

INTERMEDIATE-SIZED FILAMENTS OF THE PREKERATIN TYPE IN MYOEPITHELIAL CELLS

WERNER W. FRANKE, ERIKA SCHMID, CHRISTA FREUDENSTEIN, BEATE APPELHANS, MARY OSBORN, KLAUS WEBER, and THOMAS W. KEENAN

From the Division of Membrane Biology and Biochemistry, Institute of Experimental Pathology, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany; the Max Planck Institute for Biophysical Chemistry, D-3400 Göttingen, Federal Republic of Germany; and the Mammary Biology Laboratory, Department of Animal Sciences, Purdue University, West Lafayette, Indiana 47907

ABSTRACT

Myoepithelial cells from mammary glands, the modified sweat glands of bovine muzzle, and salivary glands have been studied by electron microscopy and by immunofluorescence microscopy in frozen sections in an attempt to further characterize the type of intermediate-sized filaments present in these cells. Electron microscopy has shown that all myoepithelial cells contain extensive meshworks of intermediate-sized (7–11-nm) filaments, many of which are anchored at typical desmosomes or hemidesmosomes. The intermediate-sized filaments are also intimately associated with masses of contractile elements, identified as bundles of typical 5–6-nm microfilaments and with characteristically spaced dense bodies. This organization resembles that described for various smooth muscle cells. In immunofluorescence microscopy, using antibodies specific for the various classes of intermediate-sized filaments, the myoepithelial cells are strongly decorated by antibodies to prekeratin. They are not specifically stained by antibodies to vimentin, which stain mesenchymal cells, nor by antibodies to chick gizzard desmin, which decorate fibrils in smooth muscle Z bands and intercalated disks in skeletal and cardiac muscle of mammals. Myoepithelial cells are also strongly stained by antibodies to actin.

The observations show (*a*) that the epithelial character, as indicated by the presence of intermediate-sized filaments of the prekeratin type, is maintained in the differentiated contractile myoepithelial cell, and (*b*) that desmin and desmin-containing filaments are not generally associated with musclelike cell specialization for contraction but are specific to myogenic differentiation. The data also suggest that in myoepithelial cells prekeratin filaments are arranged—and might function—in a manner similar to the desmin filaments in smooth muscle cells.

KEY WORDS myoepithelial cells · mammary gland · intermediate filaments · keratin · desmin

Myoepithelial cells represent a special anatomical and functional situation that is reflected in their paradoxical name. On the one hand, they are

highly contractile cells, some of which are stimulated by special hormones, and seem to function in a manner similar to true muscle cells. On the other hand, unlike muscle cells, they are not mesenchymal components or derivatives but are true epithelial cells that grow together with other epithelial cells and are permanently separated from the connective tissue by the basal lamina (3, 8; for an extensive review of normal and disordered states of various types of myoepithelial cells see reference 36). Myoepithelial cells have been described in secretory alveoli and in ducts of various glands such as the sweat and odor glands of the skin (for reviews see references 14 and 51), lacrimal glands, the Harderian gland (for references, see reference 9), the various types of salivary glands (59, 72; for a review see reference 80), and mammary glands. In the lactating mammary gland, where they are especially frequent, they have been extensively studied both with respect to their morphology and function, especially the oxytocin-induced contraction important for milk let-down (61, 63, 74), and with respect to their differentiation, maintenance, and changes during lactation and involution (5, 37, 43, 53–57, 69, 75). Myoepithelial cells show an organization reminiscent of that observed in smooth muscle cells. They contain large amounts of actin-containing microfilament bundles, as well as tropomyosin and myosin (2, 12, 15, 30), “dense bodies” (56, 57), and endocytotic vesicles (37, 54–57).

Of particular interest in these cells are the intricate networks of filaments of intermediate thickness (7–11 nm) that morphologically resemble the arrays of the 10-nm filaments described in smooth muscle (56, 57, this study). Such intermediate-sized filaments in smooth muscle cells have been shown to contain one major protein of apparent molecular weight 50,000–55,000 which is called “desmin” (44–47) or “skeletin” (62). This protein has been located in fibril arrays of smooth muscle cells *in situ* and *in vitro* (11, 16, 47, 62), in postmitotic myoblasts and myotubes of skeletal muscle (6, 16), in cultured cardiac muscle cells (44), and in the intercalated disk-associated filaments of isolated cardiac myofibers (47) and frozen sections of heart tissue (cf. reference 25). It has also been reported in subsurface plaques and in the Z lines of skeletal and cardiac muscle (44–47). We and others have recently shown that a great diversity of epithelial cells, including normal and transformed cells in culture, are characterized by extensive arrays of intermediate-sized filaments that contain preker-

atin-like proteins (“cytokeratins”; 19–21, 23, 24, 26–28; cf. references 29, 52, 70, and 71). Also, various epithelial cells growing in culture contain a third type of constitutive filament protein of ~57,000 mol wt (“vimentin,” 25; cf. references 23 and 28). This third type of intermediate-sized filament is the major, if not exclusive, class of filaments found in most nonmuscle mesenchymal cells (24; see also references 6, 25, 27, 34, 38, 40, 49, 66, 68, and 71).

In our study we have been concerned with elucidating the nature of the intermediate-sized filaments in myoepithelial cells, using immunofluorescence microscopy and electron microscopy. We show that the predominant (or exclusive) intermediate-sized filaments present in myoepithelial cells are of the prekeratin type and that desmin filaments are not detected in these cells. These observations show that the myoepithelial cell, although it is functionally analogous to a smooth muscle cell, is the product of a true epithelial differentiation different from myogenic differentiation. Our observations also suggest that keratin-like filaments might function in contractile epithelial cells in a manner similar to that of the desmin-containing filaments in true muscle structures.

MATERIALS AND METHODS

Materials

Lactating mammary gland tissue from rats (Sprague-Dawley) and cows (Holstein) was obtained as previously described (10, 22, 41, 81). Small pieces of internostril skin tissue from the muzzle of freshly killed calves and cows were similarly excised. Small pieces of salivary glands (glandula submandibularis and glandula parotis) and muscle-containing tissue (heart, large leg muscles, small intestine, and uterus wall) were taken from rats. Tissue pieces were either fixed for electron microscopy or directly frozen in isopentane cooled with liquid nitrogen and processed for immunofluorescence microscopy.

Electron Microscopy

Mammary gland tissue was fixed and processed for electron microscopy of ultrathin sections as previously described (22, 81). The other gland-containing tissues were similarly fixed, embedded, and sectioned.

Antibodies

For indirect immunofluorescence microscopy, the sections were treated with one of the following antibody preparations:

(a) Antisera raised in guinea pig against total reconstituted bovine hoof prekeratin (26–28).

(b) Specific antibodies prepared from these sera by immunoaffinity chromatography on prekeratin covalently bound to Sepharose 4B (for method see reference 78; cf. references 20, 24, and 27). The reconstituted prekeratin from bovine hoof used as affinity adsorbent is shown in Fig. 1a.

(c) Antisera against the five major polypeptides of desmosome-associated tonofilaments from bovine muzzle (13) separated by SDS polyacrylamide gel electrophoresis (Fig. 1*b*, slot 1). The protein was eluted from the excised gel slices and used as antigen (for methods, see below). Immunization was essentially as described in references 24 and 28.

(d) Antisera raised in guinea pigs against each of the five major polypeptides of desmosome-attached tonofilaments (for preparation see Fig. 1*b*), which were separated and prepared as previously described (20). The specificity of the antibodies is shown in Fig. 1*c*.

(e) Antisera made in guinea pigs against chicken gizzard desmin were prepared using modifications of the procedures described by Lazarides and Hubbard (47) and Small and Sobieszek (62). Chicken gizzard tissue was washed and minced in physiological saline buffered with 10 mM Tris-HCl, pH 7.2, and then homogenized in 1 mM EDTA, 10 mM Tris-HCl, pH 7.4, using a Polytron homogenizer (Kinematica, Lucerne, Switzerland) before centrifugation for 30 min at 3,500 g in a laboratory centrifuge. The pellet obtained was rehomogenized in the same medium and again centrifuged. The pelleted material was extracted for 1 h in cold (4°C) 1 M KCl, 10 mM Tris-HCl, pH 7.4, with gentle stirring. The pellet obtained after a further centrifugation was resuspended in 0.6 M KI (10 mM Tris-HCl, pH 7.4), briefly homogenized, and extracted with stirring for 60 min. The suspension was then centrifuged, and the pellet was washed by 30-min incubation in 1 mM EDTA, 10 mM Tris-HCl, pH 7.4, and centrifugation. The pellet was resuspended in 10 mM sodium phosphate buffer, pH 7.4, and again centrifuged. The major polypeptides seen in this preparation after electrophoresis on SDS polyacrylamide gels (for method, see reference 28) are shown in Fig. 2 (slots 2 and 7). The major band at a position corresponding to an apparent mol wt of 53,000 was excised using fluorescamine-labeled protein in the outer slots to monitor the exact position. The excised gel pieces were then cut into small (~10-mm³) blocks, incubated in the 50 mM barbital buffer used by Blomberg et al. (7) that contained 0.1% SDS and 1% mercaptoethanol, and eluted overnight with gentle stirring. The eluate was collected first by pipetting and then as the supernate of a 15-min centrifugation at ~3,500 g. The pooled extracts were then filtered through regenerated cellulose (0.45- μ m mesh size; Sartorius, Göttingen, W. Germany). Protein was precipitated by the addition of 9 vol of acetone, kept in the cold for 6–12 h, pelleted, and dried under nitrogen. This protein appeared, on gel electrophoresis, as a pure, homogeneous polypeptide band of 53,000 mol wt (Fig. 2, slot 3). Desmin from porcine uterus smooth muscle tissue, which was similarly extracted and prepared, comigrated on gel electrophoresis with gizzard desmin (Fig. 2, slots 6 and 9; cf. reference 24). Immunization was as described for prekeratin (28). In some experiments, desmin was cross-linked before immunization with 0.1% glutaraldehyde for 20 min at 4°C followed by 24-h dialysis against phosphate-buffered saline (PBS). Antibodies obtained against cross-linked desmin, however, were not different from those obtained from noncross-linked desmin. In particular, desmin antibodies that cross-reacted between avian and mammalian desmin could be produced by either procedure.

Some antisera to gizzard desmin showed specificity for avian desmin similar to the rabbit antibody preparations described by Lazarides and co-workers (44–47) and the group of Holtzer (6, 16). However, in several of our guinea pig antisera to gizzard desmin, we could demonstrate a strong cross-reaction between avian desmin and mammalian desmin by immunodiffusion with desmin purified from chicken gizzard and porcine uterus smooth

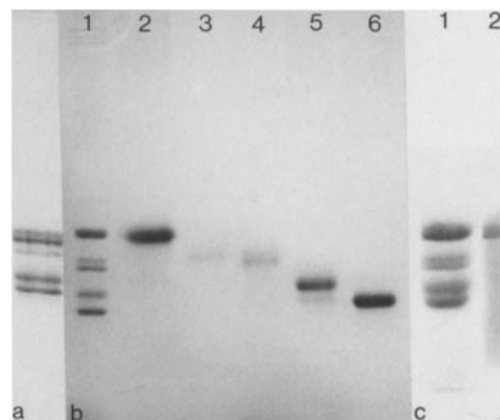


FIGURE 1 SDS polyacrylamide gel electrophoresis of the polypeptides of reconstituted bovine hoof prekeratin as used for immunization in guinea pigs and as affinity adsorbent in the preparation of monospecific antibodies to hoof prekeratin (*a*) and of desmosome-tonofilament protein from bovine muzzle (*b*, slot 1) and individual polypeptides excised therefrom, eluted and reelectrophoresed (*b*, slots 2–6; note that band 4 material, seen in slot 5, contains a minor contamination of band 5 material). *c* shows an immunoreplica that contained different antibody preparations to prekeratin preparations. Antibodies against muzzle prekeratin are of two kinds: antibodies to total prekeratin and antibodies against individual prekeratin polypeptide bands that react with several or all bands (*c*, slot 1); some of the antibody preparations to individual polypeptides react with the specific polypeptide band only. *c*, slot 2, shows the immunoprecipitate obtained with the same material as shown in *c*, slot 1, with an antiserum to the excised and purified material of band 1 (shown in *b*, slot 2).

muscle tissue (not shown here) and, specific for the desmin band, by agarose immunoreplica techniques (Fig. 2, slots 7–11; for technical details of the immunoreplica technique used, see reference 25). The antibodies to desmin did not cross-react in immunoreplica tests with vimentin or any of the prekeratin polypeptides.

Antisera against chicken gizzard desmin used in immunofluorescence microscopy on frozen sections of various tissues of chick, rat, and cow exhibited muscle specificity (Fig. 3). In cardiac tissue, Z-band equivalents were moderately stained, and intercalated disks were strongly stained (Fig. 3*a–c*; cf. reference 47). In cross-striated skeletal muscle, typical Z-line staining (Fig. 3*d–f*) similar to that described in avian tissue by Lazarides and co-workers (45–47) was seen. Smooth muscle in general was strongly decorated, often revealing the longitudinal orientation of most of the desmin-containing fibril structures in the smooth muscle cells (Fig. 3*g* and *h*; cf. reference 47). Thus, the desmin antisera described here confirm the findings of Lazarides and co-workers in avian muscle (44–47), but they also allow examination of mammalian muscle, probably due to a determinant conserved during evolution.

