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Epidermal Papilloma in an Ozark Hellbender *(Cryptobranchus alleganiensis bishopi)* from the Spring River of Northern Arkansas

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Abstract

An Ozark hellbender (*Cryptobranchus alleganiensis bishopi*) with multiple, large, warty skin lesions was collected in the Spring River, Fulton County, Arkansas, in 1994. The specimen was a female, 560 mm in total length, and had a mass of 1,947 g. Tissues were formalin-fixed, and three lesions were processed for histopathology. The normal skin at the tumor margins had a stratified squamous epidermis overlying a loose, well-vascularized, heavily pigmented dermis. Poison glands and mucous glands extended from the epidermis into the dermis. The lesions, in contrast, were masses of epidermal cells up to 100 times thicker than the normal epidermis. They consisted of long, thick, branching epidermal pegs separated by thin fibrovascular papillae. The base of the lesions and all pegs had sharp boundaries bordered by a basement membrane, ruling out invasion. Tumor cells were differentiated into scattered glandular structures suggestive of dermal glands. Cells within the pegs were poorly organized. Nuclei usually contained basophilic granules. Mitotic figures were numerous. By electron microscopy, the cells appeared to be pulling apart except where held together by desmosomes. The sum of the above observations is consistent with a pathological diagnosis of epidermal papilloma.

Introduction

Amphibian tumors, in general, are rare in wild populations (Sheremetieva, 1965; Elkan, 1976; Asashima et al., 1982; Oka et al., 1992); their occurrence was reviewed by Asashima et al. (1987). Non-invasive, papillary epidermal neoplasms (known as "epidermal papillomas") have been infrequently reported. A review of the literature dating from 1935 through 1999 revealed that relatively few studies (< 30) have addressed the occurrence of skin lesions in salamanders. Most of these investigations have focused on the Japanese newt, *Cynops pyrrhogaster* (Salamandridae), in which the presence of spontaneous skin papillomas have been well studied (Honma and Murakawa, 1967; Bryant, 1973; Pfeiffer et al., 1979, 1989; Asashima and Komozaki, 1980; Asashima et al., 1982, 1985; Asashima and Oinuma, 1982; Asashima and Meyer-Rochow, 1988; Oka et al., 1992). In this newt, the prevalence of papillomas varies both seasonally and geographically (Oka et al., 1992). In North America, a high incidence of neoplastic skin lesions is known from the tiger salamander, *Ambystoma tigrinum* (Ambystomatidae), collected in sewage lagoons in northwest Texas (see Rose, 1976, 1977, 1981; Rose and Harshbarger, 1977; Harshbarger et al., 1989).

Two studies have identified skin neoplasms within the families of primitive salamanders (Hynobiidae and Cryptobranchidae). In the Aomori salamander (*Hynobius lichenatus*) from northern Japan, the incidence of skin papillomata was 0.67% (N = 7) in 1,050 individuals examined (Asashima and Meyer-Rochow, 1988). Within the giant salamanders (Cryptobranchidae), data are available on squamous cell papillomas in a single specimen of the Japanese giant salamander, *Andrias davidianus japonicus* (Frye et al., 1989), from northern Japan.

The Ozark hellbender, *Cryptobranchus alleganiensis bishopi* (Caudata: Cryptobranchidae), is a large aquatic salamander whose range is restricted to rivers and streams in the Ozark Mountains of southcentral Missouri and northeastern Arkansas (Conant and Collins, 1998). In the following, we report the first incidence of epidermal papilloma in the Ozark hellbender, a salamander whose recent population declines (Trauth et al., 1992, 1993) have gone unresolved. This observation also represents the second report for any type of neoplasm for *Cryptobranchus* (see Cosgrove and Harshbarger, 1971).

