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Ongoing production of sensory cells in the vestibular epithelium of the chick

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Recent studies have shown that the vestibular and auditory systems of some species of birds have the capacity to generate sensory hair cells postnatally. We used a traditional technique, ^{3}H-thymidine autoradiography, and a newer method, bromodeoxyuridine immunocytochemistry, to determine whether ongoing proliferation of hair cells occurs in the intact chick vestibular epithelium. A ten-day course of ^{3}H-thymidine, bromodeoxyuridine, or both was administered to twelve-day-old chicks. Both autoradiographic and immunocytochemical labeling demonstrated ongoing production of supporting cells and Type II hair cells in all chick vestibular organs. No evidence for production of Type I hair cells was seen in this investigation. New sensory cells were distributed throughout the epithelium; there was no peripheral growth zone analogous to that found in other vertebrates. Labeled Type II hair cells were frequently seen immediately above labeled supporting cells. This observation suggests that supporting cells are precursors for new hair cells. The ongoing, postnatal regeneration of vestibular epithelial cells also suggests that this epithelium may retain the potential for repair after trauma or ototoxic damage.

Vestibular system; Hair cell; Avian; Cell proliferation; ^{3}H-thymidine; Bromodeoxyuridine

Introduction

The hair cell of the inner ear is a mechanoreceptor which transduces mechanical stimuli into neural impulses. Its morphology and function are maintained across a wide range of vertebrate classes. Some bony fishes and amphibians maintain the capacity to produce new hair cells throughout life (Corwin, 1981, 1983, 1985; Popper and Hoxter, 1984, 1990). In contrast, it has long been believed that proliferation and division of sensory hair cells in the inner ear of mammals and birds can occur only during prenatal and early postnatal development (Ruben, 1967). Recent studies, however, have shown that production of new sensory hair cells occurs in the avian inner ear. Jørgensen and Mathiesen (1988), studying the adult budgerigar (Australian parrot), were the first investigators to report on ongoing proliferation of sensory hair cells in the intact vestibular epithelium. At about the same time, several studies on the avian basilar papilla showed that while new sensory hair cells were not produced under normal circumstances, regeneration of hair cells occurred following acoustic or ototoxic trauma (Cruz et al., 1987; Cotanche, 1987; Corwin and Cotanche, 1988; Ryals and Rubel, 1988).

In the present study we utilized two labeling techniques for the identification of proliferating cells in the avian vestibular epithelium. ^{3}H-thymidine autoradiography is the classical and most accepted technique for the identification of dividing cells. The procedure, however, has the disadvantages of being time-consuming and requiring the use of radioactive materials. Additionally, we tested the feasibility of 5-bromo-2′-deoxyuridine (BrdU) incorporation as a proliferation marker for the inner ear. BrdU is a thymidine analogue incorporated into DNA during the 'S' phase of the cell cycle (Sugihara et al., 1986). Unlike ^{3}H-thymidine, BrdU is antigenically distinct from normal thymidine. Thus, it can be recognized by a monoclonal antibody, and labeled with immunocytochemical techniques.

The present study was designed to answer three questions: (i) Does ongoing production of vestibular epithelial cells occur in the domestic chicken? (ii) If so, is the pattern of proliferation similar to that seen in other vertebrates? (iii) Can dividing cells in the chick

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vestibular epithelium be identified as effectively with BrdU immunocytochemistry as with ^{3}H-thymidine autoradiography?

Materials and Methods

Subjects

Seven, twelve-day-old White Leghorn chickens (*Gallus domesticus*) received twice-daily intramuscular injections of proliferation markers for ten days. Three animals received 50 mg/kg BrdU (SIGMA, St. Louis, MO) per injection. Eleven vestibular organs from these animals were processed with immunocytochemical techniques. Two chicks were given 10.00 mCi/kg ^{3}H-thymidine (ICN Radiochemicals, Irvine, CA; specific activity 60–90 μCi/M) per injection. Six vestibular organs from these animals were processed for autoradiography. In order to validate the use of BrdU in these tissues, two chicks were given injections of both BrdU and ^{3}H-thymidine. Five vestibular organs from these animals were processed, first with immunocytochemical techniques and then for autoradiography. All animals were sacrificed either 11 or 12 days after the last proliferation marker injection.

Immunocytochemical labeling

After deep anesthesia with inhaled Halothane the chicks were perfused transcardially for 10 min with 4% paraformaldehyde in 0.4 M sodium/potassium phosphate buffer at pH 7.4. Systemic perfusion was immediately followed by intralabyrinthine perfusion, through the oval window, with the same fixative. The heads were kept in fixative for 4 h at room temperature and then stored in phosphate buffered saline (PBS), pH 7.4, for up to two weeks at 4°C. The bone surrounding the membranous labyrinth was removed, exposing the cristae, vestibule, and basilar papilla. The individual membranous organs were removed with fine-tip forceps and placed in separate glass vials.