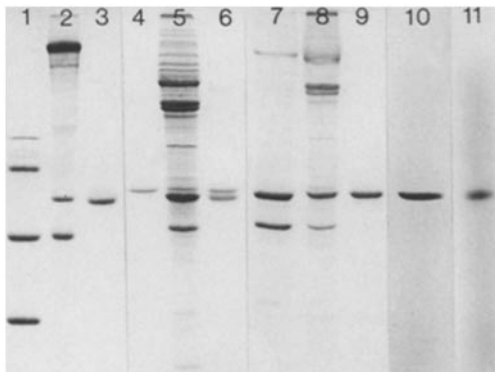


FIGURE 2 SDS polyacrylamide gel electrophoresis of cytoskeletal preparations from chicken gizzard (slots 2 and 7) and porcine uterus smooth muscle tissue (slots 5 and 8) compared with reference proteins (slot 1), purified desmin from chicken gizzard (slot 3) and porcine uterus (slot 9), murine vimentin (slot 4) and a mixture of purified vimentin and porcine desmin (slot 6), and agarose immunoreplicas of gels containing cytoskeletal proteins from chicken gizzard (slot 10) and porcine uterus smooth muscle tissue (slot 11) using guinea pig antisera against chicken gizzard desmin. Reference proteins in slot 1 are, from top to bottom, phosphorylase a, bovine serum albumin, rabbit skeletal muscle actin, and chymotrypsinogen. Note the enrichment of a polypeptide band (apparent mol wt 53,000), i.e., desmin, which is clearly of higher electrophoretic mobility than murine vimentin (apparent mol wt 57,000; slots 4–6; cf. reference 24). Note that some small amounts of vimentin are also found in cytoskeletal preparations from muscle tissue (e.g., slot 5), probably contaminating material from elements of connective tissue or blood vessels (25). Note that cytoskeletal preparations of gizzard and uterine smooth muscle after repeated extractions with high salt buffers still contain a significant amount of actin (39; the preparations shown in slots 7 and 8 have been extracted in high salt buffer one more time than those shown in slots 2 and 5). When the antibodies against chicken gizzard desmin (see Materials and Methods) are used in the agarose immunoreplica technique it can be demonstrated that an immunoprecipitate is formed only with desmin: slots 10 and 11 present the agarose immunoreplicas of slots parallel to and containing the same proteins as slots 7 and 8. The agarose overlay gel has been photographed after extensive washing (4 d) and Coomassie Blue staining (for details of preparation, see reference 25). This demonstrates immunological relatedness of mammalian and avian desmin and suggests, when seen together with published data (6, 16, 44, 45), that desmin contains some sequences highly conserved during evolution, as opposed to others which are different in birds and mammals (45).

(f) Immunoglobulins were prepared from the guinea pig antisera against chicken gizzard desmin described under (e) by ammonium sulfate precipitation followed by exhaustive dialyses against distilled water, sodium acetate buffer (0.05 M sodium acetate adjusted with acetic acid to pH 5.0), and PBS, and passage over diethylamino ethyl cellulose. Monospecific antibodies were obtained by chromatography on desmin covalently bound to CN-Br Sepharose 4B using either of the solutions previously described at low pH (78) or in PBS that contained 4 M MgCl₂. The purified gizzard desmin used as affinity adsorbent is shown in Fig. 4a. Specificity and cross-species reactivity of monospecific antibody preparations were examined by immunoreplica tests and immunofluorescence microscopy (Fig. 4b–f).

(g) Antisera raised in guinea pigs against murine vimentin purified from mouse 3T3 cytoskeletons (24) were used directly, or specific antibodies made from these sera by immunoaffinity chromatography were used (see Fig. 10). Extensive documentation of the specificity and cross-species reactivity has been presented elsewhere (25, 27).

(h) Two different rabbit antibody preparations against actin were used (42, 79), and monospecific antibodies made therefrom were as described (42).

For controls, specific preimmunization sera were used.

Indirect Immunofluorescence Microscopy

Procedures used followed those developed by Lazarides and Weber (48), using modifications described for cryostat sections (cf. references 10, 20, 23, and 24). Briefly, sections cut at temperatures between -20° and -30° C were usually fixed for 30 min in 95% ethanol at 4° C or were air-dried and fixed in acetone at -20° C for 15 min. FITC-labeled goat antibodies against rabbit IgG and rabbit antibodies against guinea pig IgG were used as second antibodies (Miles-Yeda Co., Frankfurt, W. Germany). For control of immunospecificity, antibodies were applied in some experiments in PBS containing 100 mM MgCl₂, followed by two washes in this buffer, before application of the second antibodies. Microscopy was as described (cf. reference 77).

RESULTS

Electron Microscopy

Our electron microscopic observations on myoepithelial cells of lactating mammary gland of rat and cow confirm and extend observations by previous authors (33, 37, 43, 53–57, 61, 69, 74). The central part of the myoepithelial cells, which contains the nucleus, is characteristically located in angular niches interspersed between the basal portions of secretory epithelial cells (a typical position is shown in Fig. 5). Large cytoplasmic extensions project from this region, and the tentaclelike processes of adjacent myoepithelial cells are in intimate contact with one another, and, thus, form a “basket” of myoepithelial cell strands surrounding the entire alveolus (see above references for details). The epithelial layer containing the myoepithelial cells is separated from the mesenchymal space by the basal lamina (Fig. 5a and b), and hemidesmosomes with attached tonofibril-like bundles of

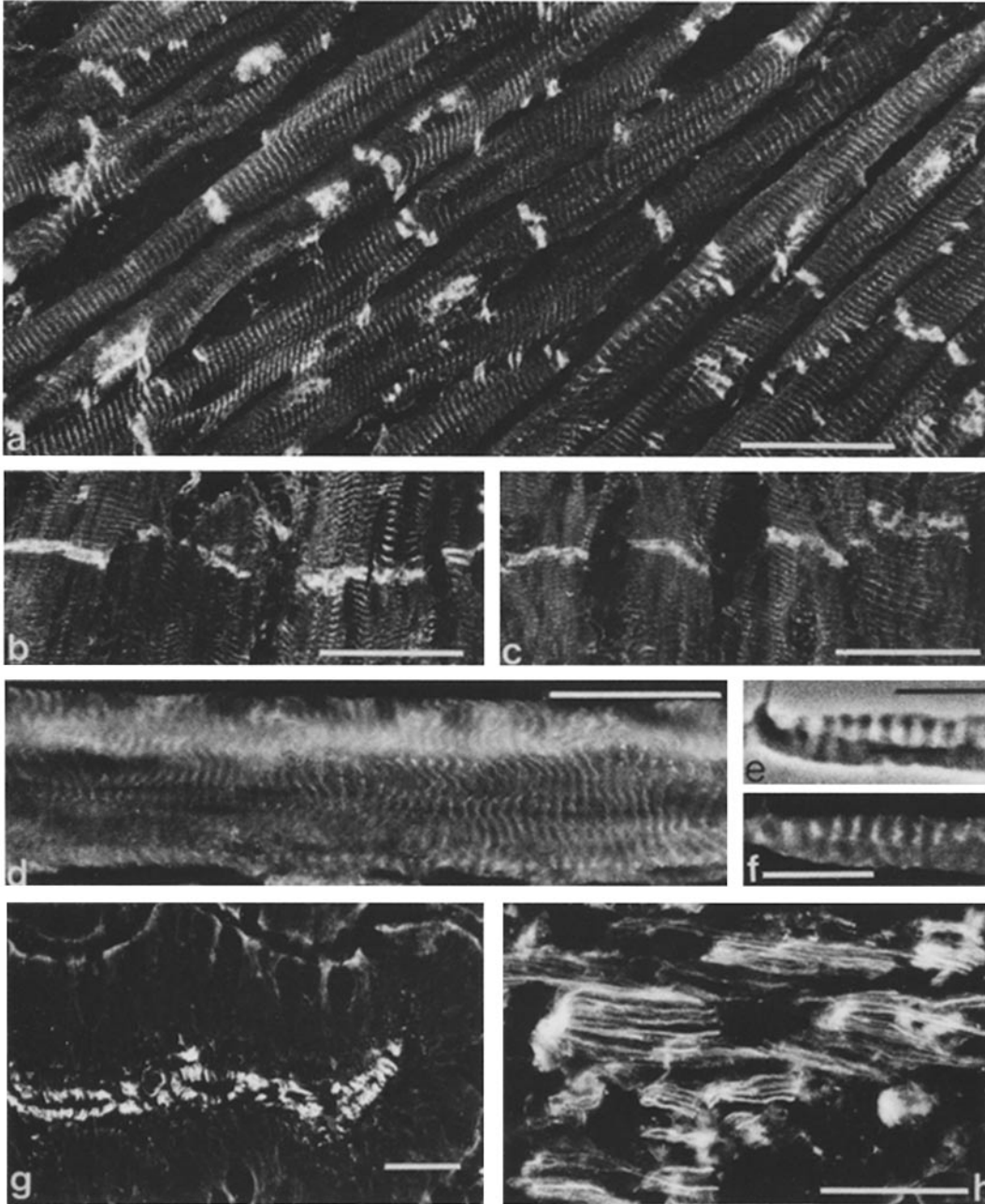


FIGURE 3 Immunofluorescence micrographs of frozen sections of various rat tissues showing the cross-reaction of mammalian and avian desmin by the decoration of specific muscle structures with guinea pig antibodies against chicken gizzard desmin (see text). Sections through cardiac muscle (*a-c*) reveal strong decoration of Z bands and, at especially high intensity, in intercalated disks (*a* presents a survey; *b* and *c* show details of intercalated disks). At higher magnification, intercalated disks sometimes display, in oblique and grazing sections, their composition by small plaques $\sim 0.3 \mu\text{m}$ in diameter (e.g., in the right of *c*). Pronounced decoration of Z lines is also seen in cross-striated leg muscle (*d* presents a survey; *e* and *f* show the same myofibril in phase contrast [*e*] and epifluorescence [*f*] optics in order to facilitate the identification of the localized bands as Z lines). Strong decoration of smooth muscle cells is shown in *g* and *h* in inner portions of the lamina propria of the villi of the small intestine as seen in cross section (*g*) and in mostly longitudinal section (*h*). Bars, $30 \mu\text{m}$ (*a-d*, *g*, and *h*) and $10 \mu\text{m}$ (*e* and *f*).

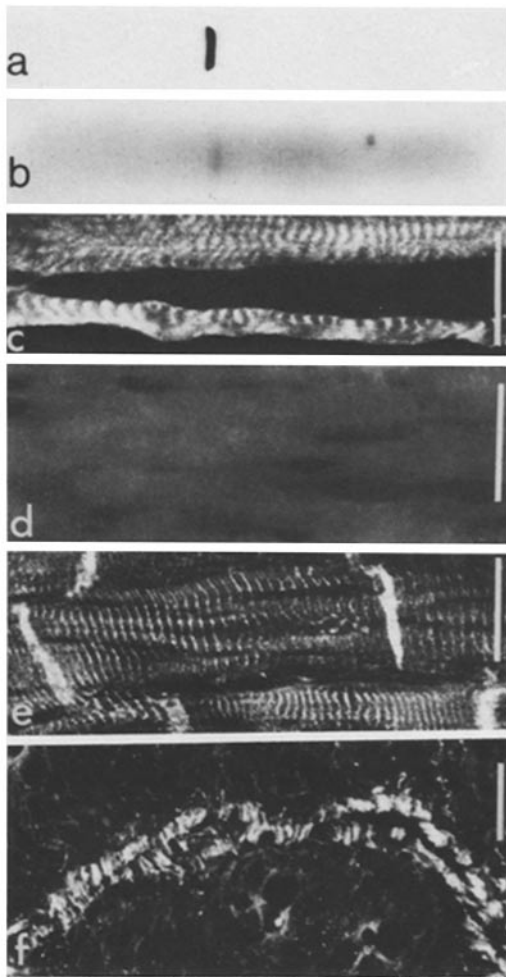


FIGURE 4 Monospecific antibodies to chicken gizzard desmin (*a*, SDS polyacrylamide gel electrophoresis showing the purity of the protein coupled to Sepharose as affinity adsorbent; *b*, immunoreplica showing the cross-reaction of the affinity-purified antibodies to avian desmin with mammalian desmin, using the same porcine uterus smooth muscle material as shown in Fig. 2, slot 8) stain Z lines in frozen sections of rat leg muscle (*c*), which is not seen with monospecific antibodies to total bovine hoof prekeratin (*d*). Monospecific antibodies to desmin also stain intercalated disks and Z lines of rat cardiac muscle (*e*) and smooth muscle cells of the lamina propria of villi of the rat intestine (*f*). Bars, 20 μ m.

intermediate-sized filaments are common (Fig. 5 *b*; cf. references 1, 53–57, 69, 74, and 75). Myoepithelial cells are interconnected by tight junctions, fasciae adhaerentes, gap junctions (not shown here but see references 53 and 69), and by typical desmosomes with attached tonofibrils (Fig. 6 *a*; cf.

references 53–57, 69, and 75). The occurrence of intermyoepithelial desmosomes in fully lactating rat mammary gland alveoli is of special significance because, in this and other rodent species, typical desmosomes with associated tonofibrils are not maintained during lactation (53–59; observed also in this study for the rat; however, see also reference 33), in contrast to other species such as the cow (81). Interestingly, typical symmetric desmosomes are consistently observed between myoepithelial cells and secretory epithelial cells; these desmosomes would, therefore, seem to be an exception to the principle that typical desmosomes disappear in lactating secretory epithelial cells of rodents.