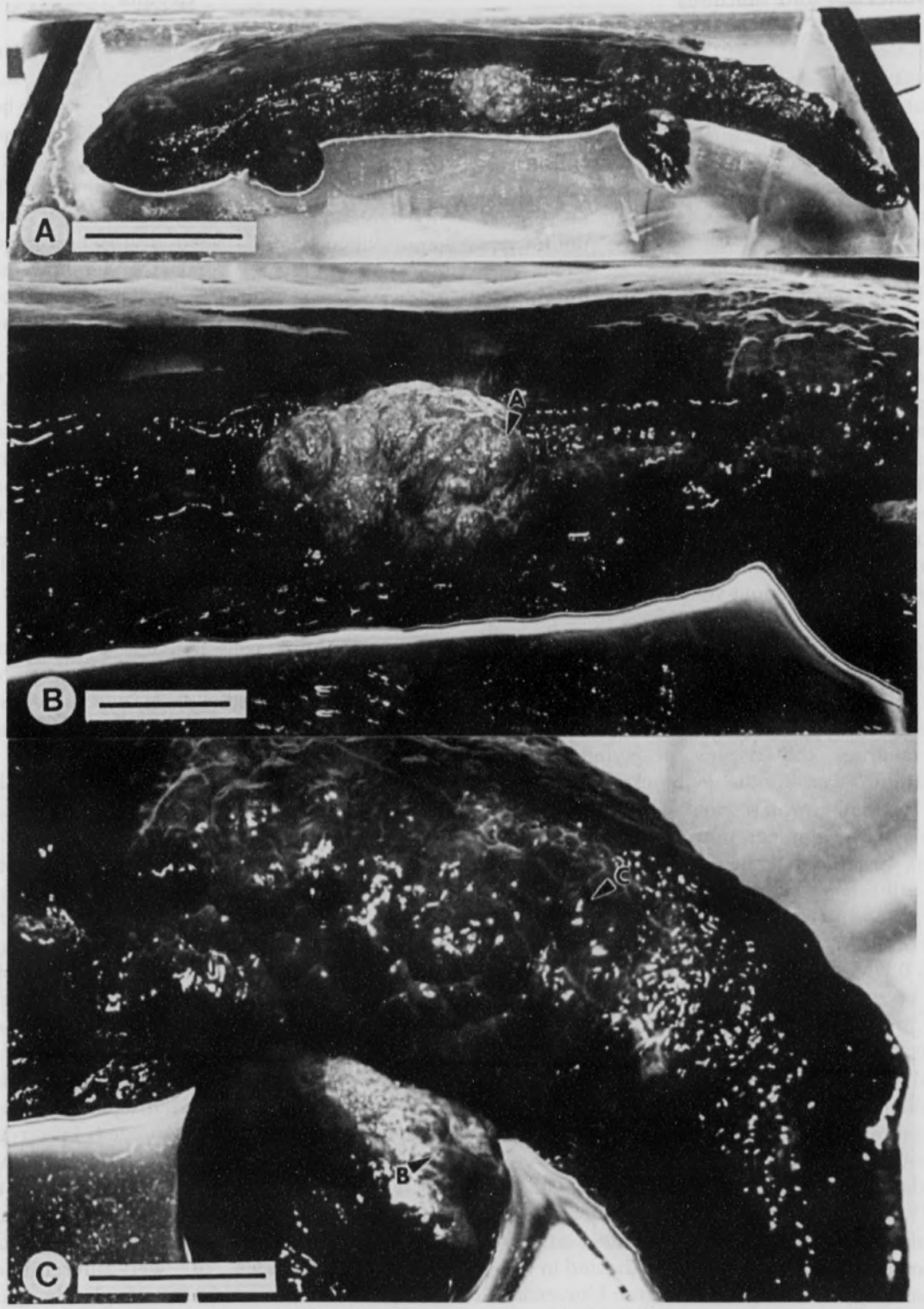

Fig. 1. Photographs of a living adult Ozark hellbender (*Cryptobranchus a. bishopi*) illustrating major skin lesion areas on the body and tail. A. Left lateral view of salamander showing a massive globular tumor mass. Line = 100 mm. B. Close up of the enlarged tumor embedded within rugose skin. Line = 25 mm; A indicates region from which tissue section shown in Fig. 3A was obtained. C. Rump and left thigh revealing large continuous papillomal region. Line = 25 mm; B and C indicate tissue sections shown in Fig. 3B and C, respectively.