Poly / rec media embedded tissue. Half of the vestibular organs were washed three times in PBS and immersed in 1 N hydrochloric acid (HCl) for 30 min to hydrolyze the DNA and expose the BrdU antigen (Sugihara et al., 1986). These organs were again washed three times in PBS, dehydrated in a graded acetone series, and embedded in Poly/Rec Media (Polysciences, Warrington, PA), a resin chosen for its permeability to immunolabeling antibodies. The organs were later sectioned at 3 μm and mounted on chrome-alum subbed slides. Tissue sections were placed in 100% acetone for one hour to remove the embedding medium. They were then rehydrated and incubated in pronase, 10 units/ml (Calbiochem–Behring, La Jolla, CA) for 10 min, to expose the antigenic sites (Sugihara et al., 1986). The sections were successively incubated in 3.6% normal horse serum for 1 h, a 1:300 dilution of monoclonal antibody to BrdU (B-44, Becton-Dickinson, Mountain View, CA) for 2 h, a 1:222 dilution of biotinylated horse-anti-mouse antiserum (Vector, Burlingene, CA) for 1 h, and avidin-biotin complex (Vector, Burlingene, CA) for 1 h. All reagents were diluted in PBS with 0.5% bovine serum albumin. The slides were washed three times in PBS after each reagent, except that no wash was performed after the incubation in the normal horse serum. All incubations were carried out at room temperature. The antibody complex was visualized using 0.01% hydrogen peroxide and 0.75 mg/ml diaminobenzidine in Tris buffer, pH 7.6, for 10 min, and the slides were again washed three times in PBS. The sections were then counterstained with 0.1% Fast Green FCF in 1.0% acetic acid for 60 s, dehydrated in a graded series of alcohols and xylenes, and coverslipped with DPX.

Spurr's embedded tissue. The remaining half of the vestibular organs were dehydrated in serial alcohols (35, 70, 95, and 100% ethanol) and propylene oxide, and embedded in Spurr's plastic (Polysciences, Warrington, PA). Serial semi-thin sections (2.5–3.0 μm) were placed on a drop of 0.05% poly-vinyl-alcohol (DuPont) on acid-washed slides and allowed to dry. The sections were then deplasticized by submersion in a saturated solution of sodium ethoxide, rehydrated by reversing the alcohol series, and partially digested with HCl (1 N, 30 min) and pronase (5 units/ml, 10 min) to expose the antigenic sites on the DNA. The rest of the procedure followed the steps described above.

Autoradiographic labeling

After deep anesthesia with inhaled Halothane the chicks were perfused transcardially for 10 min with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at 4°C, pH 7.4. Systemic perfusion was followed immediately by intralabyrinthine perfusion through the oval window with the same fixative, and the heads were kept in fixative overnight at 4°C. The vestibular end-organs were removed as described above. The organs were washed three times in PBS, dehydrated in a graded series of methanols and acetones, embedded in Polybed media (Polysciences, Warrington, PA), sectioned at 3 μm and mounted on acid-washed chrome-alum subbed slides. The slides were dipped in Kodak NTB2 Nuclear Track Emulsion (1:1 dilution) and allowed to expose for six to 12 weeks at 4°C. They were then developed with Kodak D-19 developer for 4 min and fixed in Kodak Fix for three and one half minutes. Slides were lightly counterstained with toludine blue, dehydrated in a graded series of alcohols and xylenes, and coverslipped with DPX.

Double labeling

Tissue from animals injected with both BrdU and ^{3}H-thymidine was processed for both immunocytochemical and autoradiographic labeling. This tissue was fixed, embedded in Poly/Rec Media, sectioned and labeled for BrdU as described in the immunocytochemical labeling protocol. One exception is that the slides were not immediately counterstained or coverslipped. Instead, they were dipped in emulsion and developed as described in the autoradiographic labeling protocol. Finally, the tissue was counterstained with Fast Green FCF, dehydrated, and coverslipped with DPX.

Analysis of supporting / hair-cell pairs

In order to assess the spatial relationship between newly proliferated cells that differentiated into hair cells and those that became support cells, we analyzed the relative number of occurrences in which labeled pairs of cells were vertically aligned in the sensory epithelium.