In myoepithelial cells, the occurrence of densely stained and densely fasciated bundles of tonofibrils does not seem to be confined to desmosome-attached tonofibrils but is seen, to variable degrees, throughout the cytoplasm. In the lactating cow, the density of such bundles is often as high as in true keratinizing epithelia (Fig. 6 *c*; cf. reference 81). These bundles of intermediate-sized filaments also extend into the numerous, often extremely attenuated cell processes (Fig. 4 *c*) and may contribute to the mechanical stability of the organization of the basket of normal myoepithelial cells (regarding isolated myoepithelial cells, see also reference 61). They are also found in the basal feelike projections of myoepithelial proliferates present in fibroadenomas (1), in mammary gland involution (43, 56, 57), and in hormone-induced myoepithelial hyperplasia and dedifferentiation (69).

Most of the cytoplasm of myoepithelial cells is occupied by extended masses of densely interwoven filaments, the majority of which seem to be myofilaments, that are oriented parallel to the longitudinal axis of the cells (for a survey, see Fig. 7 *a*). These myofilament masses also extend into the numerous fine cell processes. Within these filamentous aggregates, one recognizes both thin filaments of 5–6-nm diameter, identified as myofilaments that are similar to those observed in, for example, smooth muscle (Fig. 7 *b* and *c*; cf. references 2, 15, 30, 36, 37, 43, 54, 56, and 57), and numerous filaments of 7–11-nm diameter identified as intermediate-sized filaments (Figs. 6 *b* and 7 *b* and *c*). These intermediate filaments, which in their structure and mode of arrangement resemble the 10-nm filaments of smooth muscle, form a loose meshwork and most often show orientations parallel to those of the myofilaments (Fig. 7 *a–c*).

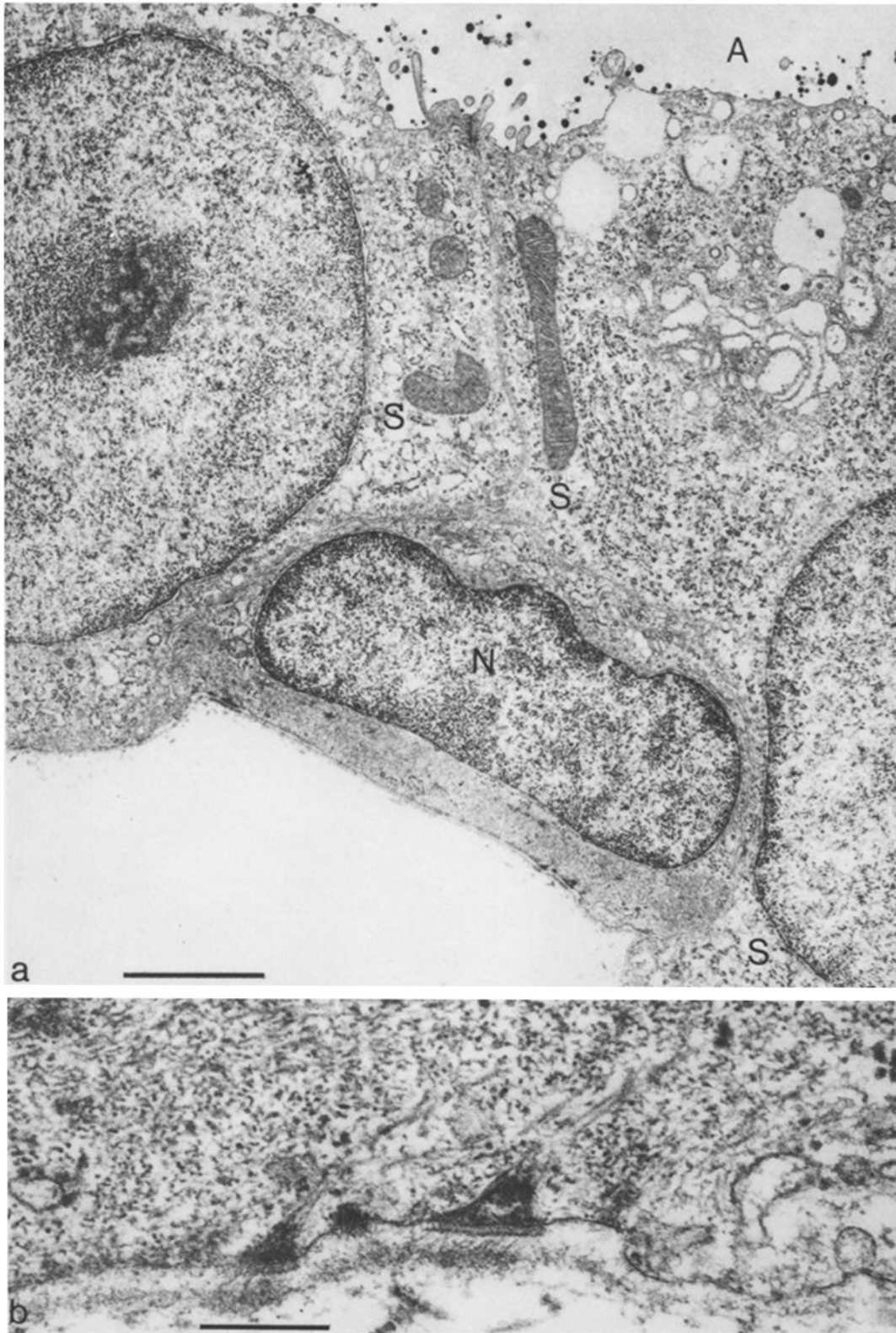


FIGURE 5 Survey electron micrograph (*a*) of an ultrathin section of alveolus of lactating rat mammary gland showing the characteristic location of the central portion of a myoepithelial cell (*N*, nucleus of the myoepithelial cell) inserted in basal niches of adjacent milk secretory cells (*S*). *b* presents, at higher magnification, the high density of cytoplasmic filaments and typical hemidesmosomal plaques with attached intermediate-sized filaments (at the outer plasma membrane bordering on the fuzzy structured basal lamina). *A*, alveolar lumen. Bars, 2 µm (*a*) and 0.2 µm (*b*). *a*, × 11,000; *b*, × 100,000.

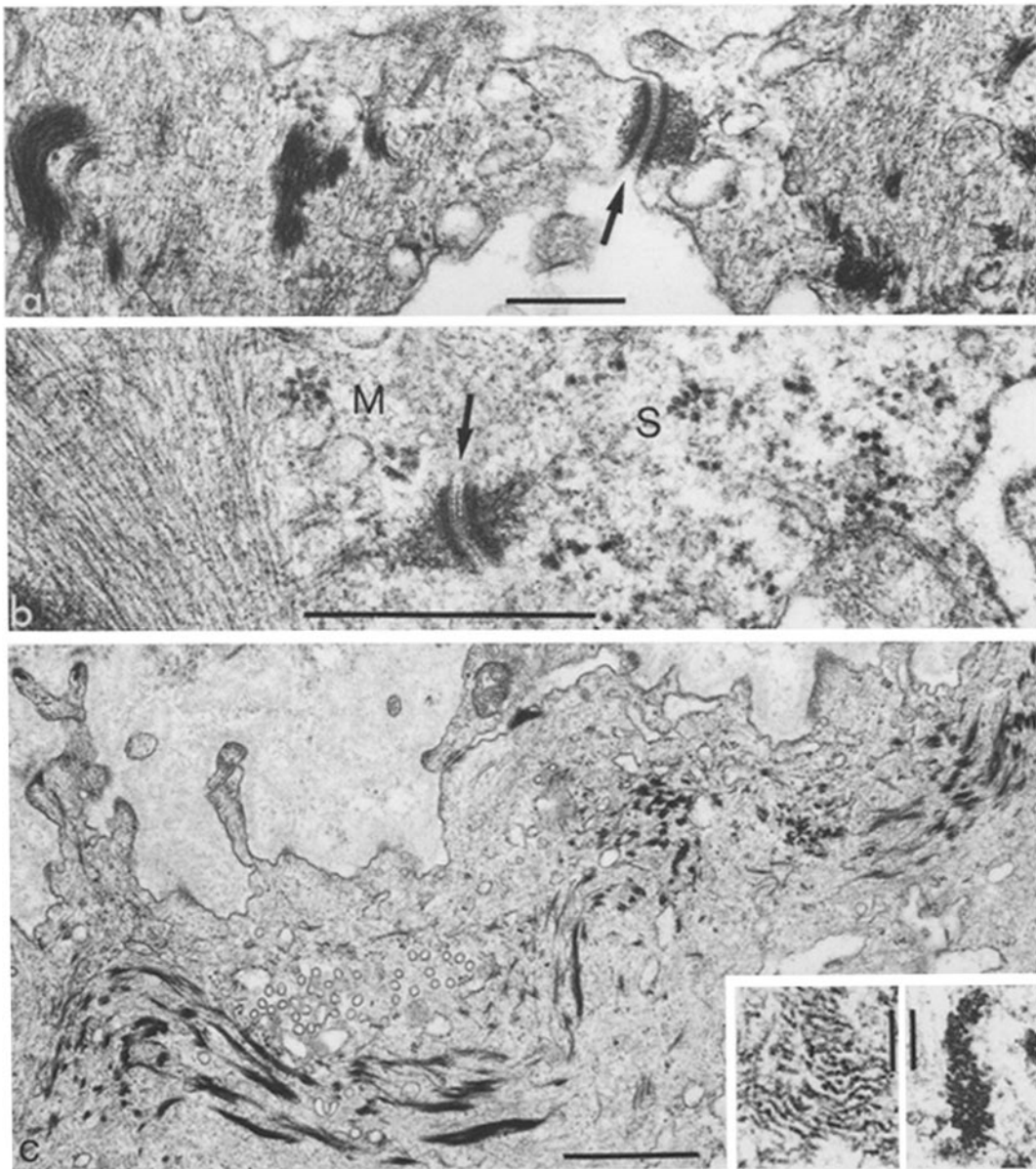


FIGURE 6 Electron micrographs of sections through myoepithelial cells of lactating mammary glands of cow (*a* and *c*) and rat (*b*) showing the abundance of intermediate-sized filaments in these cells, either in association with typical desmosomes between adjacent myoepithelial cells (*a*) and between myoepithelial and milk secretory epithelial cells (*b*) or lying virtually free in the cytoplasm (*a-c*). Besides relatively loosely packed bundles of intermediate-sized filaments (e.g., left part of *b*, left *inset* in *c*), densely stained, tightly packed bundles of filaments, which closely resembled prekeratin bundles of keratinizing epithelia (dense aggregates in *a* and *c*; *inset* in *c*) are frequent in myoepithelial cells, especially in those of bovine udder. Sections obliquely grazing the basal surface of the alveolus (*c*; the upper part of the figure shows basal lamina material) display the high density in cow udder myoepithelial cells of keratinlike filaments, some of which extend into the attenuate processes. Note also the abundance of surface membrane caveolae. Bars, 0.5 μm (*a* and *b*), 1.0 μm (*c*), and 0.1 μm (*insets* in *c*). *a*, $\times 53,000$; *b*, $\times 78,000$; *c*, $\times 18,000$; *insets* in *c*, $\times 80,000$.