Materials and Methods

On 3 October 1994, one of us (PD) collected a female *C. a. bishopi* possessing multiple skin lesions from the Spring River (Fulton County), Arkansas, in the vicinity of Bayou Access (T21N, R5 W, Sec. 33). The specimen was returned to the lab and photographed (Fig. 1); this was soon followed by the removal of small tissue samples from several skin lesion areas (dorsal tail surface, left lateral body surface, and left rear leg surface). The animal was fed live minnows and/or small crayfish and remained alive in a chilled aquarium until being sacrificed (in a 10% chloretone solution) on 15 February 1995. (The preliminary biopsied sites had successfully healed during the approximately 4.5 months in captivity.) Upon death, additional tissue samples were taken throughout the body; all samples were fixed in either 10% formalin or 2% glutaraldehyde (the latter for studies using transmission electron microscopy–TEM). The hellbender was fixed in 10% formalin, preserved in 70% ethanol, and deposited into the Arkansas State University herpetological collection as voucher specimen ASUMZ 20081. At the time of death, the salamander measured 560 mm in total length, 392 mm in snout-vent length, and had a mass of 1,947 g.

Routine histological techniques (Presnell and Schreibman, 1997) were employed to prepare the tissue samples for light microscopy (LM). In brief, these steps included dehydrating tissues in a graded series of ethanol concentrations, clearing the tissues in xylene, and embedding in paraffin. Paraffin tissue blocks were then oriented so that sectioning would yield either sagittal or transverse sections that were cut into serial strips (at a thickness of 8μm). Sectioned tissue was affixed to slides using Haupt's adhesive, stained with hematoxylin, counterstained with eosin (H&E), and then enclosed with glass coverslips. For TEM, tissue samples were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer for 2 hr and postfixed in 1% osmium tetroxide. After four rinses in cacodylate buffer, the tissues were passed through a graded series of acetones, infiltrated, embedded in Mollenhauer' Epon-Araldite Mixture number 2 (Dawes, 1988), and sectioned with a diamond knife. Sections were picked up on copper grids for examination and were stained with uranyl acetate (3% aqueous) and lead citrate. TEM images were recorded on Kodak SO 163 electron image film with a JEOL 100 CXII TEM-SCAN electron microscope. A voucher set of stained tissue slides is deposited at the Registry of Tumors in Lower Animals (RTLA) located in the Department of Pathology at George Washington University Medical Center (Washington, D.C.) and was assigned the accession number RTLA 6035.

Results

Gross Morphology.--Clusters of papillary epidermal neoplasms (epidermal papillomas) were scattered along much of the body surface of the tumor-bearing Ozark hellbender. Conspicuous anterior body lesions included those incorporating the right eye and several patchy, light-colored ovoid lesion clusters atop the head (Fig. 2). A large, highly-vascularized, globular, flesh-colored tumor (approximately 50 X 35 mm in size) protruded from the left dorsolateral, rugose surface of the body approximately midway between the fore and hind leg. Posterior to this large lesion was a broad, continuous papillomatous area on the rump. The rump patch appeared as a low, smooth, multilobate cluster of lesions; the tumorous skin appeared lighter in pigmentation compared to the surrounding normal skin. The papillomatous dorsal surface of the left hind leg was similar in appearance to the rump region.

