleton probably provides the strength required in cells of a free surface subjected to mechanical or fluid forces. Perhaps the lens and iris epithelia lack keratins because they are not exposed to such forces. The endothelial cells of blood vessels were also exceptional in that they were not stained by the antiserum to keratins.

Though it is difficult to make quantitative estimates from immunofluorescence, it seems very likely that the keratin content of the nonkeratinocyte epithelial cell types is much lower than that of keratinocytes. The term "keratinocyte" should continue to be reserved for the cells of stratified squamous epithelia, because in addition to having a higher keratin content they are able to make crosslinked envelopes (1, 36), structures not so far demonstrated in any other cell type.

Other reservations may apply to the nature of the keratins present in nonkeratinocyte epithelial cells. The antiserum used

Table 1. Immunofluorescent staining of cultured cells by anti-keratin antiserum

Cell type	Relative amount of stained filaments
Human epidermal cells	+++
Human corneal epithelial cells	+++
Human nasopharyngeal epithelial cells	+++
Human nasopharyngeal carcinoma (D562)	++
Human cervical adenocarcinoma (HeLa)	+
Rabbit epidermal cells	++
Rabbit corneal epithelial cells	++
Rabbit kidney-derived epithelial cells	+
Rabbit intestine-derived epithelial cells	+
Rabbit bladder-derived epithelial cells	+
Rabbit trachea-derived epithelial cells	+
Mouse teratomal keratinocyte line (XB2)	++
Rat kangaroo kidney epithelial line (Pt K2)	+
Human diploid skin fibroblasts	−
Rabbit diploid fibroblasts (skin, bladder, etc.)	−
Rabbit skin fibroblast line (NBL-11)	−
Mouse fibroblast line (3T3)	−
Hamster fibroblast line (NIL)	−
Baby hamster kidney fibroblast line (BHK)	−
Rabbit lens epithelial cells	−
Human adrenal cortex adenocarcinoma (SW-13)	−
Mouse melanoma line (M-3)	−
Mouse neuroblastoma line (A2)	−
Rat hepatoma line (H4)	−

in the present study was prepared against human epidermal keratins, known to consist of a group of related components (37, 38). Recently these keratins have been fractionated electrophoretically and antisera have been prepared against single keratins of homogenous molecular weight between 46,000 and 65,000 (ref. 38; A. Vidrich and T.-T. Sun, unpublished data). These keratins, though structurally related, can be distinguished chemically and immunologically (38). When tissue sections and cultured cells of the kinds described above were tested with antisera to the 46,000 and 63,000 molecular weight keratins prepared by Fuchs and Green (38) and to the 55,000 and 65,000 molecular weight keratins prepared by Vidrich and Sun (unpublished), it was found that epithelial cells were stained by these antisera. Nevertheless, in view of the diversity of the crossreacting keratins within epidermal cells and the known differences between those of epidermis and those of cultured keratinocytes (2, 38), it is possible that the different epithelia do not contain identical keratins, even though all may be stained by antisera to single purified keratins.

This work was essentially completed in December 1977. During the past year, several reports from the laboratories of W. Franke, K. Weber, and M. Osborn have described immunofluorescent staining of some cultured cell types—including HeLa, Pt K2, mammary epithelial cells, and human kidney epithelial cells—by guinea pig antisera to cow keratins (11, 39–42). In general our results are in close agreement.

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