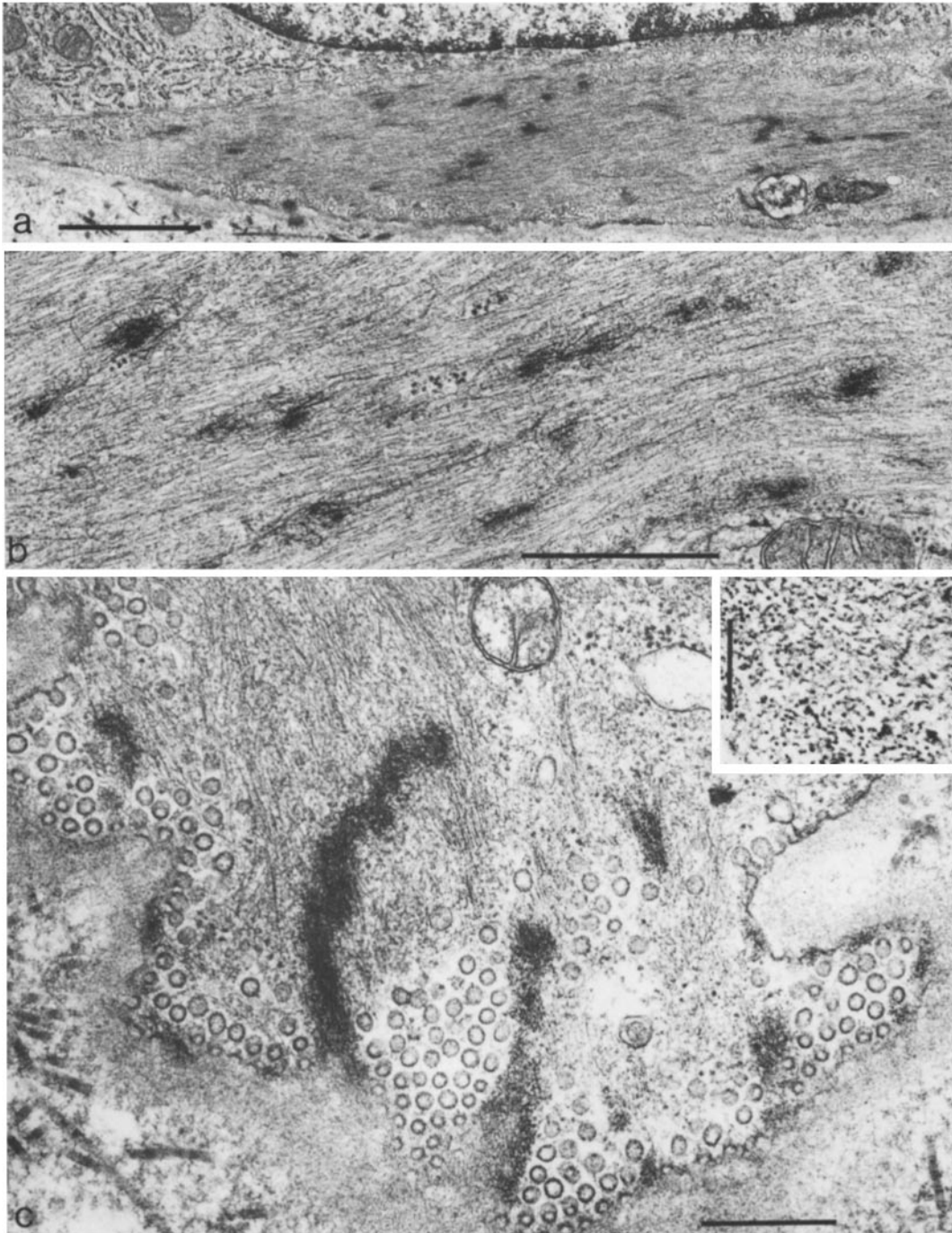


FIGURE 7 Electron micrographs of myoepithelial cells of lactating mammary gland of rat (*a* and *b*) and cow (*c*) showing structural details of cytoplasmic organization that resemble smooth muscle cells. *a* presents a survey illustrating the predominance of filament masses and “dense bodies” (the nucleus in the upper part is in an adjacent secretory epithelial cell). The smooth musclelike architecture of filament bundles and dense bodies is shown at higher magnification in *b*. *c* shows, in a section grazing the basal surface of a myoepithelial cell (basal lamina and collagen fibrils are seen in the bottom part), the connections of some of the dense bodies to the plasma membrane and the high density of surface membrane caveolae and endocytotic vesicles, respectively. The *inset* in *c* illustrates the high density of typical thin myofilaments in myoepithelial cells. Bars, 1 μm (*a*), 0.5 μm (*b* and *c*), and 0.1 μm (*inset* in *c*). *a*, $\times 20,000$; *b*, $\times 58,000$; *c*, $\times 40,000$; *inset* in *c*, $\times 100,000$.

They also seem to converge upon, and be attached to, characteristic and conspicuous dense bodies (Fig. 7; cf. references 37, 43, 56, 57, 69, and 75) that strongly resemble the dense bodies observed in smooth muscle cells (cf. references 11, 60, 62, 64, 76). In the cell periphery, at the side oriented toward the lamina propria, these intermediate-sized filaments often appear to be anchored at specific surface membrane-attached dense bodies (Fig. 7c) or at typical hemidesmosomal plaques (Figs. 5b; see above). At higher magnification, two forms of lateral association of intermediate-sized filaments present in myoepithelial cells can be distinguished, i.e., relatively loosely and very tightly fasciated bundles (for a comparison, see the two inserts in Fig. 6c). The latter form closely resembles typical bundles of prekeratin filaments in keratinizing epithelia (cf. references 19, 28, 50, 51, and 81) and in cultured PtK cells (21, 52). The ultrastructure of the intermediate-sized filaments present in myoepithelial cells is identical to that described for other cells and includes, in cross sections, the presence of an unstained, apparently hollow core (cf. references 13, 19, 21, 38, 40, 50–52, 62, 66–68, and 76).

The periphery of the myoepithelial cells, especially on the side facing the basal lamina, shows numerous, small, apparently uncoated plasma membrane “caveolae” or peripheral vesicles (Fig. 7a and c). This organization, which is often taken as an indication of intensive endocytosis (cf. references 37, 43, 54, 57, 69, and 75), is also well known in endothelial and muscle cells (for references, see references 23, 25, 31, 64, and 73).

In the modified sweat glands of bovine muzzle and in the salivary glands of rats, the organization of filaments, dense bodies, desmosomes, and hemidesmosomes is essentially similar to that described for mammary gland. The ultrastructure of sweat gland myoepithelial cells has been amply described in the literature (for reviews, see references 14 and 51). The specific organization of the modified sweat glands present in bovine muzzle skin differs, however, from that of other sweat glands in that there are very few “clear cells” but large numbers of typical mucoid “dark cells.” The dark cells are characterized by well-developed, ergastoplasm-like stacked cisternae of rough endoplasmic reticulum and masses of large (0.5–3- μ m) secretory vesicles containing an intensely stained, proteinaceous granule. In fact, the overall fine structure of the dark cells of mucoid sweat glands of bovine muzzle closely resembles that described

by Fortney (18) for those of the glabrous skin of the foot and toe pads of the opossum. The ultrastructure of endpieces and duct intercepts of the submaxillary gland of the rat and other mammals has been described in detail in the literature, including special descriptions of the myoepithelial components (32, 59, 72, 80).

Immunofluorescence Microscopy

MAMMARY GLAND: When frozen sections of lactating rat or cow mammary gland are examined by immunofluorescence microscopy using antisera and monospecific antibodies to actin, a strong and preferential staining of the myoepithelial cells is observed (Fig. 8). This abundance of actin in myoepithelial cells, especially in comparison with the adjacent milk secretory cells, is in agreement with previous reports regarding mammary glands and other myoepithelia-containing glands (2, 12, 15, 30). This immunofluorescence microscopy with actin antibodies is a useful technique to specifically stain the myoepithelial cells, which can be traced in both longitudinal (Fig. 8a) and cross (Fig. 8b) sections through gland alveoli. The typical basket organization of the meshwork of myoepithelial cells is best revealed in sections grazing the outer alveolar surface (Fig. 8c). Interestingly, situations have been repeatedly observed in which the pattern of arrangement of myoepithelial cells as seen in cross sections of alveoli is rather regular and seems to correspond in adjacent alveoli (Fig. 8b), an unexpected and, as yet, unexplained detail of alveolar architecture.

Only at higher concentrations of actin antibody, have we seen significant staining in gland epithelial cells, in particular along the lateral walls. The preservation of the milk secretory cells and the accessibility of the immunoglobulins to their intracellular structures have been demonstrated in control experiments routinely performed in our laboratories by immunofluorescent staining of endomembranes with antibodies to cytochrome b_5 (see micrographs in reference 10) and of intracellular secretory products with antibodies to various murine and bovine caseins (not shown here).

Immunofluorescence microscopy using the antisera and monospecific antibodies against murine vimentin (Figs. 9a and b and 10) and galline desmin (Fig. 9c and d) does not show immunospecific staining either of the milk secretory cells or of myoepithelial cells, although it should be emphasized that the antibodies against chicken

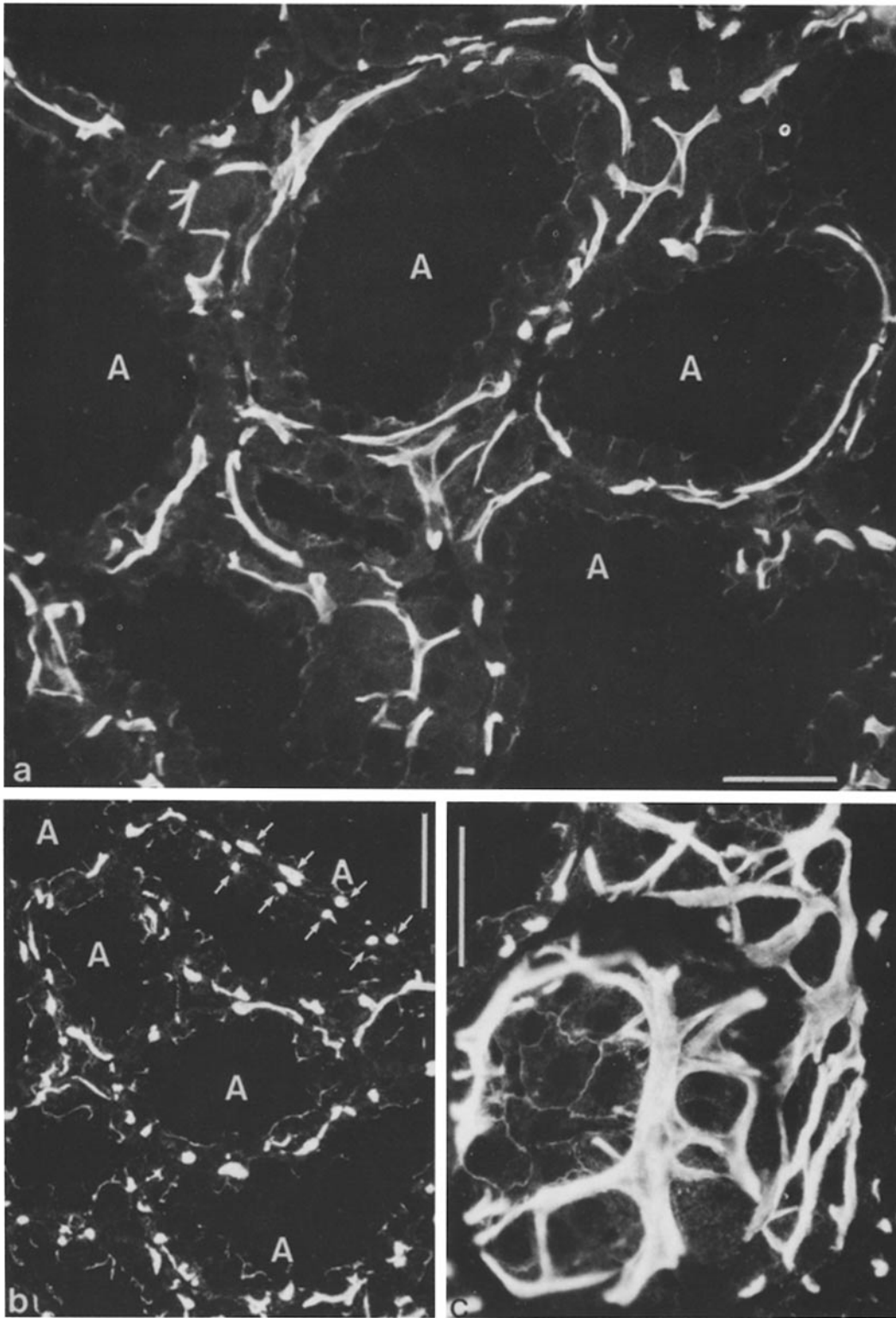


FIGURE 8 Immunofluorescence microscopy of frozen sections through lactating mammary gland of rat (A, alveolar lumina) as revealed after decoration with antibodies to actin. Note the high concentration of actin in myoepithelial cells, as compared to secretory cells, which is especially clear in sections including cell portions that surround a large part of the alveolus (a). The typical location of myoepithelial cells, in particular their central cell body portions, in basal niches of the alveolar epithelium is best seen in cross sections through alveoli (b), which sometimes show a regular spacing of neighboring myoepithelial cells (arrows in b). The basketlike overall arrangement of the myoepithelial cells, with their many branches and tentaclelike processes, is best seen in sections grazing the outer alveolar surface (c). The same staining is obtained with the monospecific antibodies to actin described by Jockusch et al. (42). Bars, 30 μ m.