Light Microscopy.--The histology of normal skin in *C. a. bishopi* revealed a mostly homogenous epidermis 5 - 8 cell layers in height comprised of stratified squamous (Fig. 2B) and a highly-vascularized dermis embedded with conspicuously- large, epidermal mucous glands and granular (poison) glands. Pigmented melanocytes (chromatophore cells) were evident lying along the adepidermal boundary (basal lamina) as well as being scattered throughout the dermosubcutaneous tissue. Nuclei of epidermal cells were mostly round or oval in shape. In contrast, lesions from three regions of the tumor-bearing Ozark hellbender shown in Fig. 3 revealed a striking proliferation of the epidermal cells in masses as much as 100 times the thickness of normal skin. When viewed In cross section using LM (Fig. 3), the tumor tissue was seen to consist of long, thick, branching epidermal pegs separated by thin, fibrovascular papillae. Tumor cells were presummably derived from the strateum granulosum layer; these cells also exhibited a wide range of nuclear and cytoplasmic irregularities. Mitotic figures, observed primarily in the strateum basale cells of normal skin, were not confined to this layer in the tumor tissue. A well-defined corneal layer was absent on the surface of the large tumor (Fig. 3A). In some instances, irregularly- grouped epidermal glands appeared to be squeezed into regions between proliferating cell pegs (Fig. 3B and C). Many of these secretory glands had hypertrophied to as much as eight times their normal diameter. The growth pattern of tumor cells within some epidermal pegs was that of individually-segregated masses of well-differentiated glandular compartments, reminiscent of glands lying within the dermis. In most cases, the base of the lesions as well as individual epidermal pegs had well-defined limiting boundaries bordered by a basement membrane. In one area tumor cells had breached the basement membrane and

