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The Modulation of Cell Adhesion Molecule Expression and Intercellular Junction Formation in the Developing Avian Inner Ear

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The cells that constitute the membranous labyrinth in the vertebrate inner ear are all derived from a single embryonic source, namely, the otocyst. The mature inner ear epithelia contain different regions with highly differentiated cells, displaying a highly specialized cytoarchitecture. The present study was designed to determine the presence of adherens-type intercellular junctions in this tissue and study the expression of cell adhesion molecules (CAMs) associated with these junctions, namely, A-CAM and L-CAM, in the developing avian inner ear epithelia. The results presented here show that throughout the early otocyst, A-CAM is coexpressed with L-CAM. The formation of asymmetries between sensory and nonsensory areas in the epithelium is accompanied by the modulation of CAMs expression and the assembly of intercellular junctional complexes. A-CAM and L-CAM display reciprocal expression patterns, the former being expressed mostly in the mosaic sensory epithelium, while L-CAM becomes conspicuous in the nonsensory areas but its expression in the sensory region is markedly reduced. Adherens-type junctions and numerous desmosomes are found in the junctional complexes of early otocyst cells. The former persist to maturity of the various inner ear epithelia, whereas desmosomes disappear from junctional complexes of hair cells but remain in the intercellular junctional complexes of all other cell types in the membranous labyrinth. Thus, adherens type intercellular junctions comprise the only defined cytoskeleton-bound junction in mature hair cells. A-CAM-positive cells are also found in the region of the acoustic ganglion in early developmental stages but not in the mature neural elements.

INTRODUCTION

The epithelium of the inner ear of vertebrates presents anatomically complex regions containing a multitude of structurally and functionally distinct cell types (Hirokawa, 1978; Tanaka and Smith, 1978; Chandler, 1984a). Among these are simple epithelial cells with secretory or supportive functions as well as the various cells forming the sensory elements of the organ of Corti (or basilar papilla in avian species). The latter assemble into a highly organized mosaic epithelium in which sensory hair cells are interspaced by support cells, forming together a tightly packed and sealed honeycomb-shaped mixed epithelium (Tanaka and Smith, 1978; Ginzberg and Gilula, 1979). Along the subapical borders of the cells, at the level of the cuticular plate, junctional complexes have been described, consisting of apical tight junctions and, next to them, microfilament-associated *zonulae adherentes* (Ginzberg and Gilula, 1979). In contrast to contacts between neighboring support cells, the heterotypic junctions between hair cells and support cells are apparently devoid of desmosomes and gap junctions (Ginzberg and Gilula, 1979; and this report).

One of the attractive features of the inner ear epithelia is that all their diverse cellular constituents are apparently derived from the single simple epithelium of the otocyst. During embryonic development, this apparently uniform epithelium undergoes spatially and temporally regulated differentiation events giving rise to a multitude of different anatomical domains and diverse cell types of the mature inner ear (Knowlton, 1967; Cohen and Fermin, 1978; Meier *et al.*, 1978a,b; Chandler, 1984b).

Recent attempts to understand the molecular and cellular bases for tissue organization have focused much attention on a family of surface glycoproteins designated cell adhesion molecules (CAMs) (Thiery *et al.*, 1982; Edelman, 1983, 1985a; Obrink, 1986) or cadherins (Takeichi, 1987). It has been suggested that the selective expression of CAMs leads to the specific and selective cellular interactions and thus plays an important role in cell and tissue morphogenesis (Thiery *et al.*, 1982; Edelman, 1985a; Takeichi *et al.*, 1985a; Takeichi, 1987).

L-CAM, also named uvomorulin (Hyafil *et al.*, 1980) or E-cadherin (Hatta *et al.*, 1985), is a calcium-dependent CAM which localizes in adherens-type junctions of epithelial tissues and different cultured cells (Hyafil *et al.*, 1980; Gallin *et al.*, 1983; Boller *et al.*, 1985; Volk *et al.*, 1987). Another calcium-dependent CAM, namely, A-CAM (Volk and Geiger, 1984, 1986a,b), was shown to

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be present in the actin- and vinculin-associated adherens-type junctions in adult avian cardiac muscle and lens tissue and to be transiently expressed in a large variety of epithelia in early chick embryos (Duband *et al.*, 1987). Antibody inhibition experiments indicated that this molecule is involved in the intercellular interactions between a variety of cultured cells (Volk and Geiger, 1986a,b).

Recent studies on CAM expression in the developing avian inner ear have indicated that the level of L-CAM expression in this epithelium varied markedly in different regions of the developing epithelium and that the borders between regions expressing different ratios of L-CAM to N-CAM mark the future borders between the various structural and functional domains of this tissue (Richardson *et al.*, 1987). Notably, this study has shown an apparent decrease in L-CAM expression during maturation of the basilar papilla, the sensory region of the avian cochlea.

In the present study we have attempted to characterize the expression of A-CAM in the developing and mature inner ear of the chick, and to determine its involvement in the formation of intercellular junctions in this tissue.

We presently report that distinct and reciprocal changes in the levels of A-CAM and L-CAM expression were observed. At the otocyst stage both CAMs were coexpressed in the epithelium, while following differentiation A-CAM disappeared from the nonsensory ear epithelia and was retained only in the basilar papilla. L-CAM, on the other hand, decreased to barely detectable levels in the basilar papilla, whereas throughout the nonsensory regions of the membranous labyrinth it was prominently present. This reciprocal mode of selective expression of A-CAM and L-CAM and its possible involvement in the induction of molecularly and mechanically distinct intercellular junctions are discussed.

MATERIALS AND METHODS

Tissues. Chicken embryos of selected developmental stages or chick hatchlings were obtained from a local hatchery. The embryos were staged according to the criteria of Hamburger and Hamilton (1951). Inner ears from the following stages were used in this study: Stage 12 (otic pit), stage 17–19 (otocyst), stage 26–34 (morphogenesis of cochlear duct), stage 35–40 (differentiating cochlear epithelium). Apparently mature epithelium was obtained from hatchlings as well as from 2-day-old chicks.

Dissection of the tissue was carried out in the fixative solution (3% formaldehyde in 0.1 *M* phosphate buffer, pH = 7.4). For immunohistochemical analysis, we fixed for 1 or 2 hr whole heads (until stage 17), complete inner ears (stage 26–34), or isolated cochleas with the bony capsule removed (all later stages). Following a brief wash in the same buffer, the tissue was infiltrated with 30% sucrose (in PBS) overnight. Samples were embedded in Tissue-Tek (Lab-Tek Products) and frozen in liquid nitrogen. Cryosections of 4–6 μm were cut at −23°C using a Frigocut 2800 cryostat (Jung Reichert, FRG) and collected on gelatin-coated glass slides.

Whole mounts of dissected cochleas of 2-day-old hatchlings, enabling surface view of the cells, were prepared as follows: The inner ear was dissected out, immersed in PBS, and gently separated from the bony capsule. The tegmentum vasculosum was removed all along the cochlear duct in order to expose the surface of the basilar papilla. The tectorial membrane was then partially dissected away, revealing the surface of the hair cells. The remaining tissue, consisting of the basilar papilla with the adjacent ligaments, blood vessels, and neural elements, was fixed in methanol (−20°C, 5 min) and acetone (−20°C, 20 min) and immunolabeled using the same procedure described here for the cryosections.