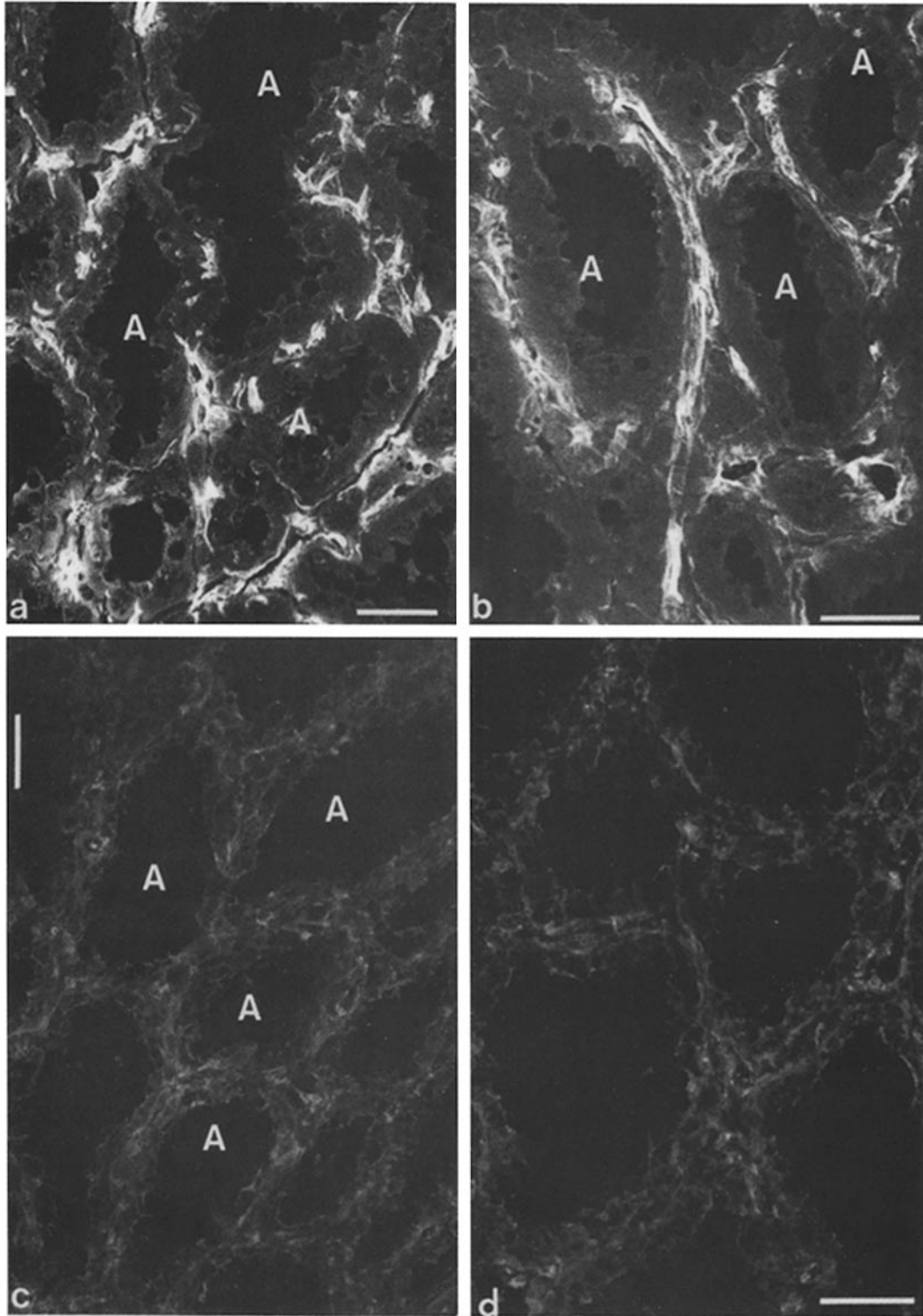


FIGURE 9 Immunofluorescence microscopy of frozen sections of lactating rat mammary gland (same as in Fig. 8) as revealed after reaction with antibodies to vimentin (*a* and *b*) and desmin (*c* and *d*). The antibodies to vimentin do not stain secretory epithelial and myoepithelial cells but do intensely decorate fibroblasts, capillary endothelial cells, and other cells of the mesenchymal space (*a* shows most fibroblasts in cross section; *b* shows most fibroblasts oriented in the section in parallel to the cell axes). Antisera (*c*) and monospecific antibody preparations (*d*) against desmin, which intensely stain muscle cell structures (cf. Fig. 4), do not significantly decorate any of the epithelial and mesenchymal cell types contained in this gland tissue. Bars, 30 μ m.

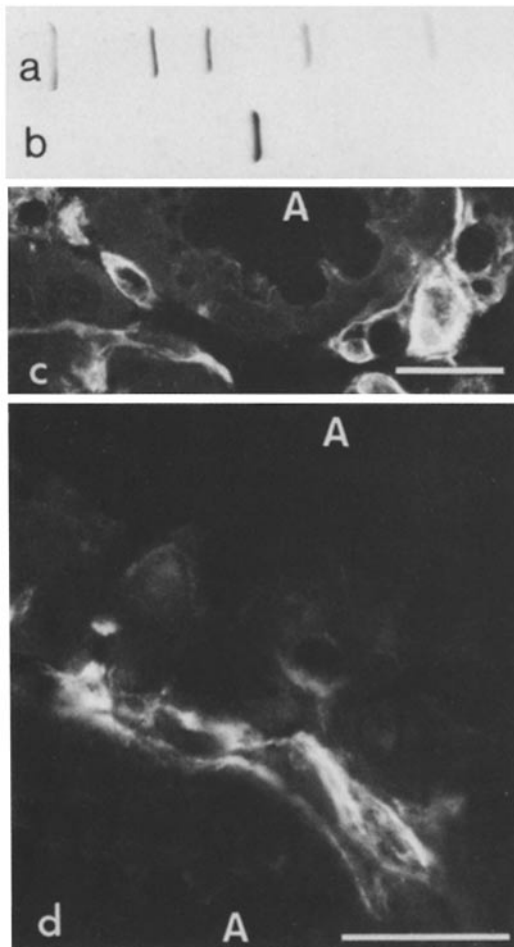


FIGURE 10 Immunofluorescence microscopy of frozen sections of lactating rat mammary gland (A, alveolar lumina) using monospecific antibodies to murine vimentin (a, reference proteins in SDS polyacrylamide gel electrophoresis, from left: myosin heavy chain, phosphorylase a, bovine serum albumin, actin; b, purified vimentin from mouse 3T3 cytoskeletons used as affinity adsorbent in the preparation of monospecific antivimentin). Specific staining is seen only in cells of the mesenchymal space (c and d), which can appear either somewhat dilated (c) or tightly interposed between adjacent alveoli (d). Note the absence of staining in epithelial cells. Bars, 20 μ m.

gizzard desmin used here show strong positive staining of mammalian muscle structures (Figs. 3 and 4). The antibodies to vimentin specifically decorate, however, cells of the lamina propria of the alveoli, including fibroblasts and capillary endothelial cells (Figs. 9 a and b and 10 c and d). An indistinct background staining of myoepithelial

cells was observed with some of the vimentin antibodies (e.g., preparation GP_{4M} as described in reference 27) but was considered not to reflect immunospecific antibody binding because it was selectively diminished when incubations were performed at elevated ionic strength (PBS, 100 mM MgCl₂; see Materials and Methods).

When frozen sections of rat mammary gland are examined by immunofluorescence microscopy using antibodies against prekeratin (that is, with any of the antibody preparations described under a-d in Materials and Methods), a strong and rather selective decoration of the myoepithelial cells is found (Fig. 11). The very weak reaction in the milk secretory cells is in agreement with electron microscopic observations showing an absence of bundles of cytokeratin-like filaments and of desmosome-attached tonofibrils in the milk secretory cells of rodents (see above), which makes lactating rat and mouse mammary gland an especially useful object for demonstrating the specificity of the prekeratin-richness of the myoepithelial cells. The typical arrangements of the myoepithelial cells in interconnected meshworks surrounding the alveolar bases can be seen in Fig. 12 a and b, which presents different aspects of grazing sections to the alveolar surface, i.e., sections which include relatively large portions of such cells. At higher magnification, such grazing sections sometimes reveal densely packed fibrils containing prekeratin-like material that seem to run through the whole body of the cell forming either a meshwork structure or orienting themselves parallel to the longitudinal axis of the cell.

When frozen sections of bovine udder are examined by immunofluorescence microscopy with the antibodies mentioned above, an essentially similar result is obtained. Myoepithelial cells of udder are not specifically stained with antibodies to vimentin and desmin but are strongly decorated by antibodies to actin and prekeratin (Fig. 12 c-f). In contrast to the mammary gland tissue of rodents, however, the staining of milk secretory cells is usually more intense, and sometimes individual alveoli can be found that show a moderate positive staining of all epithelial cells with antibodies to prekeratin. This observation by immunofluorescence microscopy is in agreement with the finding that milk secretory cells to cow's udder not only maintain conspicuous desmosome-tonofibril complexes during lactation but also can show accumulation of filaments of prekeratin-like appearance, together with occasional structures resem-

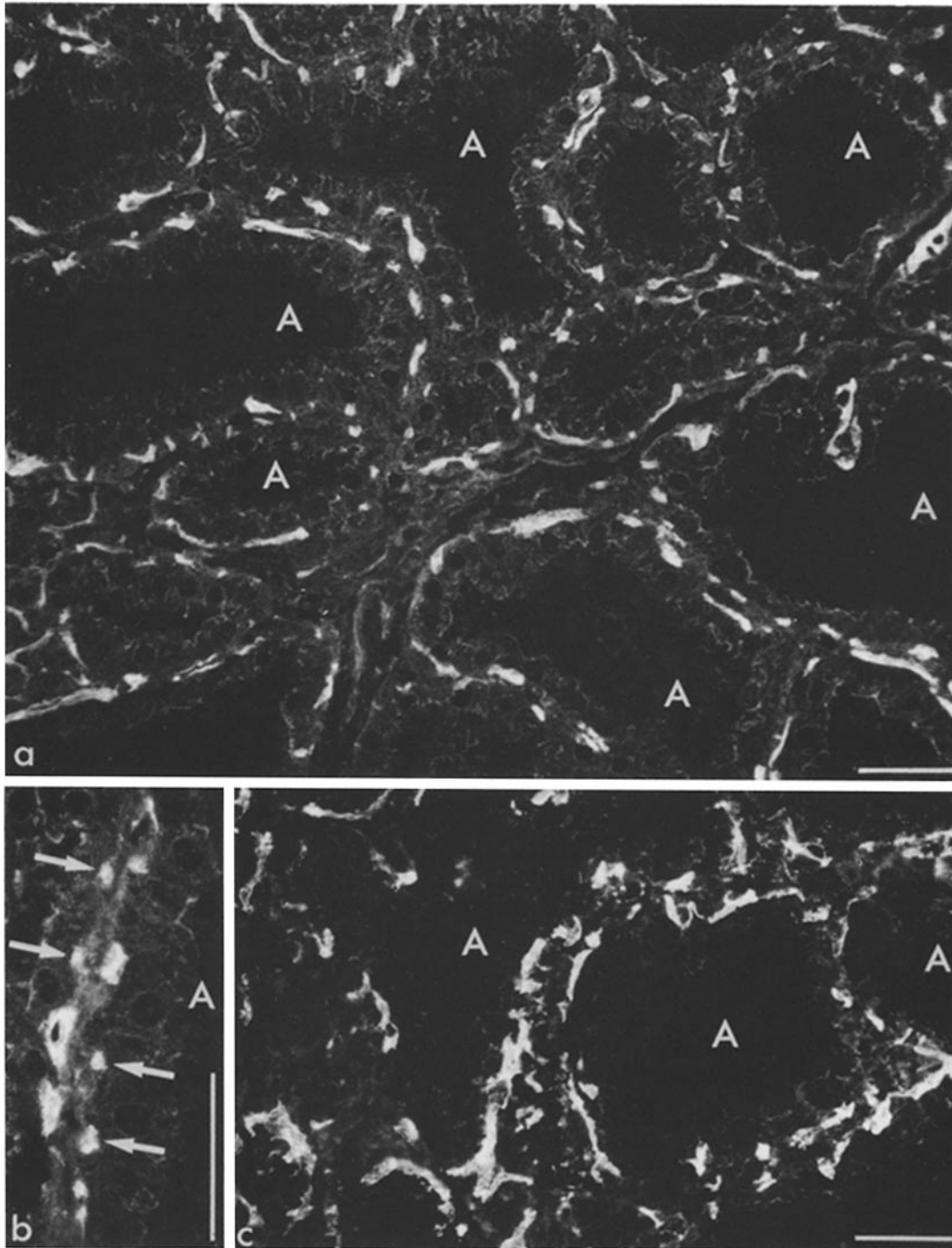


FIGURE 11 Immunofluorescence microscopy of lactating rat mammary gland (same preparation as in Figs. 8 and 9) showing the strong decoration of myoepithelial cells with antibodies to bovine hoof prekeratin when examined with total antisera against prekeratin (*a* and *b*) and specific antibodies to prekeratin prepared from IgG fraction by immunoaffinity chromatography (*c*; for details, see text). The typical regular spacing of central cell portions of myoepithelial cells is seen at higher magnification in *b* (arrows). The milk secretory epithelial cells of rat show only a very weak reaction, the significance of which is not clear when compared with the nonspecific background staining seen with control sera. Bars, 30 μ m.