A Chi-square contingency table (Edwards, 1967) was constructed to determine if the occurrence of labeled supporting/hair-cell pairs was significantly more common than would be expected if the labeled supporting cell and hair cell nuclei were distributed randomly throughout the epithelium. Ten tissue sections, spaced 50 μm apart, were examined from a lateral ampulla labeled with BrdU; similarly, ten sections from a utricular macula labeled with BrdU were examined. A labeled supporting/hair-cell pair was considered to exist only when the two labeled nuclei actually overlapped in a line perpendicular to the epithelial surface. Thus the maximum possible width of a pair would be the combined width of two nuclei which barely overlapped each other. Each tissue section was therefore considered to consist of segments whose width was defined as twice the average width of a labeled nucleus; the number of segments in each tissue section was calculated by dividing the width of the tissue section by the segment width. Each segment could potentially contain either 1) no labeled nuclei, 2) a labeled supporting cell nucleus only, 3) a labeled hair cell nucleus only, or 4) a labeled supporting/hair-cell pair. The observed probability of each of these possibilities could be readily calculated by dividing the actual number of events by the total number of epithelial segments examined in each organ. For example, the observed probability of a labeled supporting cell in any segment was calculated by dividing the number of labeled supporting cells observed by the total number of segments examined.

The observed probability of a labeled supporting/hair-cell pair in any segment was calculated by dividing the number of such pairs observed by the total number of segments examined. The predicted probability of a labeled supporting/hair-cell pair in any segment (assuming random distribution of the labeled supporting cells and hair cells) was calculated by multiplying the observed probability of a labeled supporting cell by the observed probability of a labeled hair cell.

Results

Normal vestibular anatomy

The anatomy of the vestibular labyrinth is highly conserved across vertebrate classes (Ramprashad et al., 1986). There are two types of vestibular sensory organs: the cristae ampullaris which detect angular acceleration, and the maculae (the otolithic organs) that may detect gravitational and centripetal accelerations as well as vibrational stimuli (Iurato, 1967). The chick vestibular labyrinth contains three cristae ampullaris: anterior, posterior, and lateral, and three maculae: the utricle, the saccule, and the lagena (Retzius, 1894; Tanaka and Smith, 1978).

The cellular morphology of the sensory epithelium is essentially identical in all the vestibular end-organs, consisting of supporting cells and two types of hair cells (Wersäll, 1956; Iurato, 1967; Lindeman, 1969). Low power photomicrographs of the chick vestibular epithelium are shown in Figs. 1 and 2. The supporting cells extend from the basement membrane to the lumenal surface of the sensory epithelium. The basal cytoplasm of the supporting cells forms a continuous sheet along the basement membrane which is pierced at intervals by nerve fibers. The cytoplasm of the supporting cells reaches the surface of the epithelium, enveloping the sensory hair cells. The supporting cell nuclei form a fairly uniform row along the basement membrane, and are confined to the lower portion of the epithelium.

The two types of sensory hair cells, designated Type I and Type II, are primarily distinguished by the morphology of their innervation (Wersäll, 1956; Iurato, 1967; Jørgensen and Cristensen, 1989). Type I hair cells are enclosed in a nerve calyx which surrounds all but the apical portion of the cell, and synaptic connections are observed at intervals throughout the interface between the hair cell and calyx. One to several hair cells may be enveloped by a single calyx. Type II hair cells receive small boutonal nerve endings around the basal portion of the cell. A small number of hair cells have synaptic connections both with an abutting calyx and with bouton endings. The nuclei of both Type I and Type II hair cells are located in the upper two-thirds of the sensory epithelium, above the row of supporting cell nuclei (Fig. 3).

Labeling of supporting cell nuclei and hair cell nuclei

Labeling of supporting cell nuclei and Type II hair cell nuclei is seen using both ^{3}H-thymidine autoradiog-

raphy and BrdU labeling. A section from a crista ampullaris processed for BrdU immunolabeling and a section from a crista ampullaris processed for autoradiographic labeling are shown in Fig. 1. In the tissue processed immunocytochemically, labeled nuclei are seen as the dark round areas in the epithelium (reaction product). In the tissue processed for autoradiography, labeled supporting cells and hair cells are identified by the presence of exposed silver grains over their nuclei. A crista ampullaris labeled with both BrdU immunocytochemistry and ^{3}H-thymidine autoradiography is shown in Fig. 4. Dividing nuclei are labeled with both techniques; the large, dark immunocytochemically labeled nuclei are overlain with silver grains. Essentially all labeled nuclei are identified by both techniques; there was no indication that either method is more sensitive.

Pattern of proliferation

Cell types. It was not possible to distinguish Type I from Type II hair cells unequivocally in the tissue processed for BrdU immunocytochemistry (Figs. 1A and 2A). In the tissue processed for autoradiography, labeling was observed only in the nuclei of supporting cells and Type II hair cells (Figs. 1B, 2B, and 3). At the survival times examined in this study, no labeled Type I hair cell nuclei were observed in any organ.

Distribution of labeled nuclei. Labeled nuclei were distributed throughout all areas of all vestibular organs, including the superior, lateral, and posterior ampullae, and the utricular, saccular, and lagenar maculae. Although no formal measurement of the distribution of labeled nuclei was attempted, there was no obvious anterior-posterior or medial-lateral distribution of labeled nuclei in any of the organs examined.