Antibodies. Anti-A-CAM monoclonal antibody ID 7.2.3 was prepared as previously described (Volk and Geiger, 1984) and used as hybridoma supernatant throughout this study. Rabbit antibodies to L-CAM were prepared and used as described (Gallin *et al.*, 1983). FITC-conjugated phalloidin (Molecular Probes) was used to decorate filamentous actin. Secondary antibodies were DTAF- or rhodamine-conjugated affinity-purified goat antibodies to mouse Fab or to rabbit IgG containing an average of three fluorophores per IgG molecule.

Immunohistochemical labeling. To block nonspecific binding sites, sections were treated with 5% normal goat serum for 1 hr prior to introduction of the first antibody. All antibody preparations used contained 5% normal goat serum. The sections were incubated with the first antibodies for 1–2 hr at room temperature, washed twice (10 min each time) with PBS, and incubated with the secondary antibodies for 40 min. Slides were washed with PBS, dehydrated in absolute ethanol for 5 min, and mounted in Entellan. Sections were examined with a Zeiss Axiophot photomicroscope equipped for epifluorescence, and photographed on Kodak Tri-X film.

Electron microscopy. For transmission electron microscopic (TEM) analysis, tissues were dissected as described and fixed promptly at room temperature in 2% glutaraldehyde in 0.1 *M* cacodylate buffer, pH = 7.2, containing 0.5 m*M* $CaCl_2$. The tissue was postfixed for 1 hr in 1% osmium tetroxide, dehydrated in alcohols, and embedded in Epon (Polybed 812, Polysciences). Semi-

thin sections were cut with glass knives and stained with toluidine blue. Thin sections were cut in an LKB Nova ultramicrotome (LKB, Sweden) using a diamond knife, stained with lead citrate and uranyl acetate, and viewed in a Philips 410T electron microscope at 80 kV.

RESULTS

The expression of A-CAM, L-CAM, and actin and their subcellular localizations were determined in the various epithelia of the inner ear at various stages of development. For the sake of clarity we distinguish here four major developmental stages, as follows:

Development of the otocyst. This stage includes the formation and invagination of the otic pit and its closure and detachment from the ectoderm. The epithelium of the otocyst, at this stage, is largely uniform, without apparent differentiation of the sensory cell primordium. A transverse section shown in Fig. 1A presents the typical relative orientations of the neural tube and the otocyst with the acoustic ganglion and the surrounding mesenchyme. Actin is detected in increased quantities along the apical borders of the epithelial cells (Fig. 1B). Immunohistochemical labeling of embryos displaying otic placodes or pits revealed faint, yet significant labeling for A-CAM and an intense L-CAM staining (Fig. 1D), essentially as shown earlier (Richardson *et al.*, 1987; Duband *et al.*, 1988). Further examination of a large number of embryos indicated that the level of A-CAM in the otic epithelium gradually increased until it appeared to be homogenously and extensively expressed throughout the epithelium (Fig. 1C). It is noteworthy that A-CAM levels in the otocyst at that stage were still lower compared to those of the nearby neuroepithelium. Labeling of the same sections for both A-CAM and L-CAM indicated that the two exhibit a similar pattern of distribution in the otocyst. The acoustic ganglion cells were positively labeled for A-CAM (Fig. 1C) yet showed no fluorescence with anti L-CAM (Fig. 1D).

Electron microscopic examination of the otocyst revealed well-organized subapical junctional complexes (Fig. 2A). These junctions contained the typical spatial arrangement of tight junctions, adherens junctions, and desmosomes (Fig. 2B). Notably, the latter were quite abundant during that phase of development. This organization of junctional elements was uniformly detected throughout the entire epithelium of the otocyst.

Morphogenesis of the cochlear duct. At about 4.5 days of embryonic development, the first stages of cochlear development became apparent. This was manifested by the extension of an elongating duct in a ventromedial direction. Cryosections through the proximal end of the early cochlear duct, followed by double labeling for A-CAM and L-CAM, revealed the presence of both molecules within the epithelial cells. Often, this labeling was, however, nonhomogenous, showing apparently complementary staining patterns for the two molecules (Figs. 3A and 3B). Longitudinal cryosections of cochleas from 6-day-old embryos showed that A-CAM staining was consistently more extensive in the area of the presumptive sensory epithelium (Fig. 4A), while L-CAM levels were apparently reduced in this region (Fig. 4C). Moreover, A-CAM antibodies also labeled the adjacent acoustic ganglion, in contrast to L-CAM staining, which was essentially negative. Comparison of the pattern of labeling obtained for the two CAMs with the actin patterns in the same sections (Figs. 4B and 4D) indicated that the latter was predominantly found along the apical junctions, while the former were distributed throughout the basolateral membranes. The electron microscopic examination of the nondifferentiated cochlear ducts indicated that the overall cellular morphology and junctional topology were similar to those described above for the otocyst.

Differentiation of the epithelia. The next major step in the development of the cochlea involves differentiation of the otic epithelia, leading to the appearance of various support cells and distinctive hair (sensory) cells. The latter are detected exclusively in the basilar papilla whereas nonsensory cells are interposed between the hair cells and line the other cavities throughout the labyrinth. Processes of neurites could be seen approaching the areas of the sensory cells, as previously described (Rebillard and Pujol, 1983; Whitehead and Morest, 1985a,b).

Immunofluorescent labeling for A-CAM indicated that the protein was widely distributed throughout the various epithelial components of the cochlea, including the sensory epithelia and the tegmentum vasculosum (Fig. 5A). The intensity of L-CAM staining was significantly reduced in the basilar papilla, however, extensive labeling was detected in nonsensory areas (Fig. 5B).

Electron microscopic examination of cochlear tissues of the same developmental stage revealed distinctive hair cells with apparent stereocilia and initial signs of cuticular plate formation. The hair cells were spaced by presumptive support cells (Fig. 5C). The heterotypic junctional complexes between hair cells and support cells contained extensive tight junctions and *zonulae adherentes* (Fig. 5D). Desmosomes were occasionally detected, though at a remarkably lower frequency than that found in earlier stages. It should be emphasized that around the 11th–12th embryonal day a marked deterioration of desmosomal structures was noted in the heterotypic intercellular contacts formed between hair and support cells. In cochleas of 14-day-old chick embryos desmosomes could no longer be detected in hair