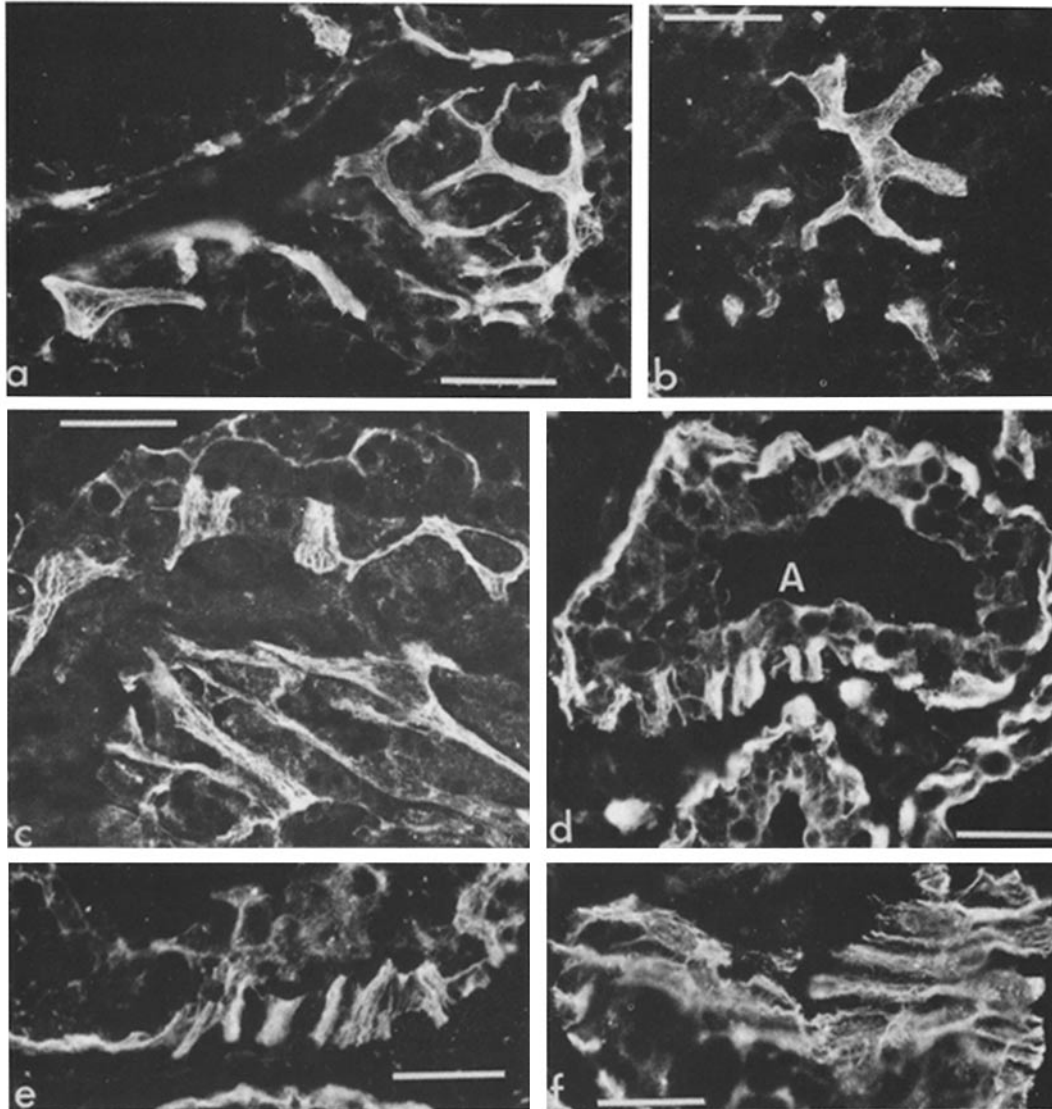


FIGURE 12 Details of the reaction of antibodies to total prekeratin from bovine hoof (*a-c*) and polypeptide components IV (*d*) and I from desmosome-attached tonofilaments of bovine muzzle (*e* and *f*) on frozen sections of lactating mammary gland of rat (*a* and *b*) and cow (*c-f*) as seen by immunofluorescence microscopy. *a* and *b* show various forms of branching of myoepithelial cells and the intercellular connection of myoepithelial cells included in sections grazing outer alveolar surfaces, sometimes displaying the typical basket meshworks of the interconnected "myoepithelium" (*a* and *b*). Occasionally, distinct fibrillar arrays decorated by prekeratin antibodies can be recognized in "flat-sectioned" myoepithelial cells (*a* and *b*). Note that, in the bovine tissue, in addition to the strong staining of the myoepithelial cells, some moderate staining is sometimes seen in milk secretory cells, especially in the lateral cell cortices. *e* and *f* show the positive reaction of myoepithelial cells to antibodies to prekeratin polypeptide component I seen in cross (*e*) and grazing (*f*) sections. Bars, 20 μ m.

bling keratohyalin granules, indicative of some "keratinization" in this species, even in fully active milk secretory cells (81).

Although similar intensities of decoration of

myoepithelial cells have been seen with all the various prekeratin antibody preparations described in Materials and Methods (see also Fig. 1 *a-c*), the positive decoration of myoepithelial

cells with antisera raised specifically against the purified band I polypeptides from desmosome-attached tonofilaments of bovine muzzle (for preparation, see Fig. 1 *b* and *c*) is especially noteworthy (Fig. 12 *e* and *f*). In contrast to antibodies raised against the other four polypeptides, antibodies to component I, which do not cross-react with the other four polypeptides in immunoreplica tests (Fig. 1 *c*, slot 2), stain epidermal stratum spinosum and myoepithelial cells of alveoli and ducts of some glands (e.g., serous endpieces of salivary glands) but not of others (e.g., eccrine sweat glands of bovine muzzle). Antibodies to component I also do not significantly decorate the extended cyto-keratin fibril systems present in various cultured epithelial cells, including PtK and HeLa cells (cf. reference 20).

SWEAT GLANDS: When sweat glands and modified mucoid sweat glands of bovine muzzle are examined by immunofluorescence microscopy using the antibodies described above, results essentially the same as those for mammary gland are obtained. Myoepithelial cells in alveoli and ducts of modified sweat glands are strongly decorated by antibodies to actin (data not shown) and prekeratin (Fig. 13) but not by antibodies to desmin and vimentin (not shown). Besides myoepithelial cells, secretory cells, duct epithelial cells, and the epithelial cells of sebaceous glands also show a significant, though much less intensive, decoration by antibodies to prekeratin (Fig. 13 *a-c*). In cross sections of sweat gland alveoli, the distribution of myoepithelial cells again often displays a somewhat regular spacing of their central cell portions (Fig. 13 *b*). Sections across alveoli that include large portions of myoepithelial cells (Fig. 13 *c*) and sections grazing alveolar surfaces (Fig. 13 *d*) often reveal the fibrillar suborganization of the prekeratin-positive material, which is usually oriented parallel to the myofibrillar structures.

SALIVARY GLANDS: Myoepithelial cells of the various salivary glands give identical results. In Figs. 14 and 15, immunofluorescence micrographs of one representative, the mid-portion of the submandibular gland of the rat, are shown. Fig. 14 gives a survey of this part, illustrating the density of myoepithelial cells seen after reaction with antibodies to bovine prekeratin. The regular distribution of myoepithelial elements (Fig. 14 *b*) and the branches of their cellular processes, which are often in contact with those of adjacent myoepithelial cells and thus form the typical basket arrays characteristic of this gland, are shown at higher

magnification in Fig. 14 *b-f* (for the typical anatomy of the myoepithelial basket of terminal portions of salivary glands, see references 3, 8, 32, 59, 72, and 80). Not infrequently, the cellular branches and processes can be relatively thick, sometimes almost as thick as the central, i.e., nucleus-containing, portion (Fig. 14 *f*). Higher magnification sometimes reveals arrays of fibrils decorated by prekeratin antibodies that are in an intricate three-dimensional meshwork (Fig. 14 *g*) or form parallel fibrils (Fig. 14 *h*).

Intercalated pieces and ducts of salivary glands also contain myoepithelial cells, though they are comparatively widely spaced. The cytoplasm of these cells is again strongly decorated by antibodies to actin (not shown) and prekeratin (Fig. 15 *a-c*) but not by antibodies to desmin (Fig. 15 *d*) and vimentin (not shown here). The duct epithelial cells are also moderately well stained with antibodies to prekeratin, usually along their lateral walls but, in contrast to the myoepithelial cells, do not display internal fibrillar organization when observed at higher magnification (Fig. 15 *a-c*). Again, an indistinct moderate staining of myoepithelial cells of this tissue was observed with some preparations of vimentin antibodies. But this was found to be greatly reduced in the high-salt controls for immunospecificity (see above) and was therefore considered not to be immunospecific.

DISCUSSION

Epithelial cells, including normal and transformed cells in culture, contain—or have the potential to form in certain development stages—desmosomes with attached tonofilaments as well as bundles of intermediate-sized filaments lying virtually free in the cytoplasm. The predominant and characteristic type of intermediate-sized filament in epithelial cells can be identified by its polypeptide pattern and by its reaction with antibodies to prekeratin (23, 24, 26–29; cf. reference 71). To emphasize differences in occurrence and possibly also in function in cells which are not committed to keratinization (26, 28), we have recently proposed the term “cytokeratin” for these prekeratin-like components which appear widely in the epithelial cells of diverse vertebrates. It is not yet clear whether maculae adhaerentes and prekeratin-like filaments are strictly limited to epithelial cells because similar structures have also been reported in cardiac muscle (for references, see references 4, 17, and 65) and in myocytes of beating chicken embryo “mini hearts” growing *in vitro* (35). However, with

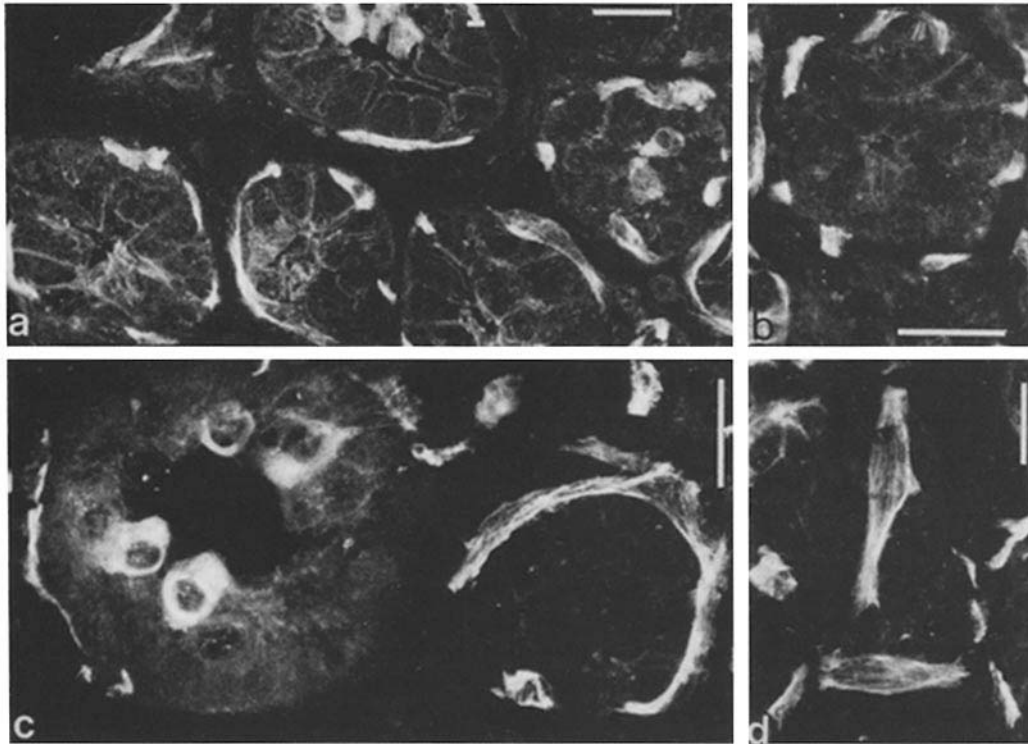


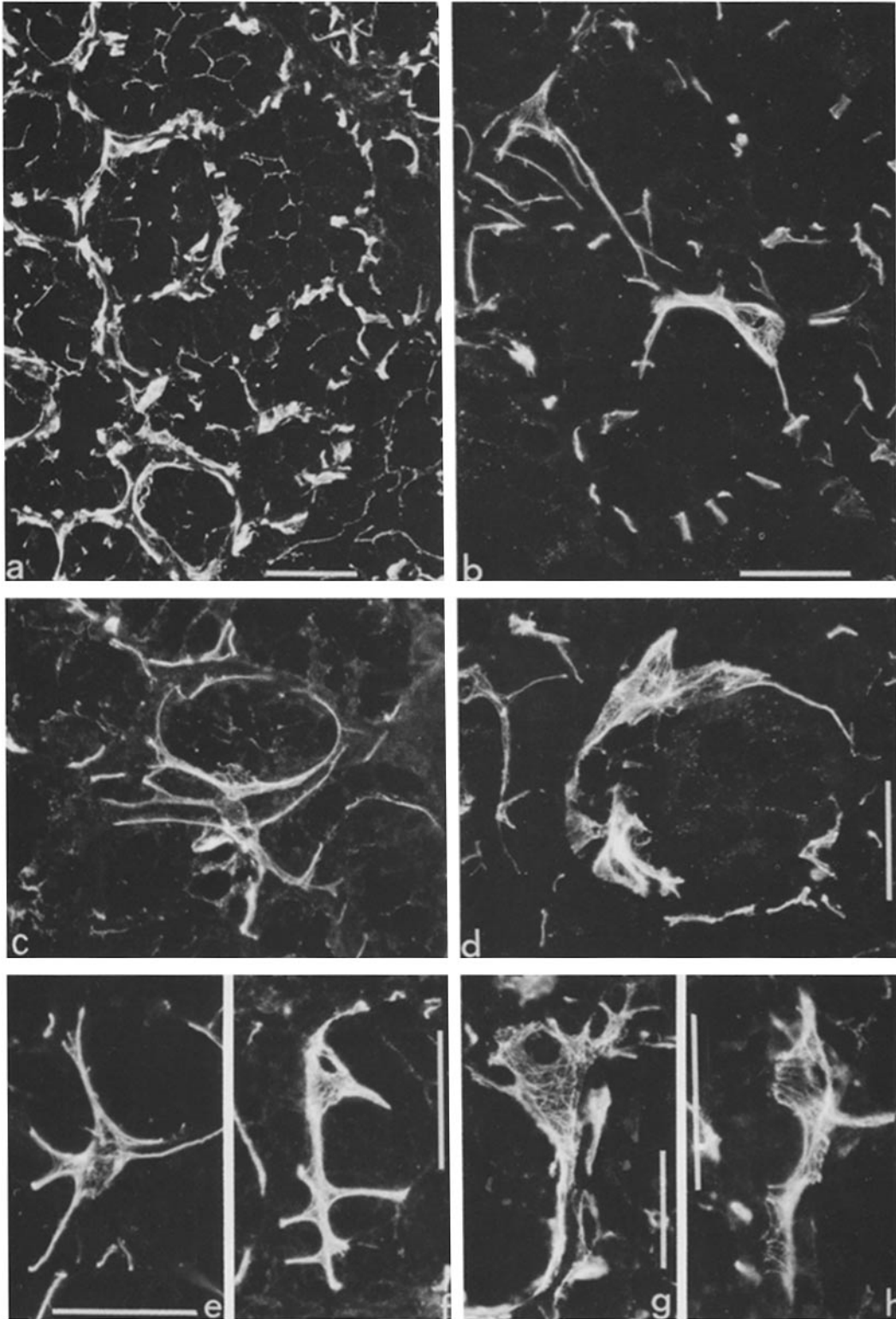
FIGURE 13 Immunofluorescence microscopy of frozen sections through packages of modified sweat glands of bovine muzzle tissue after decoration with antibodies to bovine hoof prekeratin. *a* presents a survey of several terminal gland regions containing only mucoid dark cells. Note the intense and selective staining of the myoepithelial cells that is seen in a cross-sectioned alveolus in *b*, also demonstrating the rather regular spacing of myoepithelial cell portions along the alveolar circumference. *c* and *d* show intracellular fibril arrays in myoepithelial cells contained in a cross section of an alveolus (right part of *c*) and in a grazing section of the outer alveolar surface (*d*). Duct epithelia show occasional cells in which masses of keratinlike fibril bundles are accumulated. Such cells can be identified by electron microscopy and by immunofluorescence microscopy using antibodies to prekeratin (left part of *c*). Bars, 20 μ m.