Supporting / hair-cell pairs. Two representative sections of utricular maculae, one processed with immunocytochemical techniques and the other for autoradiography, are shown in Fig. 2. Labeled supporting cell nuclei are identified by their position along the basement membrane, while labeled hair cell nuclei are identified by their position in the upper two-thirds of the epithelium. The frequent occurrence of labeled hair cell nuclei directly above labeled supporting nuclei is easily appreciated from this figure. We refer to these as supporting/hair-cell pairs. In most sections more than one half of the labeled hair cell nuclei are associated with labeled supporting cell nuclei.

In the tissue sections from the lateral crista ampullaris, a total of 403 segments were examined. There were 38 labeled supporting cells (observed probability = 0.094), 24 labeled hair cells (observed probability = 0.060), and the predicted probability of a labeled supporting/hair-cell pair was $0.094 \times 0.060 = 0.0056$. The

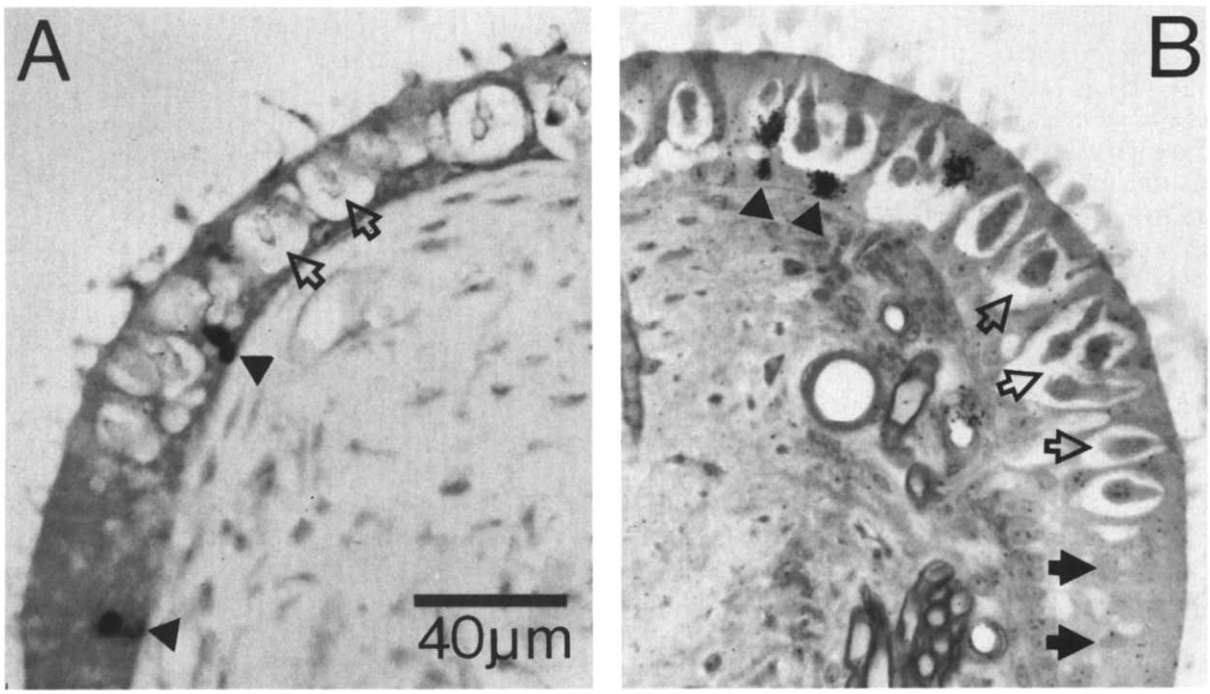

Fig. 1. Normal vestibular sensory epithelium in the chick, and ongoing level of proliferation. Supporting cell nuclei are seen in a fairly uniform row on the basement membrane, below hair cell nuclei. Type I hair cells are enveloped by nerve calyces (open arrows); Type II hair cells have bouton type nerve endings (solid arrows). A) Cross section of a crista ampullaris processed for BrdU-immunostaining. Labeled nuclei are recognized by the darkly stained reaction product on the cell nuclei (arrowheads). B) Cross section of a crista ampullaris processed for autoradiography. Exposed silver grains over nuclei indicate incorporation of ^{3}H-thymidine into the DNA of these nuclei during the 'S' phase of the cell cycle (arrowheads). Two supporting cells and as many Type II hair cells are labeled.