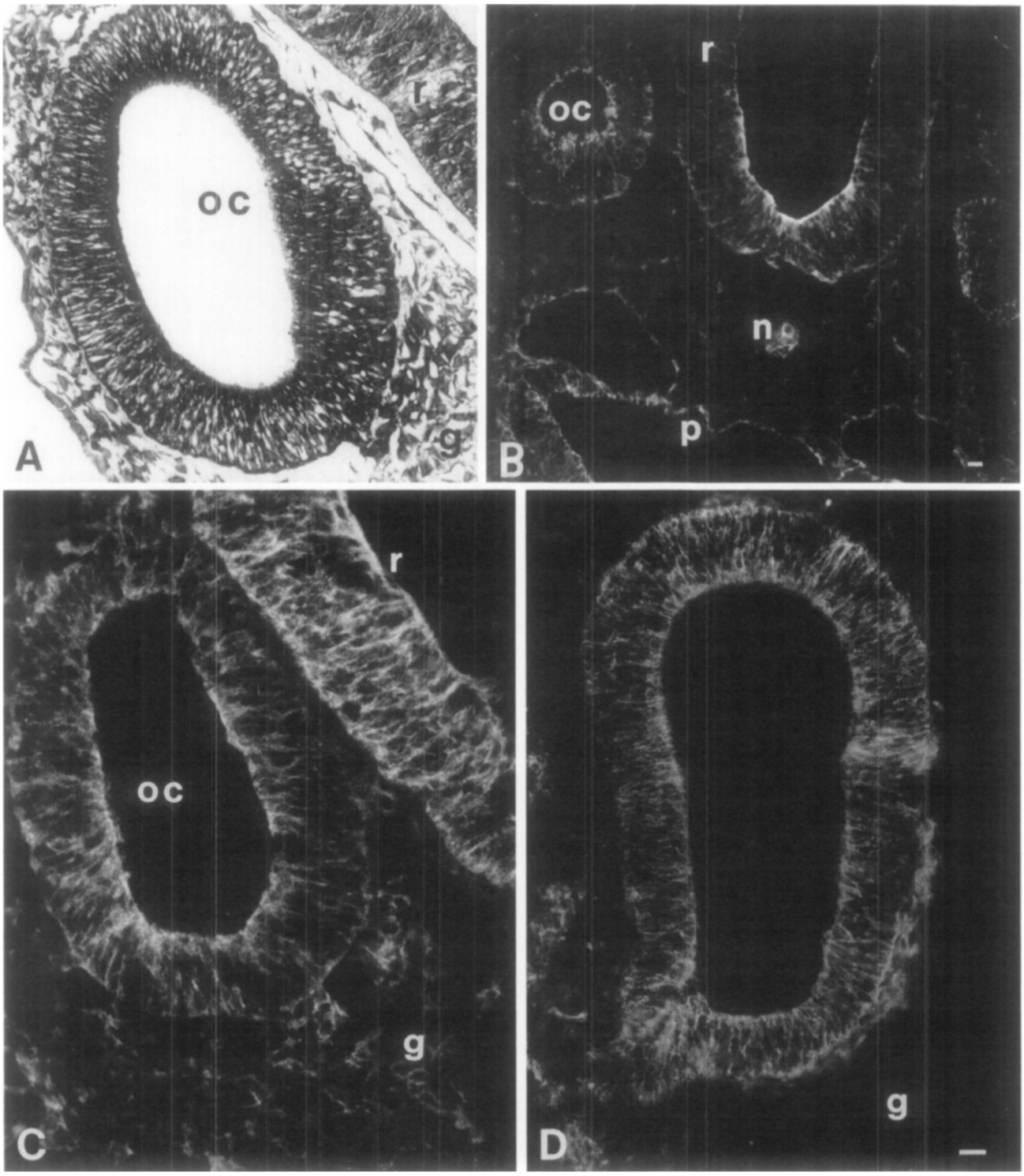

FIG. 1. Epon-embedded section (A) or cryosectioned (B–D) otocysts of 3-day-old (stage 17–19) embryos stained with toluidine blue (A) or immunostained for actin (B), A-CAM (C), and L-CAM (D). Anti-A-CAM immunofluorescence (C) is more intense in the epithelium of the rhombencephalon than in the otocyst. Several cells in the area of the acoustic ganglion are also positive for A-CAM, but not for L-CAM. The distribution of L-CAM (D) and A-CAM (C) in the otocyst epithelium is generally similar. oc, otocyst lumen; r, rhombencephalon; g, otic ganglion; n, notochord, p, pharynx. Bar = 10 μm.

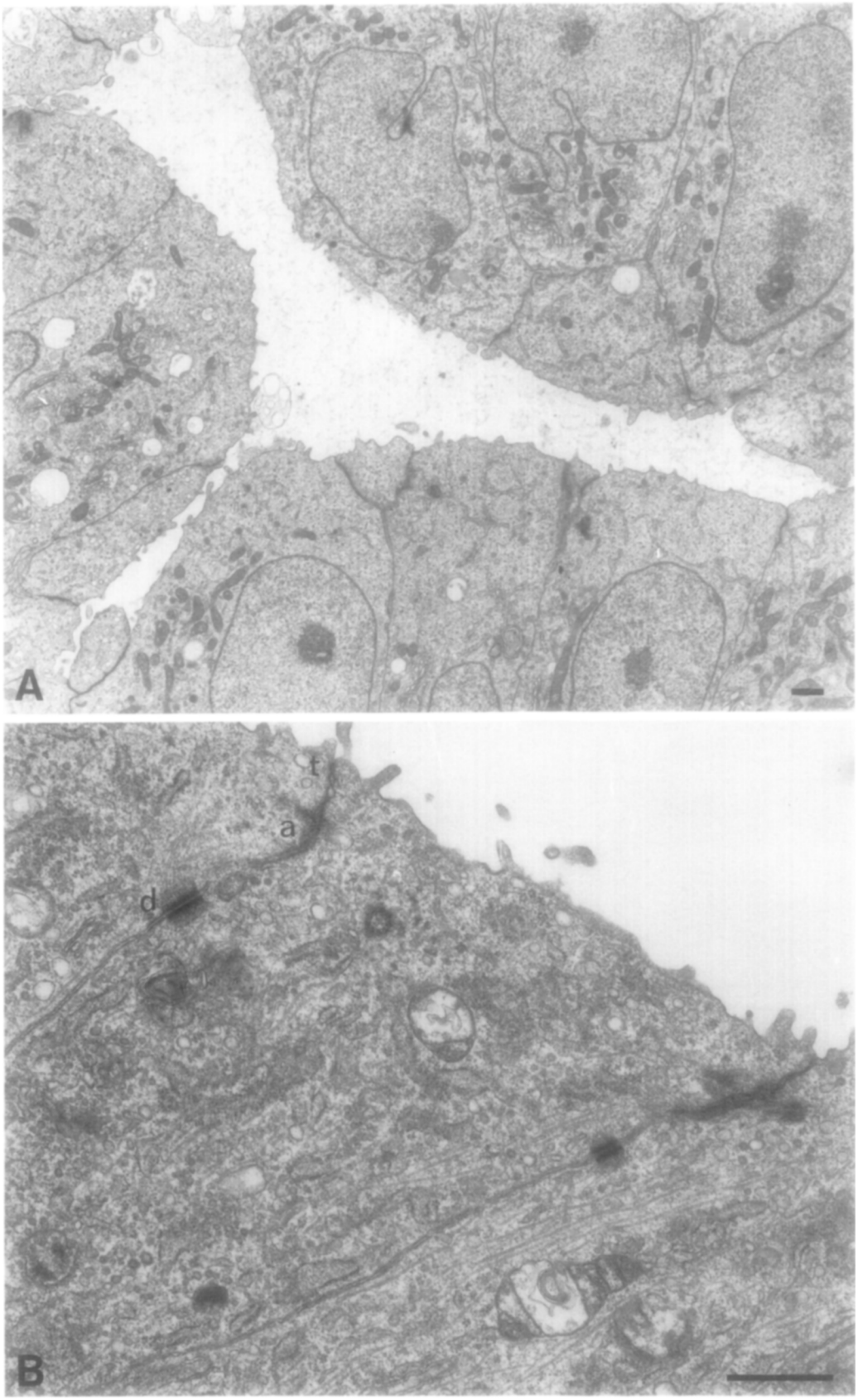

FIG. 2. Electron photomicrographs of the area bordering the lumen of 3-day-old embryos' otocysts, showing that cells around the lumen (A) appear similar. It can be seen at a higher magnification (B) that the subapical intercellular contacts throughout the epithelium contain junctional complexes including tight junction, adherens junction, and desmosomes. t, tight junction; a, adherens junction; d, desmosome. Bar = 1 μm.