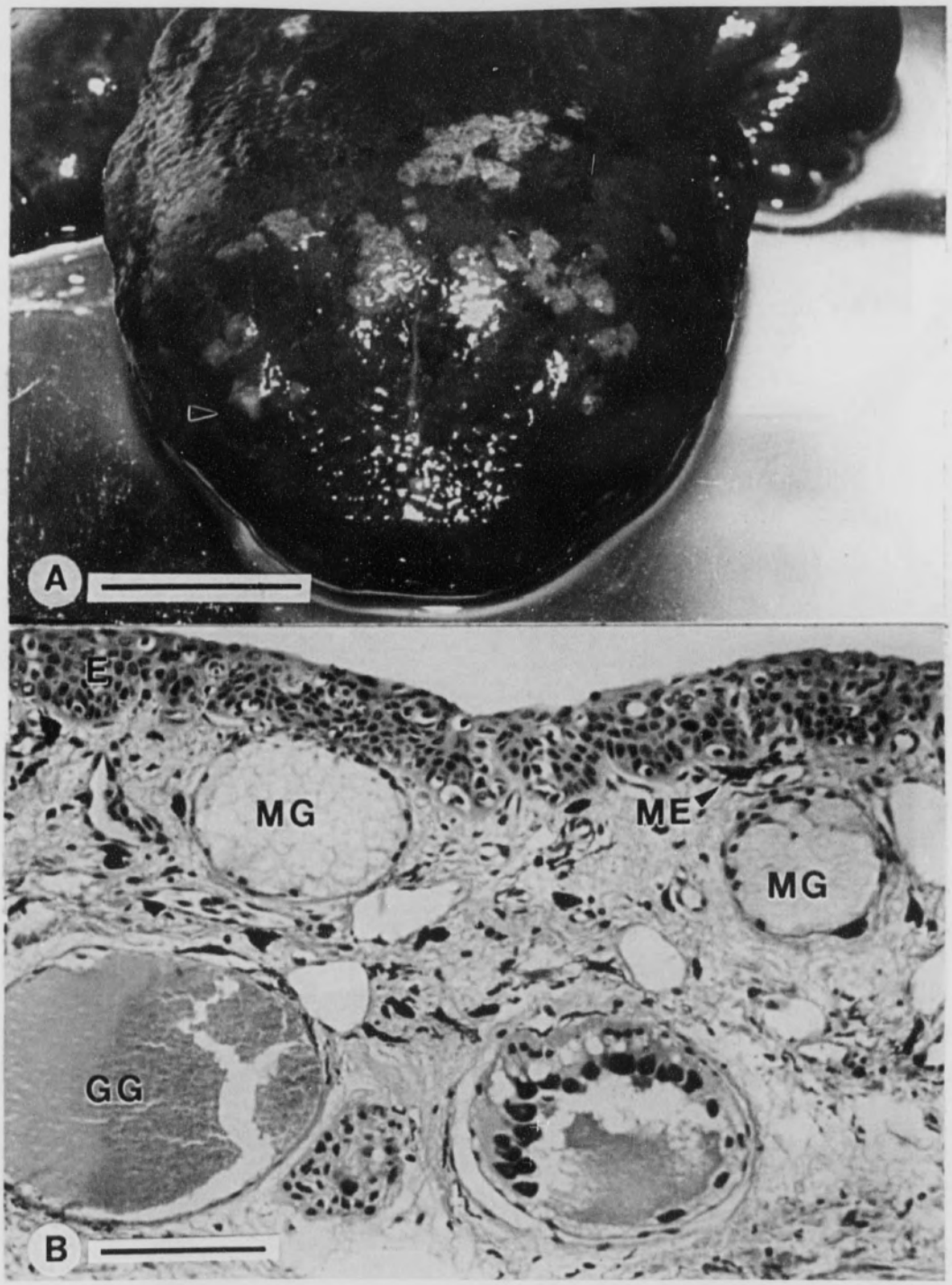

Fig. 2. Head region (A) and cross section of normal skin (B) of *Cryptobranchus a. bishopi* shown in Fig. 1. Line in A = 25 mm; arrow points to right eyeball enveloped by tumorous tissue. Line in B = 100 µm; E = epidermis; GG = granular gland; ME = melanocyte; MG = mucous gland.