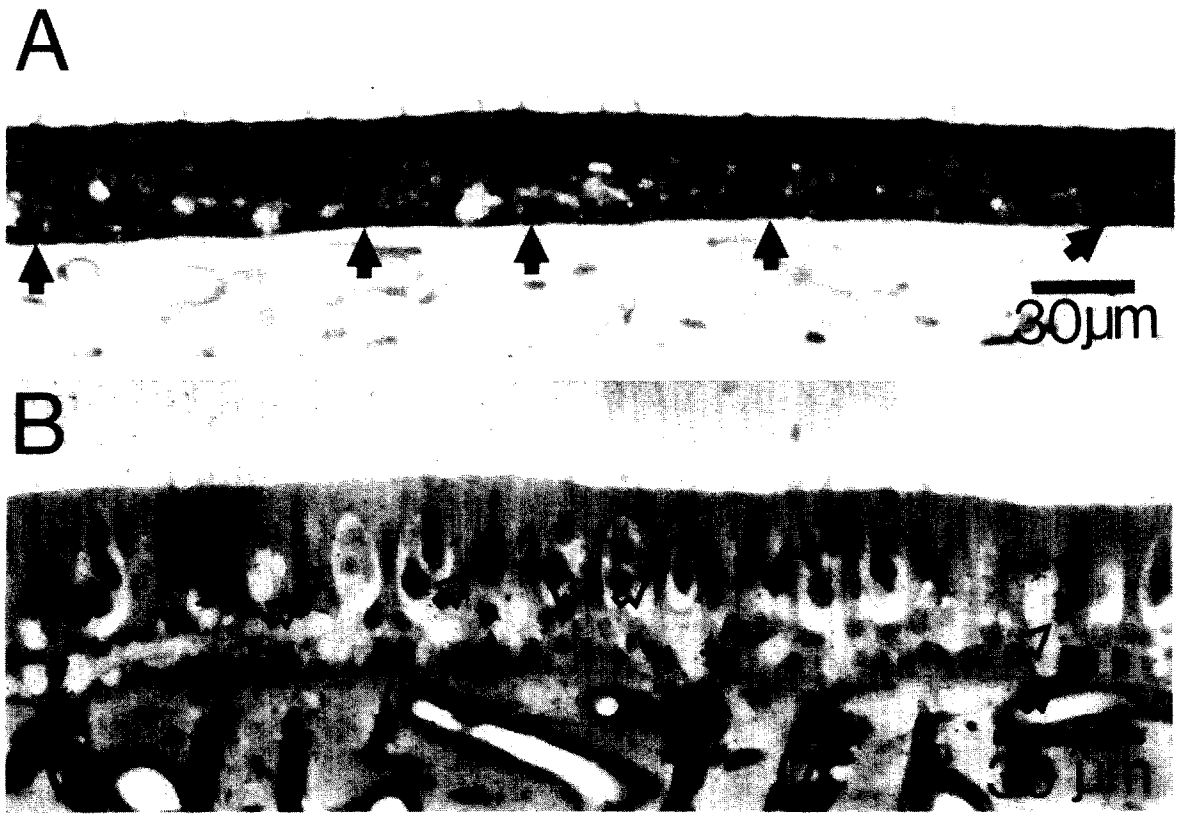

Fig. 2. Avian utricular macula. Note similarity of epithelial anatomy in vestibular organs (see Fig. 1). A) Tissue processed for BrdU-immunostaining. B) Tissue processed for autoradiography. Labeled supporting cell nuclei (solid arrows, located adjacent to the basement membrane) and Type II hair cell nuclei (open arrows, in the upper two thirds of the epithelium) are labeled with silver grains or the reaction product. Note the distribution of labeled nuclei throughout all areas of the epithelium, and the frequent occurrence of labeled hair cell nuclei immediately above labeled supporting cell nuclei.

number of such pairs actually observed was 14 and the observed probability was therefore 0.035 ($P < 0.001$).

Statistical analysis of the relative occurrence of supporting/hair-cell pairs revealed an occurrence much greater than predicted by random assessment. In the tissue sections from the utricular macula, a total of 459 segments were examined. There were 46 labeled supporting cells (observed probability = 0.10), 31 labeled hair cells (observed probability = 0.068), and the predicted probability of a supporting/hair-cell pair was $0.10 \times 0.068 = 0.0068$. The number of pairs actually observed was 17 and the observed probability was 0.037 ($P < 0.001$).

Discussion

Ongoing proliferation in the vestibular system of adult budgerigars has previously been reported (Jørgensen and Mathiesen, 1988). In the present study we utilized a traditional technique, ^{3}H-thymidine autoradiography, and a newer method, BrdU immunocytochemistry, to document cell proliferation in the vestibular epithelium of young chickens. We will first discuss the usefulness of BrdU labeling relative to ^{3}H-thymidine autoradiography, and then we will address the observed pattern of cell division.