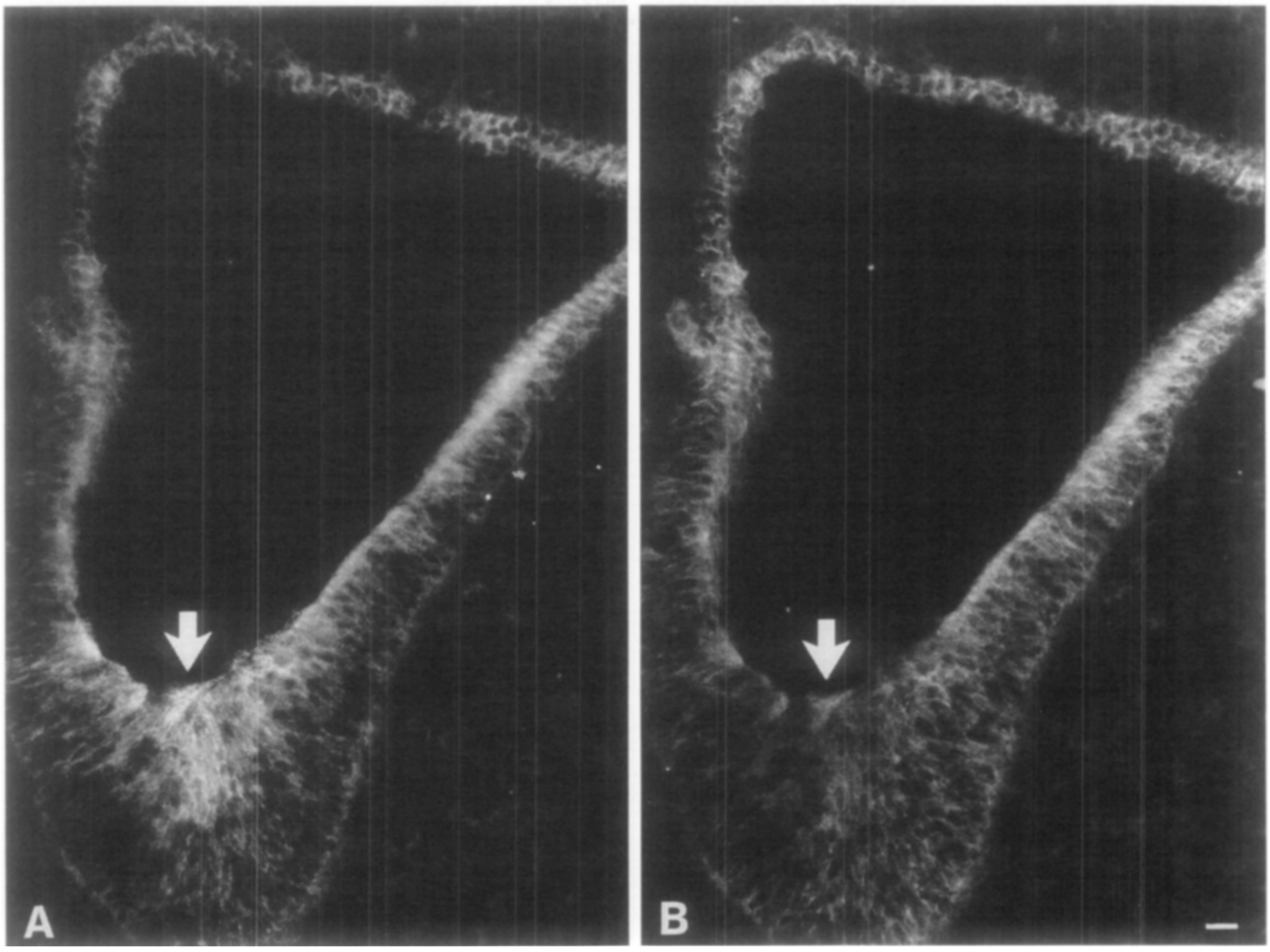

FIG. 3. A cryosection of an early cochlear duct growing out from the otocyst of a 4.5-day-old embryo (stage 23), double immunolabeled for A-CAM (A) and L-CAM (B). Both molecules are present in the apical as well as the lateral aspects of the cells. Note reduction of L-CAM in the presumptive sensory area, where an apparent increase of A-CAM staining can be observed (arrows). Bar = 10 μm.

cell junctions. It was nevertheless found that desmosomes with their distinctive morphology were present in contacts formed by neighboring support cells.

Epithelia of the mature cochlear duct. In 18-day embryos the development of the various cellular elements of the cochlea was essentially complete. Staining for A-CAM (Fig. 6A) revealed intense labeling of the basilar papilla, mostly restricted to the cell apices. The labeling was particularly associated with the hair cell region, easily identified by the intense actin staining at the cuticular plate and the stereocilia (Fig. 6B). Furthermore, at that stage, A-CAM could no longer be detected in the nonsensory epithelia of the inner ear. In contrast, L-CAM staining in the basilar papilla was extremely faint, while in the nonsensory epithelia it was extensively expressed (Fig. 6C). There again, actin staining of the hair cells facilitated identification of the basilar papilla (Fig. 6D). This mutually exclusive pattern of expression of A-CAM and L-CAM was retained in the mature ear.

A higher resolution view of A-CAM and L-CAM distribution along the reticular lamina was obtained by double-labeling of permeabilized-fixed surface preparations of the basilar papilla for both CAMs. As shown in Fig. 7A, A-CAM was prominently present along the heterotypic hair cell-support cell junctions as well as along the relatively small homotypic junctions formed by adjacent support cells. L-CAM could hardly be detected in the basilar papilla (Fig. 7B) at that stage. For comparison, we also show a phalloidin-stained preparation displaying actin labeling of the stereocilia and the intercellular interphases (Fig. 7C).