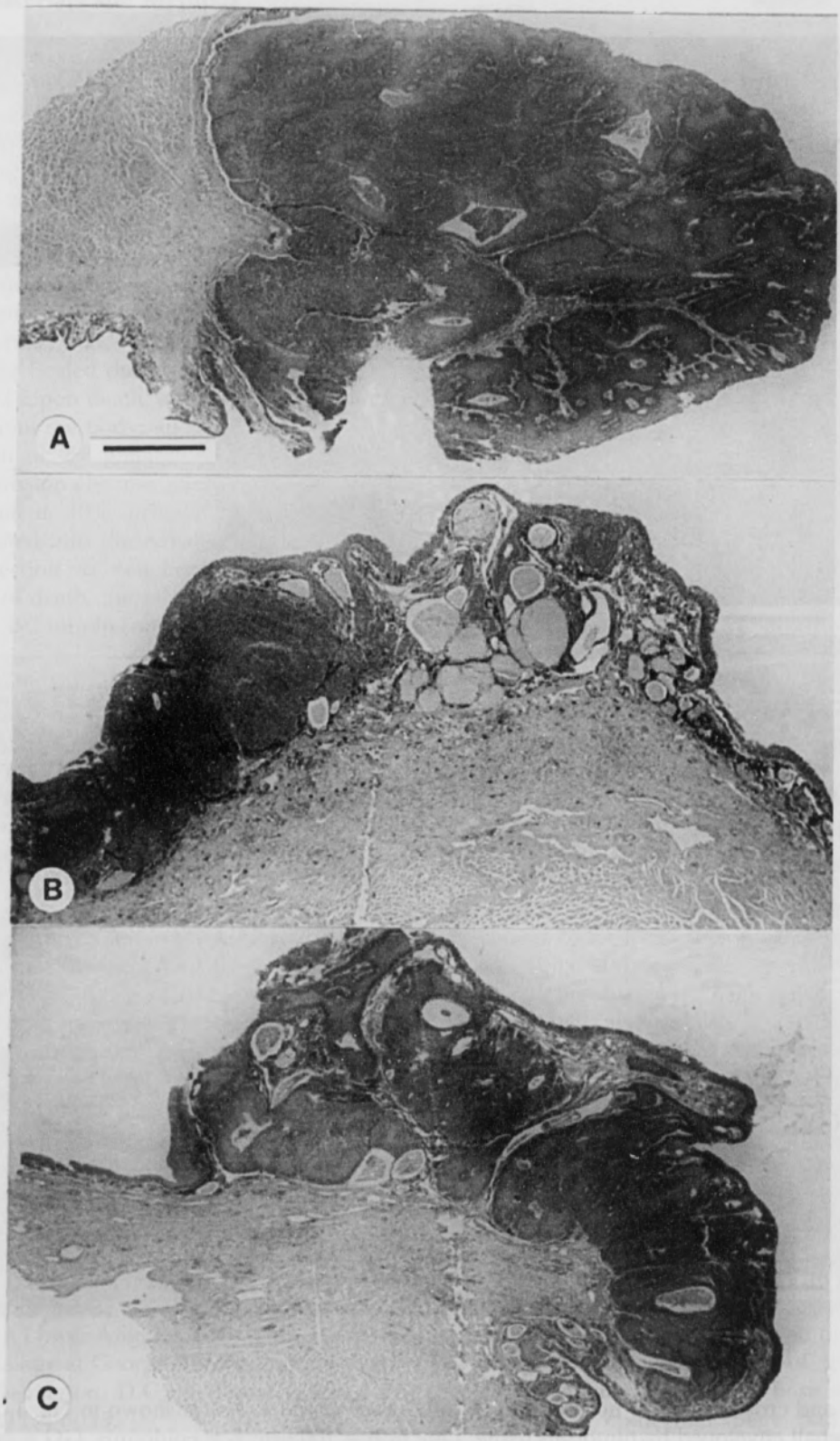

Fig. 3. Photomicrographs of neoplastic lesions from the dorsolateral body wall (A), left thigh (B), and rump region (C) of *Cryptobranchus a. bishopi* . Line in A = 2 mm for A - C.