BrdU labeling

Both BrdU immunocytochemistry and ^{3}H-thymidine autoradiography effectively labeled dividing cell nuclei. In tissue exposed to the two markers, the same nuclei were labeled by both techniques. ^{3}H-thymidine has been previously used to label proliferating cells in the avian inner ear. The results from this study indicate that BrdU immunolabeling is as effective as ^{3}H-thymidine autoradiography in identifying dividing cells in the avian labyrinth. BrdU labeling can also be used to identify regenerating hair cells in the chick basilar papilla following acoustic trauma (Roberson and Rubel, unpublished observations).

BrdU immunocytochemistry has three distinct advantages over ^{3}H-thymidine autoradiography. BrdU immunocytochemical labeling may be developed in a single day, while tissue prepared for autoradiography must be allowed to expose the emulsion for several weeks or months. Secondly, although BrdU is a potential mutagen and teratogen, it nonetheless requires

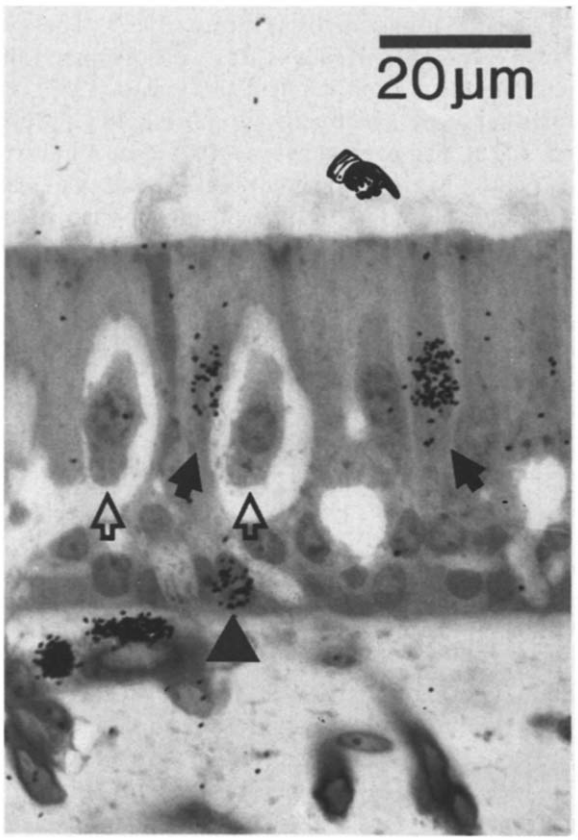

Fig. 3. High power photomicrograph of the avian utricular macula. This tissue section was obtained from a bird allowed to survive 12 days after the last tritiated thymidine injection. Two unlabeled Type I hair cells (open arrows) are displayed. At least one, and possibly two Type II hair cells (solid arrows) are labelled. Pointer aims at stereocilia of labeled Type II hair cell. In addition, one supporting cell has also incorporated the proliferation marker (arrowhead). Labeled cells to the left of arrowhead are endothelial cells which form part of the stroma.

considerably fewer precautions to handle than radioactive materials. Finally, BrdU immunocytochemistry produces a high contrast label that is easily visualized under low magnification, thus allowing rapid examination of large areas of tissue. This feature is particularly useful for quantitative studies.

The primary disadvantage of BrdU labeling is the relatively poor quality of the tissue-sections which are obtained. Tissue prepared for BrdU labeling must be fixed for a relatively short time because excessive fixation creates cross-linkages which render the BrdU antigen inaccessible to the antibody. The plastic in which the tissue is embedded must be chosen for its permeability to the antibody rather than for optimal tissue preservation. Finally, the tissue must be treated with HCl and pronase prior to the immunocytochemical labeling. HCl uncoils the DNA helix and pronase presumably breaks fixation cross-linkages, but these treatments also disrupt other cellular elements. Of the two processing methods for BrdU immunocytochemistry used, embedding the tissue in Spurr's before exposing it to HCl and pronase, yielded better quality histology. This procedure allows HCl and pronase direct access to the cell nuclei while the embedding material provides some protection to fine structures such as the stereocilia.

The tissue processed for autoradiographic labeling rendered the best histology. In this case, the labeling is produced by the emission of radioactive decay products. There are thus no constraints upon the choice of fixative or embedding media, nor is any pre-treatment of the tissue necessary.

A second undesirable effect of BrdU was that it appeared to interfere with normal growth. At the end of the 10 day course of injections, birds which had received BrdU weighed 10 to 15% less than those which had received only ^{3}H-thymidine. Other workers who have used BrdU to label mitotic cells in other systems have not reported the growth stunting seen in our experiments (Miller and Nowakowski, 1988). Most of the previous studies have used lower doses of BrdU; it is possible that lower doses would have resulted in adequate labeling of nuclei in the vestibular sensory epithelium with less growth inhibition.