Electron microscopy at this stage revealed mature cochlear epithelia. Adherens type junctions were abundant both in the sensory (Figs. 8A and C) and the nonsensory (Fig. 8B) cells. The fine structure of the heterotypic junctions in the basilar papilla (i.e., the ones formed between hair cells and support cells) was revealed in high power electronmicrographs showing the presence of both tight and adherens junctions. Desmo-

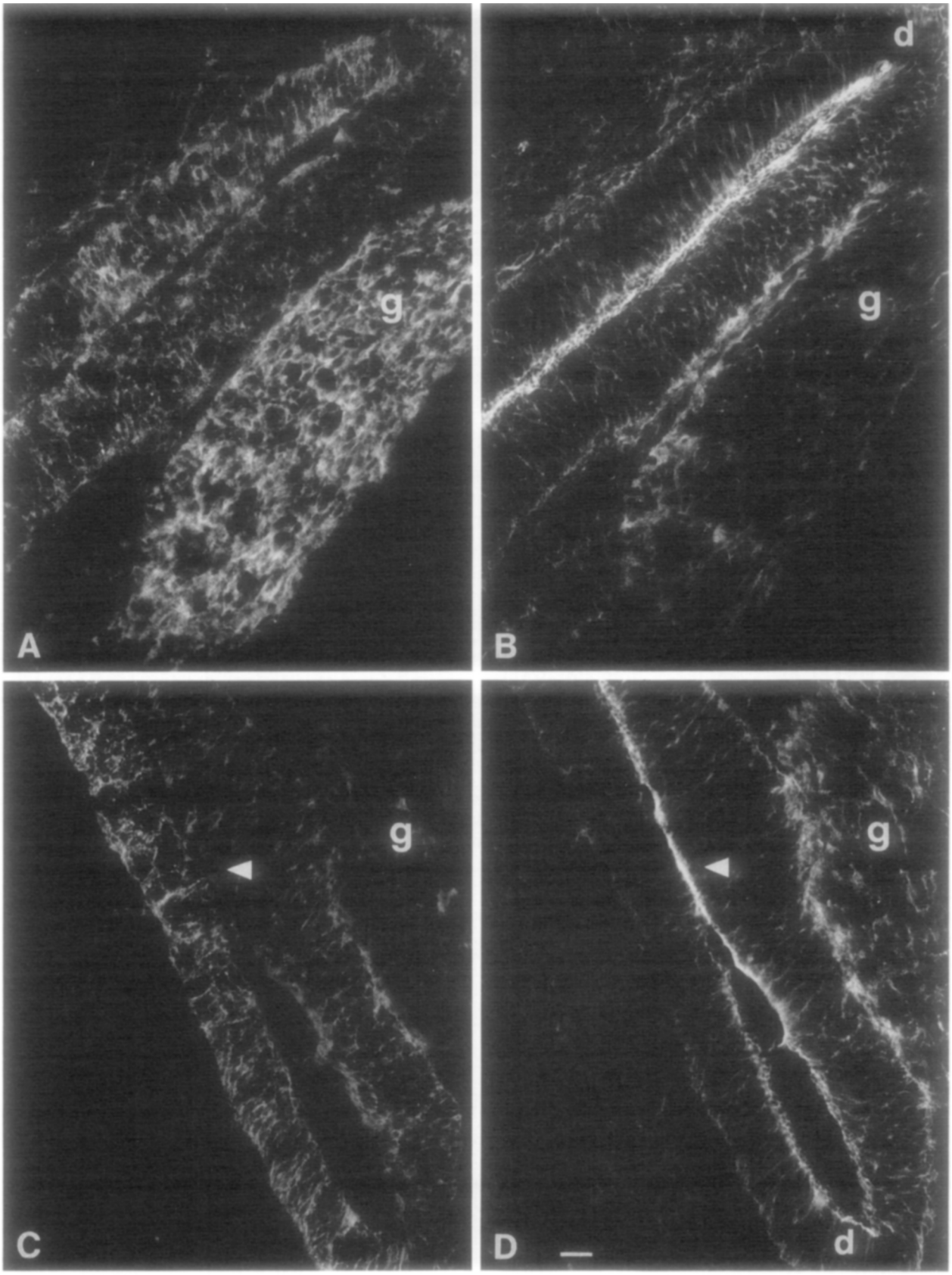

FIG. 4. Longitudinal cryosections of elongating cochlear duct of 6-day-old embryos (stage 28) double-labeled for A-CAM and actin (A, B) or L-CAM and actin (C, D). A-CAM staining is distributed throughout distal and proximal areas of the duct, in the region where the ganglion innervates the epithelium and the sensory cells form as well as in the opposite part of the duct. L-CAM distribution is considerably reduced (arrowhead in C) in the area where hair cells form (arrowhead in (D)). The ganglion is positive for A-CAM staining but not for L-CAM staining. g, ganglion; d, distal end of duct. Bar = 20 μm.