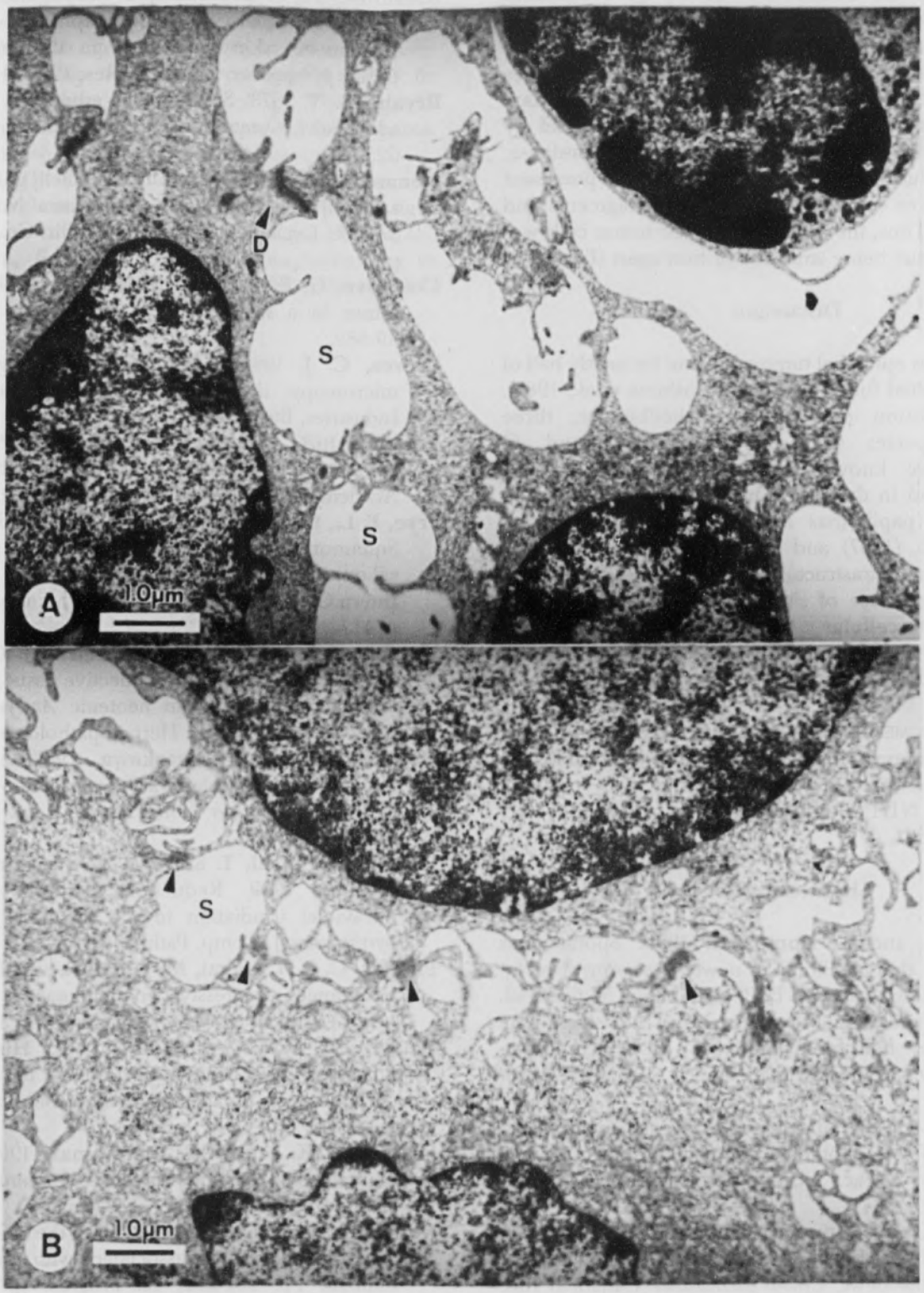

Fig. 4. Electron micrographs of tumorous epidermal cells (A) and normal epidermal cells (B) in *Cryptobranchus a. bishopi.* In A, large intercellular spaces (S) are numerous; in contrast, B reveals small intercellular regions. D = desmosome in A (indicated by pointers in B).

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were invading the connective tissue, in a manner characteristic of squamous cell carcinoma.

Electron Microscopy.--The ultrastructure of epidermal cells of tumors revealed enlarged intercellular spaces between cells (Fig. 4). Subcorneal cell clusters characteristically exhibited cytoplasmic pedicles joined by desmosomes (Fig. 4A and B). Although electron-dense, cytoplasmic inclusions were observed, most cells possessed evacuated spheres containing membranous fragments and cellular debris. Thus, the appearance of the tumor cells was suggestive of tissue being stretched or torn apart (Fig. 4A).

Discussion

Spontaneous epithelial tumors account for nearly half of all tumors reported for amphibians (Asashima et al., 1987). With the addition of the Ozark hellbender, three salamander species (including *A. tigrinum* and *C. pyrrhogaster*) are known to exhibit papillomas. The neoplasms found in the Ozark hellbender were similar to the epidermal papillomas described and illustrated by Asashima et al. (1987) and Pfeiffer et al. (1989) for *C. pyrrhogaster.* Our ultrastructural analysis in *C. a. bishopi* indicated the presence of similar intercellular spaces and associated intercellular bridges with desmosomal attachments; these cytoplasmic characteristics are also described for tumor cells in *C. pyrrhogaster* (Pfeiffer et al., 1989).

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