A third unwanted property of BrdU is that it has the effect of blocking expression of the differentiated phenotype in many different cell lineages. For example, increasing concentrations of BrdU have been shown to result in the production of progressively fewer chicken erythrocytes, and addition of BrdU to a cultured mouse myoblast line blocked myogenic differentiation (Tapscott et al., 1989).

Pattern of cell proliferation in vestibular epithelium

Proliferation of supporting cells and hair cells is seen in the sensory epithelium of all chick vestibular end-organs, but the pattern of proliferation is quite different from that reported in other vertebrates. The differences include the identity and anatomical location of precursor cells, as well as the types of new cells produced.

Labeled nuclei are distributed throughout the epithelia, without aggregation in any particular area. In otolithic organs from sharks, proliferation is primarily seen in a 'growth zone' at the periphery of the organs (Corwin, 1981). No such zone is apparent in the current study, nor was 'annular' addition reported in budgerigars (Jørgensen and Mathiesen, 1988) or in the adult oscar (Popper and Hoxter, 1990).

A consistent event seen in this study is the occurrence of labeled Type II hair cell nuclei directly above a labeled supporting cell nuclei. The abundance of these pairs suggests that the supporting cells may be precursors for the new vestibular hair cells. Alternatively, new cells may be derived from a population of stem cells distinct from the supporting cell population,

as has been described in an adult fish (Presson and Popper, 1990). While no morphologically distinct population of precursor cells could be distinguished by light microscopy, it is possible that more detailed studies using electron microscopy or immunological markers may reveal more than one population of precursor cells.

Thus, while the precursor cells for new sensory elements in some vertebrates are a select population of cells grouped in a growth zone at the periphery of the organ, precursor cells in the chick vestibular epithelium appear to be distributed throughout the epithelium and are either supporting cells, or an uncharacterized stem cell population found within the epithelium.

Types of cells produced

In this study, only supporting cells and Type II hair cells (boutonal innervation) were labeled in the chick vestibular epithelium. No labeled Type I hair cells (calycial innervation) were observed. At least two possible explanations exist for the absence of labeling on Type I hair cells. Several lines of evidence suggest that Type I hair cells are phylogeneticaly younger than Type II hair cells. The hair cells of fish possess only bouton endings (Flock, 1964; Wersäll, 1961), and mammalian Type II hair cells are morphologically similar to the hair cells found in fish (Iurato, 1967). The cytoplasm of Type II hair cells is less well organized than the cytoplasm of Type I hair cells (Iurato, 1967), suggesting that Type I hair cells are more highly differentiated. It has also been suggested that Type I hair cells appear later in development than Type II hair cells. For example, Friedman (1959), using in vitro otocyst cultures, noted that no nerve calyces could be identified at 14–16 days of development. He suggested that the calyces may not be fully developed at this age. If Type I hair cells are more highly differentiated than Type II hair cells, perhaps the organism has lost the ability to produce these cells post-embryonically as they have become more specialized.

Alternatively, one might speculate that Type I hair cells develop from Type II hair cells. Hair cells with both types of innervation have been described (Iurato, 1967). Perhaps nerve calyces gradually envelop Type II hair cells, thus creating Type I hair cells. If so, longer survival periods after injections would be expected to yield labeled Type I hair cells. Preliminary data from a recent experiment (Weisleder and Rubel, 1991) demonstrated that chicks allowed to survive sixty days after proliferation marker injections have labeled Type I hair cells in the vestibular sensory epithelium. It is conceivable that Type I hair cells were not detected in