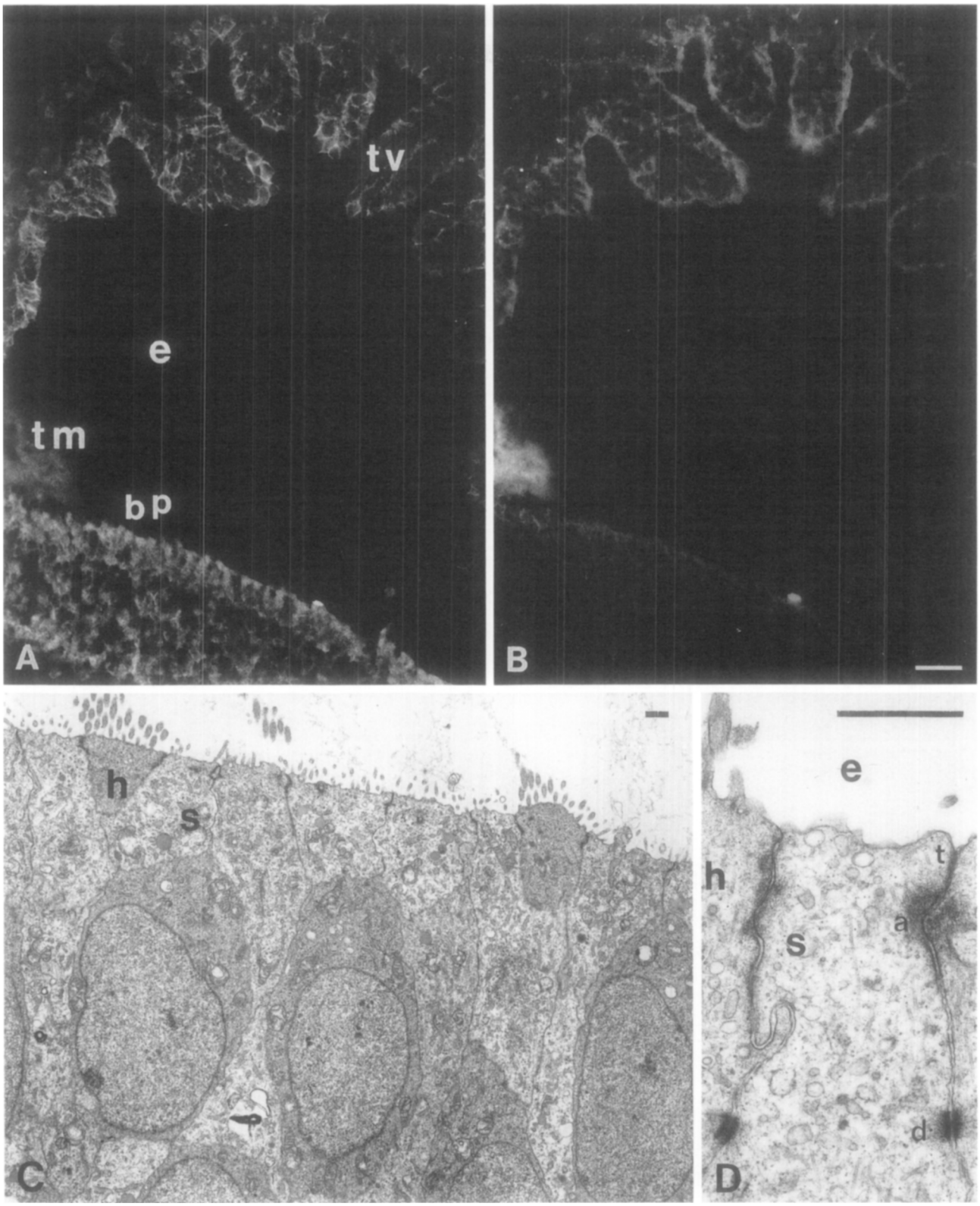

FIG. 5. Cochlear ducts of 12-day-old (stage 38) chick embryos were cryosectioned and double-labeled for A-CAM and L-CAM (A, B) or prepared for TEM (C, D). A-CAM staining is present primarily in sensory regions (basilar papilla) as well as in the tegmentum vasculosum and other nonsensory regions of the epithelium, while L-CAM labeling has decreased in the basilar papilla, relative to the marked labeling in nonsensory areas. TEM micrographs show a distinctive organization of the hair cells, featuring darker cytoplasm and recognizable stereocilia (C). A higher magnification view of the hair cell–support cell intercellular junctions (D) shows that the desmosomes are still present but their shape is markedly deteriorated. tv, tegmentum vasculosum; e, scala media (endolymph); tm, tectorial membrane; bp, basilar papilla; h, hair cell; s, support cell; t, tight junction; a, adherens junction; d, desmosome. (B) Bar = 10 μm. (C) and (D) Bars = 1 μm.

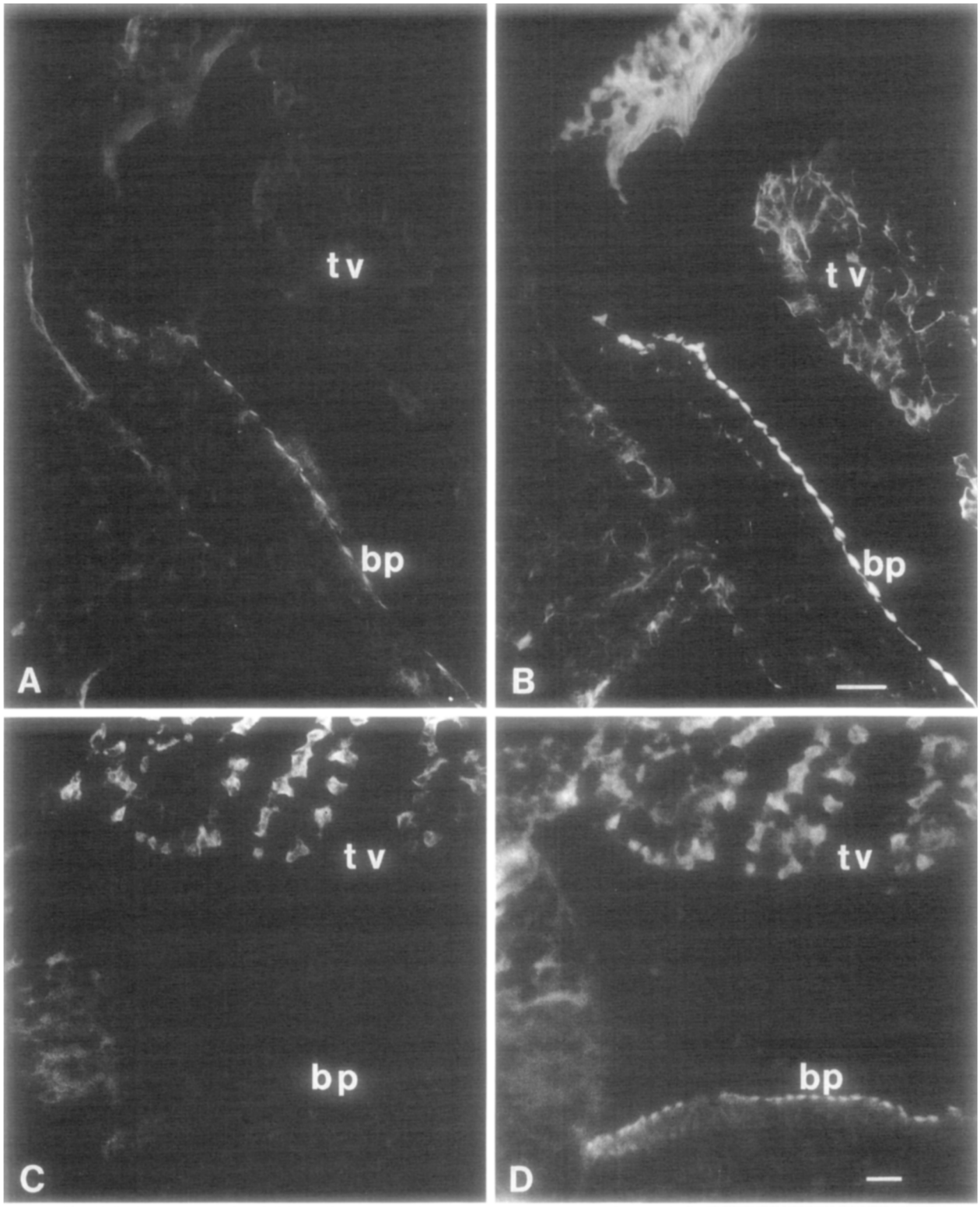

FIG. 6. Cryosections of 18-day-old embryonic cochlear ducts (stage 44), double-immunostained for A-CAM and actin (A, B) or L-CAM and actin (C, D). A-CAM expression at this stage is restricted to the apical region of the mosaic epithelium of the basilar papilla (A). The cuticular plates and the stereocilia stain intensely for actin (B). L-CAM is conspicuous in nonsensory areas, but in the basilar papilla it stains very faintly (C). tv, tegmentum vasculosum; bp, basilar papilla. Bar = 10 μm.