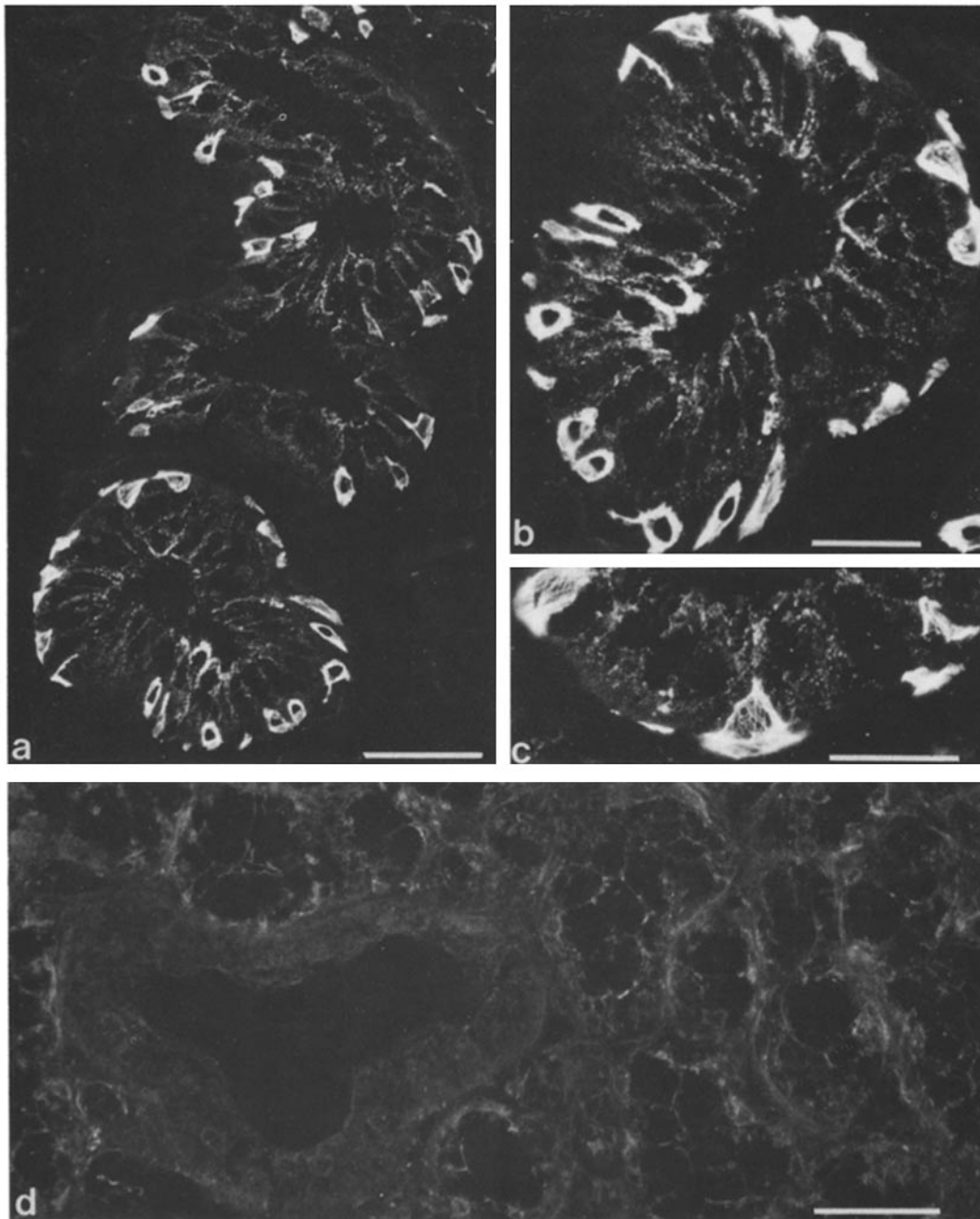
immunofluorescence microscopy using antibodies to prekeratin, we have been unable to detect cytokeratin filaments or other material cross-reacting with prekeratin in myocardium, skeletal muscle, smooth muscle, or a variety of muscle-derived cells growing in culture, including myoblasts (24; W. W. Franke and E. Schmid, unpublished data). Thus, the occurrence of typical desmosome-tonofibril complexes and the presence of a massive meshwork of cytoplasmic 7–11-nm filaments decoratable with antibodies to prekeratin in the myoepithelial cells of the three types of glands described here identify these cells as true epithelial cells and distinguish them from smooth muscle cells, which they resemble very closely in their organization and contractile function (see above).

Although our data show clearly that the cytokeratin filaments are the predominant type of

intermediate-sized filaments in myoepithelial cells, we cannot exclude the presence of small amounts of other filament proteins such as desmin and vimentin, which might escape detection because they are present in amounts below the sensitivity of the method. Likewise, we cannot rule out the possibility that some of these proteins are present in a non-filamentous form not detected by the immunofluorescence procedure used. Desmin, for example, is a component of identifiable filaments in smooth muscle cells and differentiating cardiac and skeletal muscle cells but is contained in Z-line structures in mature skeletal and cardiac muscle (44–47) in which intermediate-sized filaments cannot be resolved (for references on exceptions in abnormal situations, see reference 4).

The studies on differentiated myoepithelial cells also demonstrate that the potential to form a mas-





FIGURES 14 and 15 Immunofluorescence microscopy of submandibular salivary gland of rat (frozen section from the midportion) after reaction with antibodies to prekeratin (Figs. 14 and 15 *a-c*) and desmin (15 *d*) showing the decoration of myoepithelial cells of terminal gland portions (Fig. 14) and ducts (15 *a-c*) by prekeratin antibodies but not by antibodies to desmin (15 *d*). Survey micrographs illustrate the high frequency of myoepithelial cells in this gland (14 *a*) and often reveal, especially in cross sections through endpiece alveoli, a rather regular distribution of the myoepithelial cells (14 *b*). Some cross sections include major portions of one or several myoepithelial cells that surround the specific alveolar intercept in a ringlike fashion (14 *c* and *d*). Typical patterns of branching cell processes (14 *e* and *f*) and intracellular meshworklike or parallel arrays of cytokeratin fibrils (14 *g* and *h*) are also shown. The myoepithelial cells of ducts (15 *a-c*) are somewhat more widely spaced (note intracellular fibril meshwork in duct myoepithelial cells at higher magnification in 15 *c*). In general, duct epithelial cells (15 *a-c*) show a greater reaction with prekeratin antibodies, especially along the lateral cell walls, than secretory epithelial cells of terminal gland portions (14 *a-h*). Bars, 30 μm (14 *a* and 15 *a*) and 20 μm .

sive contractile apparatus and to specialize in contractile functions is not limited to nonepithelial cells and to mesodermal derivatives, including the various true muscle cells. Localized accumulations of contractile structures containing actin and myosin have been reported in some epithelial cells and in various other cells (12, 23, 30, 58, 60, 77; see these references for further references). In some of these situations, the occurrence of intermediate-sized filaments, putatively of a tonofilament-like character, in association with material rich in actin and myosin, has also been noted (20, 58). The situation in myoepithelial cells appears to be different, however, because here most of the cytoplasm is filled with the contractile material and the cells also contain large masses of prekeratin-containing filaments arranged in a cytoskeletal framework interspaced between the contractile filaments and the dense bodies. This structural framework of intermediate-sized filaments, although similar in ultrastructure to that present in smooth muscle cells, is composed of cytokeratins and not of desmin (for references on 10-nm filaments in muscle and on desmin, see the introduction). The mode of organization and the massive increase not only in the thin filaments (thought to be major components of the contractile apparatus) but also in the prekeratin-like intermediate filaments during myoepithelial differentiation (for example, during late gestation in mammary gland; cf. references 56 and 57) suggest that the function exerted by desmin filaments in smooth muscle is performed by intermediate-sized filaments made up of prekeratin-like proteins in myoepithelial cells. Interestingly, the cytokeratin filaments of myoepithelial cells frequently show terminal anchoring at the desmosomes and hemidesmosomes.

The observations on myoepithelial cells described here leave one with an intriguing situation with regard to the compositional and functional relationships of intermediate-sized filaments in various cells. The filaments of the vimentin, desmin, cytokeratin, and neurofilament types are distinguishable by several biochemical and immunological criteria. Yet similar structures seem to be formed in smooth muscle and myoepithelial cells by different protein subunits, i.e., desmin on the one hand and prekeratin-like proteins on the other. This suggests that these proteins may represent the analogous or homologous evolutionary development of molecules to serve at least some similar functions. Perhaps the study of very primitive contractile epithelia such as those present in

hydrozoa might help in clarifying the origin and the relationship of such structures and molecules.

We thank Mrs. C. Grund and Mr. K. Mähler for their careful technical assistance. We are indebted to Dr. B. M. Jokusch, European Molecular Biology Laboratory, Heidelberg, for the gift of her monospecific antiactin preparation and for her advice on the immunoaffinity chromatography of antibodies.

Received for publication 15 March 1979, and in revised form 19 September 1979.

REFERENCES

- AHMED, A. 1974. The myoepithelium in human breast fibroadenoma. *J. Pathol.* **114**:135-137.
- ARCHER, F. L., and V. C. Y. KAO. 1968. Immunohistochemical identification of actomyosin in myoepithelium of human tissues. *Lab. Invest.* **18**:669-674.
- BARGMANN, W. 1977. Histologie und mikroskopische Anatomie des Menschen. Georg Thieme Verlag, Stuttgart.
- BEHRENDT, H. 1977. Effect of anabolic steroids on rat heart muscle cells. I. Intermediate filaments. *Cell Tissue Res.* **190**:303-315.
- BENNETT, D. C., L. A. PEACHY, M. DURBIN, and P. S. RUDLAND. 1978. A possible mammary stem line. *Cell.* **15**:283-298.
- BENNETT, G. S., S. A. FELLINI, J. M. CROOP, J. J. OTTO, J. BRYAN, and H. HOLTZER. 1978. Differences among 100 Å-filament subunits from different cell types. *Proc. Natl. Acad. Sci. U. S. A.* **73**:4364-4368.
- BLOMBERG, F., R. S. COHEN, and P. SIEKEVITZ. 1977. The structure of postsynaptic densities isolated from dog cerebral cortex. II. Characterization and arrangement of some of the major proteins within the structure. *J. Cell Biol.* **74**:204-225.
- BLOOM, W., and D. W. FAWCETT. 1975. A Textbook of Histology. W. B. Saunders Co., Philadelphia.
- BROWNSCHIEDLE, C. M., and R. J. NIEWENHUS. 1978. Ultrastructure of the Harderian gland in male albino rats. *Anat. Rec.* **190**:735-754.
- BRUDER, G., A. FINK, and J. D. JARASCH. 1978. The b-type cytochrome in endoplasmic reticulum of mammary gland epithelium and milk fat globule membranes consists of two components: cytochrome b_5 and cytochrome P-420. *Exp. Cell Res.* **117**:207-218.
- COOKE, P. 1976. A filamentous cytoskeleton in vertebrate smooth muscle fibers. *J. Cell Biol.* **68**:539-556.
- DRENNCKHAN, D., U. GRÖSCHEL-STEWART, and K. UNSICKER. 1977. Immunofluorescence microscopic demonstration of myosin and actin in salivary glands and exocrine pancreas of the rat. *Cell Tissue Res.* **183**:273-279.
- DROCHMANS, P., C. FREUDENSTEIN, J. -C. WANSON, L. LAURENT, T. W. KEENAN, J. STADLER, R. LELOUP, and W. W. FRANKE. 1978. Structure of desmosomes and tonofilaments isolated from calf muzzle epidermis. *J. Cell Biol.* **79**:427-443.
- ELLIS, R. A. 1967. Eccrine, sebaceous, and apocrine glands. In *Ultrastructure of Normal and Abnormal Skin*, A. S. Zelikson, editor. Lea & Febiger, Philadelphia. 132-162.
- EUSEBI, V., A. PICH, E. MACCHIORLATTI, and G. BUSSOLATI. 1977. Morpho-functional differentiation in lobular carcinoma of the breast. *Histopathology (Oxf.)*. **1**:301-314.
- FELLINI, S., G. BENNETT, and H. HOLTZER. 1978. Selective binding of antibody against gizzard 10-nm filaments to different cell types in myogenic cultures. *Am. J. Anat.* **153**:451-457.
- FÖRBE, M. S., and N. SPERELAKIS. 1975. The "imaged-desmosome": A component of intercalated discs in embryonic guinea pig myocardium. *Anat. Rec.* **183**:243-258.
- FORTNEY, J. A. 1973. Cytology of eccrine sweat glands in the opossum. *Am. J. Anat.* **136**:205-220.
- FRANKE, W. W. 1971. Relationship of nuclear membranes with filaments and microtubules. *Protoplasma.* **73**:263-292.
- FRANKE, W. W., B. APPELHANS, E. SCHMID, C. FREUDENSTEIN, M. OSBORN, and K. WEBER. 1979. The organization of cytokeratin filaments in the intestinal epithelium. *Eur. J. Cell Biol.* **19**:255-268.
- FRANKE, W. W., C. GRUND, M. OSBORN, and K. WEBER. 1978. The intermediate-sized filaments in rat kangaroo PtK2 cells. I. Morphology in situ. *Cytobiologie.* **17**:365-391.
- FRANKE, W. W., M. LÜDER, J. KARTENBECK, H. ZERBAN, and T. W. KEENAN. 1976. Involvement of vesicle coat material in casein secretion and surface regeneration. *J. Cell Biol.* **69**:173-195.