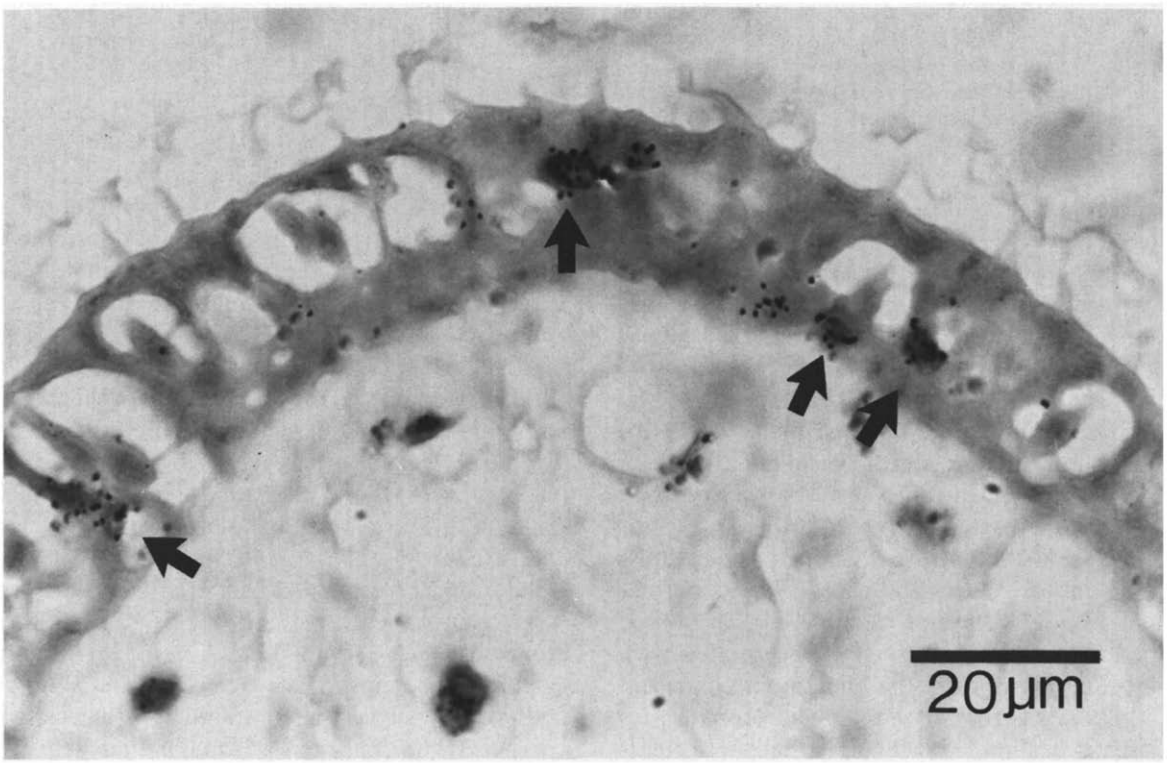

Fig. 4. A crista ampullaris processed for both BrdU immunocytochemistry and ^{3}H-thymidine autoradiography. Double-labeled hair cells and supporting cells (arrows) have nuclei displaying the reaction product toped by exposed silver grains.

the present experiment due to the short survival time following cell proliferation marker injections.

Evidence for hair cell turnover

Current evidence suggests that the vestibular supporting cells and Type II hair cells produced are replacing dying cells in the sensory epithelium, rather than increasing the total number of cells in the epithelium. Jørgensen (1981) suggests that the presence of 'light' and 'dark' * hair cells in the inner ear of an amphibian caecilian reflects turnover of the vestibular sensory epithelium. Using transmission electron microscopy he was able to differentiate several types of hair cells based on stereocilia morphology, cytoplasmic contents, and basal innervation. The light hair cells have the cytoplasmic characteristics and nuclear location typical for hair cells, but they display a continuum of stereocilia morphology from none, to thin and compact, to large and well separated. In addition, the light cells without stereocilia are not innervated basally. In contrast, the dark hair cells have a high content of vesicles and single ribosomes with a very irregular outline and many basal cytoplasmic processes. Some dark cells may contain vacuoles and show swelling of the nuclear envelope. These features are similar to those observed in degenerating tissue. Thus Jørgensen suggests that, in the amphibian caecilian vestibular sensory epithelium, the light hair cells are younger and the dark hair cells are degenerating. In the tissue from intact budgerigars of Jørgensen and Mathiesen (1988) dark staining hair cells are occasionally observed at the light microscope level. Such dark hair cells suggest that ongoing turnover of the vestibular sensory epithelium, as seen in an amphibian caecilian, may also occur in avian vestibular end-organs.

Significance of cell proliferation

The present study adds to the growing body of evidence that the supporting and sensory cells of the avian inner ear can be produced post-embryonically, either continuously (in the vestibular system) or after epithelial insult (in the auditory system). The chicks in the present study were labeled for cell division from 12 to 24 days post-hatch. Although chicks are not fully mature at this age, the adult form of the vestibular end-organs is attained before hatching, suggesting that the vestibular epithelium is essentially mature. Since Jørgensen and Mathiesen (1988) have observed ongoing production of vestibular sensory epithelial cells in the adult budgerigar, it is likely that ongoing production of vestibular sensory epithelial cells occurs throughout the life of the animal. In the avian auditory system, production of sensory cells has only been seen following epithelial insult (Corwin and Cotanche, 1988; Ryals and Rubel, 1988), and not on an ongoing basis (Jørgensen and Mathiesen, 1988; Corwin, 1981). Since the avian vestibular epithelium shows continuous production of sensory elements, it is likely that regeneration of hair cells would also be observed after trauma or toxic injury. Such observations have recently been described in a preliminary report by Weisleder and Rubel (1991). If similar regeneration of sensory cells occurs or can be induced to occur in mammals, new therapeutic options might be possible for inner ear disease due to hair cell loss.

* Traditionally, the term 'dark cell' is used to refer to a special kind of cell located on the periphery of the sensory epithelium, thought to be involved in maintaining potassium levels in the endolymph. In the context of this discussion, and following Jørgensen and Mathiesen's practice, the term is used to describe darkly stained hair cells.

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