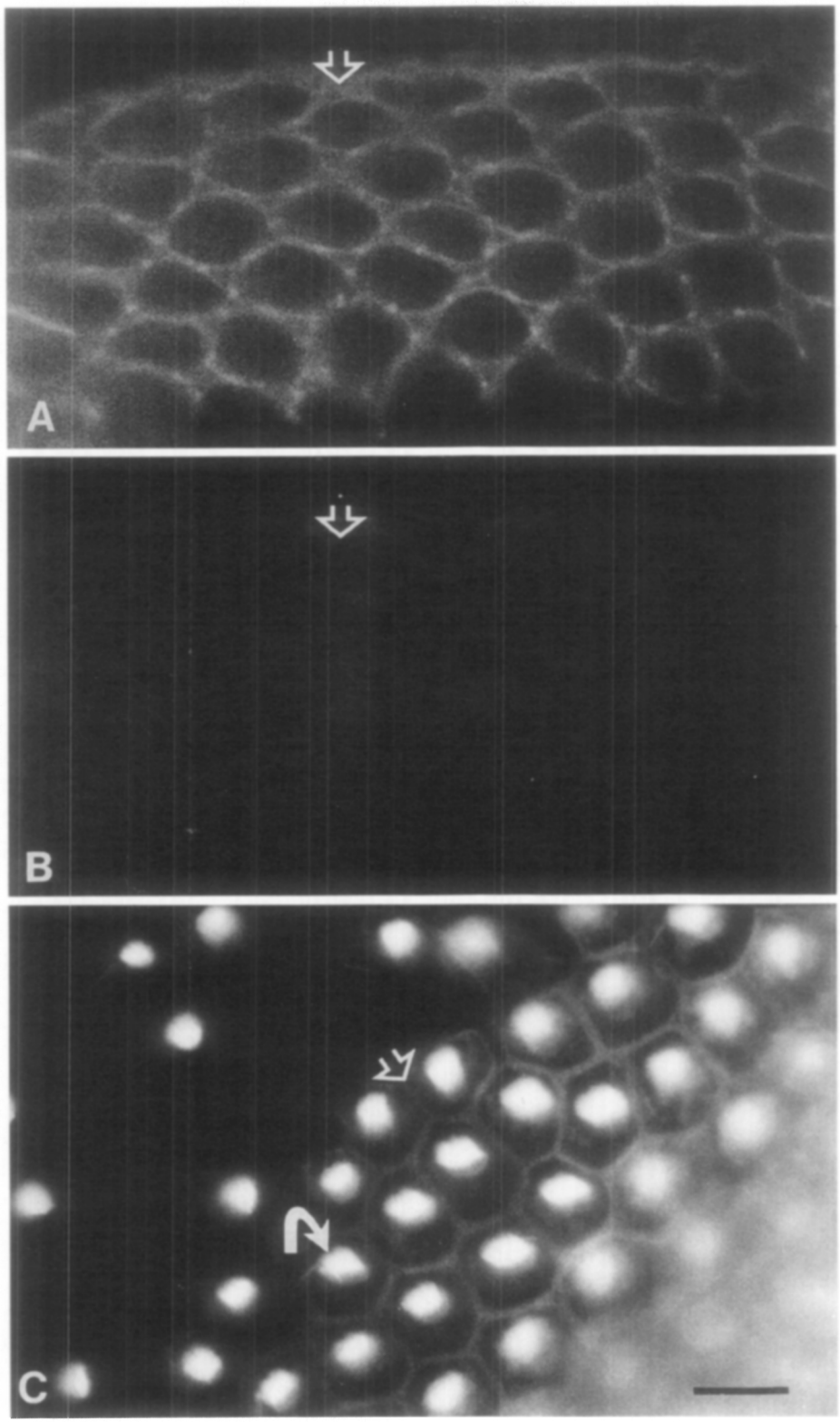

FIG. 7. Whole mounts ("surface preparations") of basilar papillas from 2-day-old hatchlings, double-stained for A-CAM and L-CAM (A, B) or for actin (C). A-CAM is apparently localized in the heterotypic junctions between hair cells and support cells (arrowhead), as well as in support cell/support cell junctions. L-CAM immunoreactivity is hardly detectable throughout the basilar papilla at that stage. Actin staining in the bundle of stereocilia (bent arrow) is intense. Actin is also detected along the junctional region at the periphery of hair cells (arrowhead in (C)), where it apparently stains the microfilament belt associated with the adherens junction. Bar = 10 μm.

somes were not present between these cells, in line with previous observations by Ginzberg and Gilula (1979). In contrast, desmosomes were abundant in the homotypic junctions between adjacent support cells (Fig. 8D) and between epithelial cells in the nonsensory regions, such as the tegmentum vasculosum (Fig. 8B).

DISCUSSION

Morphogenetic events during development have been shown to be associated with, and possibly driven by, cell–cell and cell–substrate interactions, mediated via specific adhesion molecules (Thiery *et al.*, 1982, 1985; Edelman, 1983, 1985a,b; Edelman & Thiery, 1985; Takeichi *et al.*, 1985b; Obrink, 1986; Takeichi, 1987). The system examined in this study, namely, the inner ear, presents unique patterns of differentiation, starting with the formation of the otic placode and leading to the development of the entire membranous labyrinth. Throughout this process the epithelia of the otic placode and cyst undergo dramatic diversification, forming a multitude of spatially positioned sensory and nonsensory cells. In a recent study, Richardson *et al.* (1987) have described the spatiotemporally regulated expression of N-CAM, L-CAM, and Ng-CAM as well as of extracellular matrix components in the developing inner ear of the chick. The results indicated that regions exhibiting a transition in the relative expression of N-CAM and L-CAM coincided with histogenetic borders between distinct structural and functional domains of the inner ear epithelium. Characteristically, L-CAM expression in the basilar papilla was markedly reduced during the differentiation of the tissue, in contrast to consistently high levels of N-CAM in this region. In view of the possible involvement of L-CAM in junctional interactions in some cells and tissues (Boller *et al.*, 1985; Volk *et al.*, 1987), the decrease in its expression vis-a-vis the development of conspicuous junctional complexes raised some interesting questions concerning the molecular basis for junctional interactions in this tissue.

Electron microscopic examination indicated that the chick cochlear epithelium contains well-organized junctional complexes in all stages of development (Figs. 2 and 8) (Ginzberg and Gilula, 1979). The most apical component of these junctions is the tight junction, which is followed by the more basal *zonula adherens*. The latter type of junction is generally characterized by its association with a bundle of actin filaments and by the presence of vinculin (Geiger, 1979, Geiger *et al.*, 1983, 1985a), plakoglobin (Cowin *et al.*, 1986), and possibly additional constituents in the submembrane plaque. Attempts to identify the integral membrane receptor typical for adherens junctions focused on two major proteins. Boller *et al.* (1985) have shown that L-CAM (uvomorulin) is particularly enriched along the junctional membrane of the intestinal epithelium, in regions corresponding to *zonula adherens*. Another adherens junction-associated CAM is A-CAM. In previous studies we have shown that A-CAM is associated with adherens junctions of heart and lens tissue of adult chicks (Volk and Geiger, 1986a) as well as in a large variety of embryonic epithelia (Duband *et al.*, 1988). Comparison of the expression profiles of A-CAM and L-CAM indicated that the two molecules were usually expressed in a mutually exclusive manner (Edelman, 1985b; Volk *et al.*, 1987; Duband *et al.*, 1988). Thus, after transient periods of apparent coexpression, either A-CAM or L-CAM was present exclusively. In some adherens junction-containing tissues (i.e. endothelium) neither A-CAM nor L-CAM were found, and the nature of the junctional CAM in these tissues is still unknown. Notably, A-CAM appears to bear close antigenic relationship to the Ca^{2+}-dependent CAM, N-cadherin (Hatta *et al.*, 1985). The exact molecular relationships between these molecules are now under investigation.

We show here that A-CAM is coexpressed with L-CAM in the developing otocyst and early cochlear duct. As reported earlier (Richardson *et al.*, 1987), L-CAM expression becomes nonhomogenous at the time of innervation by the acoustic ganglion and the differentiation of the various cellular constituents of the cochlear duct. Comparison with the pattern of A-CAM expression indicated that the latter stained more intensely in regions of the cochlear duct subjacent to the ganglion, where L-CAM labeling was reduced. Upon differentiation of hair cells (around Embryonic Days 9–11) L-CAM immunoreactivity in the basilar papilla significantly decreased, while A-CAM was detected along the reticular lamina as well as in the adjacent ganglion. During this stage, desmosomes in the heterotypic junctions between hair cells and support cells significantly deteriorated, until they essentially disappeared from the junctions. At a later stage, around Embryonic Days 16–18, A-CAM apparently disappeared from nonsensory constituents of the inner ear, such as the tegmentum vasculosum, as well as from the adjacent acoustic ganglion.