23. FRANKE, W. W., E. SCHMID, J. KARTENBECK, D. MEYER, H.-J. HACKER, P. BANNASCH, M. OSBORN, K. WEBER, H. DENK, J.-C. WANSON, and P. DROCHMANS. 1979. Characterization of the intermediate-sized filaments in liver cells by immunofluorescence and electron microscopy. *Biol. Cell.* **34**:99-110.
24. FRANKE, W. W., E. SCHMID, M. OSBORN, and K. WEBER. 1978. Different intermediate-sized filaments distinguished by immunofluorescence microscopy. *Proc. Natl. Acad. Sci. U. S. A.* **75**:5034-5038.
25. FRANKE, W. W., E. SCHMID, M. OSBORN, and K. WEBER. 1979. The intermediate-sized filaments of human endothelial cells. *J. Cell Biol.* **81**:570-580.
26. FRANKE, W. W., E. SCHMID, K. WEBER, and M. OSBORN. 1979. HeLa cells contain intermediate-sized filaments of the prekeratin type. *Exp. Cell Res.* **118**:95-109.
27. FRANKE, W. W., E. SCHMID, S. WINTER, M. OSBORN, and K. WEBER. 1979. Widespread occurrence of intermediate-sized filaments of the vimentin-type in cultured cells from diverse vertebrates. *Exp. Cell Res.* **123**:25-46.
28. FRANKE, W. W., K. WEBER, M. OSBORN, E. SCHMID, and C. FREUDENSTEIN. 1978. Antibody to prekeratin. Decoration of tonofilament-like arrays in various cells of epithelial character. *Exp. Cell Res.* **116**:429-445.
29. FREUDENSTEIN, C., W. W. FRANKE, M. OSBORN, and K. WEBER. 1978. Reaction of tonofilament-like intermediate-sized filaments with antibodies raised against isolated defined polypeptides of bovine hoof prekeratin. *Cell Biol. Int. Rep.* **2**:591-600.
30. GABBIANI, G., J. CSANK-BRASSERT, J.-C. SCHNEEBERGER, Y. KAPANCI, P. TRENCH, and E. J. HOLBOROW. 1976. Contractile proteins in human cancer cells. *Am. J. Pathol.* **83**:457-474.
31. GABELLA, G., and D. BLUNDELL. 1978. Effect of stretch and contraction on caveolae of smooth muscle cells. *Cell Tissue Res.* **190**:255-271.
32. GARRETT, J. R. 1977. The autonomic innervation of rabbit salivary glands studied electron microscopically after 5-hydroxy-dopamine administration. *Cell Tissue Res.* **178**:551-562.
33. GIRARDIE, J. 1968. Histo-cytomorphologie de la glande mammaire de la souris C3H et de trois autres rongeurs. *Z. Zellforsch. und Mikrosk. Anat. Abt. Histochem.* **87**:478-503.
34. GORDON, W. E., A. BUSHNELL, and K. BURRIDGE. 1978. Characterization of the intermediate (10 nm) filaments of cultured cells using an autoimmune rabbit antiserum. *Cell.* **13**:249-261.
35. GROSS, W. O., and C. MÜLLER. 1977. A mechanical momentum in ultrastructural development of the heart. *Cell Tissue Res.* **178**:483-494.
36. HAMPERL, H. 1970. The myothelia (myoepithelial cells). *Curr. Top. Pathol.* **53**:161-220.
37. HOLLMANN, K. H. 1974. Cytology and fine structure of the mammary gland. In *Lactation: A Comprehensive Treatise*. B. L. Larson and V. R. Smith, editors. Academic Press, New York. 1:3-95.
38. HOLTZER, H., S. FELLINI, N. RUBINSTEIN, J. CHI, and K. STRAHS. 1976. Cells, myosin and 100 Å filaments. In *Cell Motility*. R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory. 823-839.
39. HUBBARD, B. D., and E. LAZARIDES. 1979. Copurification of actin and desmin from chicken smooth muscle and their copolymerization in vitro to intermediate filaments. *J. Cell Biol.* **80**:166-182.
40. HYNES, R. O., and A. T. DESTREE. 1978. 10 nm filaments in normal and transformed cells. *Cell.* **13**:151-163.
41. JARASCH, E. D., G. BRUDER, T. W. KEENAN, and W. W. FRANKE. 1977. Redox constituents in milk fat globule membranes from rough endoplasmic reticulum from lactating mammary gland. *J. Cell Biol.* **73**:223-241.
42. JOKUSCH, B. M., K. H. KELLEY, R. K. MEYER, and M. M. BURGER. 1978. An efficient method to produce specific anti-actin. *Histochemistry.* **55**:177-184.
43. LASCELLES, A. K., and C. S. LEE. 1978. Involvement of the mammary gland. In *Lactation: A Comprehensive Treatise*. B. L. Larson, editor. Academic Press, New York. 4:115-177.
44. LAZARIDES, E. 1978. The distribution of desmin (100 Å) filaments in primary cultures of embryonic chick cardiac cells. *Exp. Cell Res.* **112**:265-273.
45. LAZARIDES, E., and D. R. BALZER. 1978. Specificity of desmin to avian and mammalian muscle cells. *Cell.* **14**:429-438.
46. LAZARIDES, E., and B. L. GRANGER. 1978. Fluorescent localization of membrane sites in glycerinated chicken skeletal muscle fibers and the relationship of these sites to the protein composition of the Z-disc. *Proc. Natl. Acad. Sci. U. S. A.* **75**:3683-3687.
47. LAZARIDES, E., and B. D. HUBBARD. 1976. Immunological characterization of the subunit of the 100 Å filaments from muscle cells. *Proc. Natl. Acad. Sci. U. S. A.* **73**:4344-4348.
48. LAZARIDES, E., and K. WEBER. 1974. Actin antibody: the specific visualization of actin filaments in non-muscle cells. *Proc. Natl. Acad. Sci. U. S. A.* **71**:2268-2272.
49. LETHO, V.-P., I. VIRTANEN, and P. KURKI. 1978. Intermediate filaments anchor the nuclei in nuclear monolayers of cultured human fibroblasts. *Nature (Lond.)* **272**:175-177.
50. MATOLTSY, A. 1975. Desmosomes, filaments and keratohyaline granules: their role in the stabilization and keratinization of the epidermis. *J. Invest. Dermatol.* **65**:127-142.
51. MONTAGNA, W., and P. F. PARAKKAL. 1974. The structure and function of skin. Academic Press, New York.
52. OSBORN, M., W. W. FRANKE, and K. WEBER. 1977. Visualization of a system of filaments 7-10 nm thick in cultured cells of an epithelioid line (PtK2) by immunofluorescence microscopy. *Proc. Natl. Acad. Sci. U. S. A.* **74**:2490-2494.
53. PITEKKA, D. R. 1978. Cell contacts in the mammary gland. In *Lactation: A Comprehensive Treatise*. B. L. Larson, editor. Academic Press, New York. 4:41-66.
54. PITEKKA, D. R., and S. T. HAMAMOTO. 1977. Form and function in mammary epithelium: the interpretation of ultrastructure. *J. Dairy Sci.* **60**:643-654.
55. PITEKKA, D. R., S. T. HAMAMOTO, J. G. DUAFALA, and M. K. NEMANIC. 1973. Cell contacts in the mouse mammary gland. I. Normal gland in postnatal development and the secretory cycle. *J. Cell Biol.* **56**:797-818.
56. RADNOR, C. J. P. 1972. Myoepithelial cell differentiation in rat mammary glands. *J. Anat.* **111**:381-398.
57. RADNOR, C. J. P. 1972. Myoepithelium in the prelactating and lactating mammary glands of the rat. *J. Anat.* **112**:337-353.
58. RODEWALD, R., S. B. NEWMANN, and M. J. KARNOVSKY. 1976. Concentration of isolated brush borders from the intestinal epithelium. *J. Cell Biol.* **70**:541-554.
59. RUBY, J. R., and H. B. CANNING. 1978. Ultrastructure of the acinar cells in the submandibular gland of the nine-banded armadillo. *J. Morph.* **155**:1-18.
60. SCHÖLLMEYER, J. E., L. T. FURCHT, D. E. GOLL, R. M. ROBSON, and M. H. STROMER. 1976. Localization of contractile proteins in smooth muscle cells and in normal and transformed fibroblasts. In *Cell Motility*. R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory. 361-388.
61. SCHROEDER, B. T., J. CHAKRABORTY, and M. S. SOLOFF. 1977. Binding of [³H]oxytocin to cells isolated from the mammary gland of the lactating rat. *J. Cell Biol.* **74**:428-440.
62. SMALL, J. V., and A. SOBIESZEK. 1977. Studies on the function and composition of the 10 nm (100 Å) filaments of vertebrate smooth muscle. *J. Cell Sci.* **23**:243-268.
63. SOLOFF, M. S., H. D. REES, M. SAR, and W. E. STUMPF. 1975. Autoradiographic localization of radioactivity from ³H-oxytocin in the rat mammary gland and oviduct. *Endocrinology.* **96**:1475-1477.
64. SOMLYO, A. P., A. V. SOMLYO, F. T. ASHTON, and J. VALLIERES. 1976. Vertebrate smooth muscle: ultrastructure and function. In *Cell Motility*. R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory. 165-183.
65. STAHELIN, A. 1974. Structure and function of intracellular junctions. *Int. Rev. Cytol.* **39**:191-284.
66. STARGER, J. M., W. E. BROWN, A. E. GOLDMAN, and R. D. GOLDMAN. 1978. Biochemical and immunological analysis of rapidly purified 10-nm filaments from baby hamster kidney (BHK-21) cells. *J. Cell Biol.* **78**:93-109.
67. STEINERT, P. M., W. I. IDLER, and S. B. ZIMMERMANN. 1976. Selfassembly of bovine epidermal keratin filaments in vitro. *J. Mol. Biol.* **108**:547-567.
68. STEINERT, P. M., S. B. ZIMMERMANN, J. M. STARGER, and R. D. GOLDMAN. 1978. Ten-nanometer filaments of hamster BHK-21 cells and epidermal keratin filaments have similar structures. *Proc. Natl. Acad. Sci. U. S. A.* **75**:6098-6101.
69. STRUM, J. M. 1978. Estrogen-induced alterations in the myoepithelial cells of the rat mammary gland. *Cell Tissue Res.* **193**:155-161.
70. SUN, T.-T., and H. GREEN. 1977. Cultured epithelial cells of cornea, conjunctiva and skin: absence of marked intrinsic divergence of their differentiated states. *Nature (Lond.)* **269**:489-493.
71. SUN, T.-T., and H. GREEN. 1978. Immunofluorescent staining of keratin fibers in cultured cells. *Cell.* **14**:469-476.
72. TANDLER, B. 1965. Ultrastructure of the human submaxillary gland. III. Myoepithelium. *Z. Zellforsch. und Mikrosk. Anat. Abt. Histochem.* **68**:852-863.
73. TANI, E., S. YAMAGATA, and Y. ITO. 1977. Cell membrane structure of vascular smooth muscle of circle of Willis. *Cell Tissue Res.* **179**:131-142.
74. TINDAL, L. S. 1978. Neuroendocrine control of lactation. In *Lactation: A Comprehensive Treatise*. B. L. Larson, editor. Academic Press, New York. 4:67-114.
75. TOBON, H., and H. SALAZAR. 1975. Ultrastructure of the human mammary gland. II. Postpartum lactogenesis. *J. Clin. Endocrinol. Metab.* **20**:834-844.
76. UEHARA, Y., G. R. CAMPBELL, and G. BURNSTOCK. 1971. Cytoplasmic filaments in developing and adult vertebrate smooth muscle. *J. Cell*

- Biol.* **50**:484-497.
77. WEBER, K., P. C. RATHKE, M. OSBORN, and W. W. FRANKE. 1976. Distribution of actin and tubulin in cells and in glycerinated cell models after treatment with cytochalasin B. *Exp. Cell Res.* **102**:285-297.
78. WEBER, K., J. WEHLAND, and H. HERZOG. 1976. Griseofulvin interacts with microtubules both in vivo and in vitro. *J. Mol. Biol.* **102**:817-829.
79. WEBSTER, R. E., M. OSBORN, and K. WEBER. 1978. Visualization of the same P1K2 cytoskeleton by both immunofluorescence and low power electron microscopy. *Exp. Cell Res.* **117**:47-61.
80. YOUNG, J. A., and E. W. VAN LENNEP. 1978. The Morphology of Salivary Glands. Academic Press, Inc., New York.
81. ZERRAN, H., and W. W. FRANKE. 1977. Structures indicative of keratinization in lactating cells of bovine mammary gland. *Differentiation*, **7**: 127-131.