123. *Spontaneous Progressive Skin Papilloma in Newts (Cynops pyrrhogaster)*

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Introduction. The rare occurrence of spontaneous tumors among urodeles such as newts and salamanders is thought to have some connection with the strong regenerating capacity of the animals.^{1),10)} In the Japanese newt, *Cynops pyrrhogaster*, a few tumors have been reported, e.g. nephroblastomas,¹¹⁾ lymphosarcomas,^{4),5)} and skin papillomas.^{2),7),8)} The skin papillomas as well as carcinomous tumors⁹⁾ have been stated to regress or disappear under laboratory conditions,⁸⁾ evidence for an active hyperplasia and multicentric proliferation being rather little. The present report describes three cases of progressive papillomas, two of which showing extensive multicentric proliferation.

Materials and methods. Of 3052 specimens of the newt, *Cynops pyrrhogaster*, caught in ponds, small rivers and marsh areas of Niigata and Fukushima Prefectures in Northern Japan, 10 were found to have obvious skin papillomas (0.33%). These newts bearing papillomas were kept in a room at 15–25°C, in a flat-bottomed glass vessel (26 cm diameter, 12 cm deep) containing water 1 cm deep over a gravel bottom. They were fed the aquatic oligochaetae, *Tubifex hattai*, twice a week. For light microscopy, normal and tumorous pieces of skin were fixed with Bouin's fixative, sectioned at 5 µm and stained with Delafield's hematoxylin and eosin. The mitotic indices were determined under the microscope. For electron microscopy, samples were fixed in a buffered (0.05 M phosphate buffer, pH 7.2) 2.5% glutaraldehyde solution containing 2% paraformaldehyde and 0.1% picric acid,⁶⁾ and embedded in Epon. Sections were stained with 2% uranyl acetate and lead citrate and examined with a JEM-100C electron-microscope. As in the frog skin,³⁾ in addition to the superficial cornified layers, three layers were distinguished in the newt epidermis, i.e., the surface layer (SG; stratum granulosum), the middle layer (SS; stratum spinosum), and the basal layer near the basement membrane (SGe; stratum germinativum).

Results. *Macroscopic observations:* In the 10 newts bearing skin papillomas (4 males, 6 females), the tumors occurred in various parts of the body, particularly on the back, head, hind limbs, and tail.

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Under laboratory conditions, whereas in 7 of the newts the epitheliomas eventually regressed and disappeared, i.e. in one within 4 months, in 3 within 8 months, and in 3 within a year, in the 3 others the tumors underwent a progressive hyperplasia. One of them displaying a slight hyperplasia of its tumor died in 12 months of an unknown cause, while the remaining 2 survived and exhibited a marked hyperplasia and multicentric proliferations within 3 years. One had initially a small wen-like epithelioma (Fig. 1) on the dorsal surface of the nose but it became enlarged in 8 months all over the head; at 11 months new tumors appeared in the right side of the back and at 19 months also in the right hind limb, shoulder and tail. These epitheliomas grew gradually. The newt bearing the epitheliomas at 35 months is shown in Fig. 2. The tumor on the head became especially enlarged, covering and almost destroying the eyes and nose.

Figs. 1-2. 1: A newt bearing spontaneous skin papillomas kept for 5 months under laboratory conditions. Papillomas (arrows) are present only around the nostril. $\times 4/5$. 2: The same newt after 35 months under laboratory conditions. The tumors (arrows) are visible in various parts of the body (Dorsal view). $\times 4/5$.

The other newt also displayed a marked hyperplasia of the tumor on the head and the abdomen at 11 months, with subsequent multicentric proliferation in the tail and the hind limb at 15 months. Generally speaking, the tumors were better developed on the back than on the abdomen.

Autopsy of the newts with the progressive skin papillomas revealed no macroscopic changes in the internal organs.

Light microscope observations: The normal epidermis was composed of 1–3 layers of epithelial cells (Fig. 3). The pigment granules were compactly arranged in rows under the basement membrane. The mitotic indices of the normal epidermis were 0.1–0.5% (mean range for 6 samples; about 1000 nuclei per sample were counted).

Figs. 3-4. 3: Histological section of normal newt epidermis. Note 1-3 cell layers. $\times 80$. 4: Section of skin papilloma showing epidermal hyperplasia with mitoses (arrows). $\times 115$.

By contrast, the epitheliomas consisted of 5–17 cell layers. The epithelial cells located close to the basement membrane were arranged more compactly than in the upper layers (Fig. 4). The pigment granules beneath the basement membrane were reduced in number, disappeared or scattered away. There was relatively little change in the dermal components of tumors. However, in some cases, the mucous glands were filled with tumor cells or the tumor cells had infiltrated into the dermis. The mitotic indices of the epitheliomas were 1.0–3.5% (mean range for 7 samples).

Electron microscope observations: In the three layers of the

normal epidermis, i.e., SG, SS and SGe, a large number of desmosomes were seen between the adjacent cells. Microvilli were rather few in these cells. Near the basement membrane, tonofilaments of half-desmosomes could be clearly seen. In tumorous epidermis, intercellular spaces were widened and a good many microvilli were seen on the surface. In the middle layer (SS), there were a great number of membrane-bound lysosome-like granules. Some of these granules exhibited an area of low electron density (Fig. 5).

Fig. 5. Electron micrograph of a papilloma in stratum spinosum. Numerous membrane-bound small granules are present. $\times 7,300$. Inset: Two granules showing low electron density area (arrow). $\times 30,000$.

Eight samples were carefully examined for the existence of virus-like bodies but none could be found. Unlike in the normal epidermis, the tonofilaments of half-desmosomes were rarely encountered toward the basement membrane. The pigment granules, arranged in rows near the basement membrane in the normal epidermis were scattered in tumorous areas.

Discussion. In the present studies, regression of the papillomas took place in 7 of the 10 tumor-bearing newts, but in the remaining 3 the tumors grew progressively, undergoing multicentric proliferations in the distant sites of the skin. However, the tumors were invariably restricted to the skin and never invaded into the other tissues. This appears to suggest that they were benign in nature.

So far as we are aware, there is only one article demonstrating virus-like bodies in the newt epitheliomas by means of the electron

microscope.⁸⁾ Although we could not find any such bodies in our materials, the existence of tumor viruses in the newt epitheliomas cannot be ruled out.

The changes in pigment and tonofilaments of half-desmosomes in the epitheliomas suggest that the border region of the epidermis and dermis was affected in the tumors, although the observed abnormalities were mainly restricted to the epidermal tissue. Membrane-bound cytoplasmic granules, probably lysosomes, increased markedly in tumor cells. Some of the granules exhibited an area of low electron density (Fig. 5). The number of these granules seemed to be directly related to the degree of hyperplasia.

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