It is noteworthy that in spite of the apparent association of CAMs (both A-CAM and L-CAM) with apical junctions, positive labeling was often detected along the entire lateral cell borders in early stages of development, and only later became restricted to the adherens junction area in the apical region. This phenomenon, which has also been observed in other developing epithelia (Boller *et al.*, 1985; Duband *et al.*, 1988), may be attributed to an excess of CAMs relative to other junctional constituents which remain in the extrajunctional

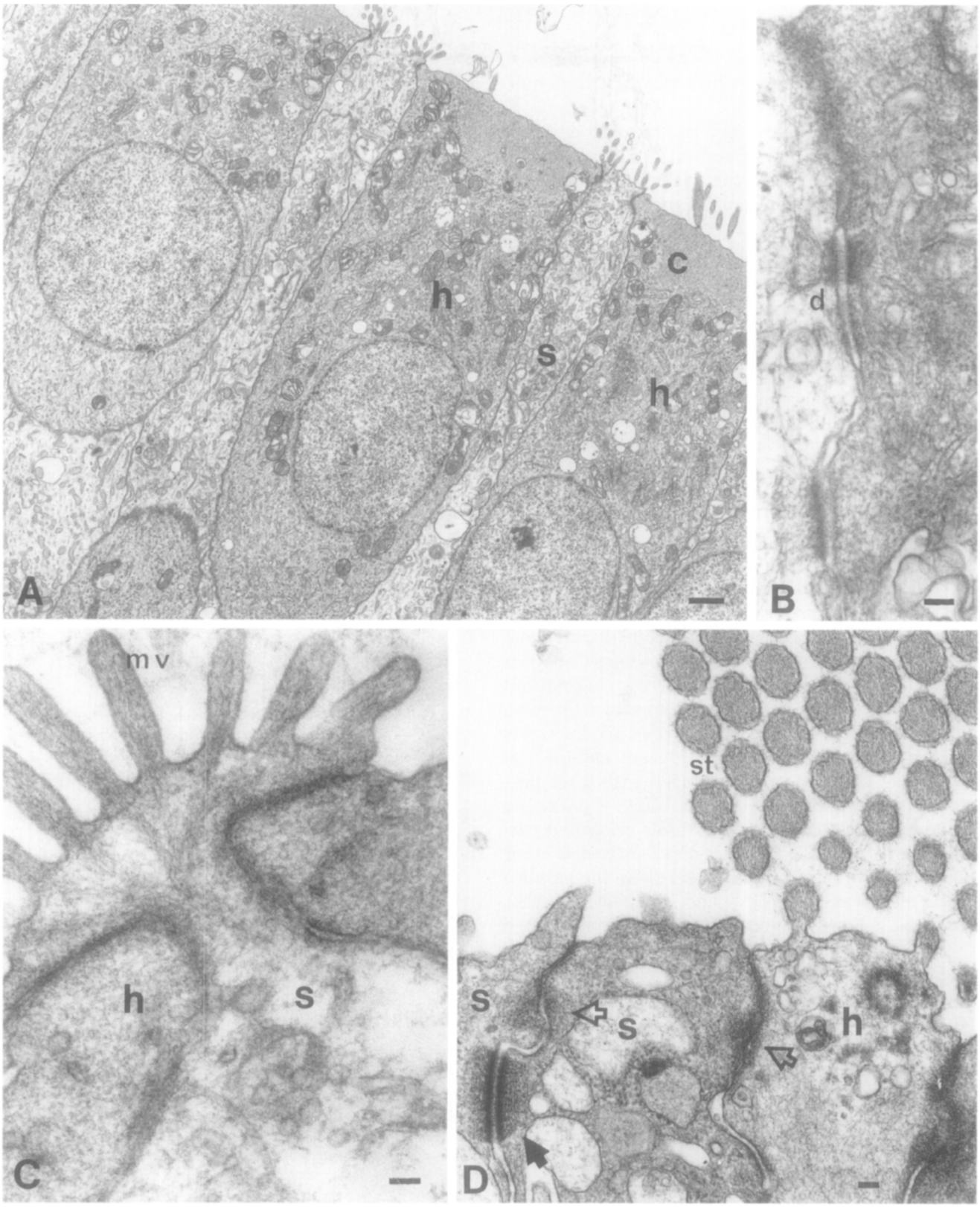

FIG. 8. Electron photomicrographs of mature avian cochlear epithelia, showing well-developed hair cells with cuticular plates and stereocilia, surrounded by supporting cells covered apically with microvilli (A). The junctions between hair and support cells in the basilar papilla do not contain desmosomes ((A) and at higher magnification (C)), but in the tegmentum vasculosum (B) and all other nonsensory epithelia of the duct, desmosomes are prominently found. Desmosomes were also seen in the basilar papilla, in junctions formed by two adjacent support cells (D). Note the well-developed adherens junction in the contact of a hair cell and a support cell (arrow) in (D). h, hair cell; s, support cell; c, cuticular plate; d, desmosome. Bars represent 1 μm in (A) and 0.1 μm in (B–D).

area, to transitional states in contact formation, or to additional cellular elements which may modulate junction assembly.

Morphogenetic processes such as invagination of the otic placode, closure of the otic pit, and the complex growth and remodeling of the various (auditory and vestibular) organs in the membranous labyrinth require an efficient and spatiotemporally regulated force-generating mechanism. The association of adherens junctions with the microfilament system raises the possibility that one of the roles of these junctions may involve the organization and regulation of the force-generating network of actin microfilaments. Such a system might participate in the establishment of mechanical forces which are necessary for the coordinated spatial modulation of the epithelium, similar to those described during neurulation and other dynamic processes (Jacobson, 1985).

The functional significance of the differential expression of A-CAM and L-CAM in the various auditory epithelia is not clear. It should be mentioned that the well developed adherens junctions in the former are the only type of cytoskeleton-bound junction present between hair and support cells. It has been shown (Gulley and Reese, 1976; Ginzberg and Gilula, 1979), and confirmed in the present study, that desmosomes disappear from the developing vertebrate hair cells, concomitant with the reduction in cytokeratin expression in them (Raphael *et al.*, 1987). Mature hair cells are known to function as mechanochemical transducers, and for that purpose they are apparently equipped with a remarkably sophisticated network of actin which is packed in the stereocilia in a complex, yet highly organized pattern (Tilney *et al.*, 1980). Actin is also localized at other sites, such as the cuticular plate and along the basolateral cell membrane (Slepecky and Chamberline, 1982, 1986). Thus, adherens junctions which are associated with potentially contractile microfilament bundles (Geiger *et al.*, 1983, 1984, 1985a,b) may play a major role in maintaining the intercellular adhesion and in the generation of transepithelial tension, properties which play an important role in inner ear physiology.

In conclusion, the present results showed that in the avian otocyst, A-CAM is coexpressed with L-CAM in the entire epithelium. The formation of asymmetries between sensory and nonsensory areas in the epithelium is correlated with the modulation of CAM expression and reorganization of intercellular junctional complexes. During that process, A-CAM and L-CAM become mutually exclusive, the former being expressed only in the mosaic-organized sensory epithelium. Concomitantly, desmosomes disappear from hair cell junctions but remain in the intercellular junctional complexes of all other epithelial cell types in the membranous labyrinth. A-CAM-positive cells were found in the region of the acoustic ganglion in early developmental stages but not in the mature neural elements.

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