Three Different Types of Tumors in Salientia

E. Elkan

(Group IX Laboratories, Shrodsells Hospital, Watford, Herts, England)

SUMMARY

Three tumors of widely differing structure occurring in Salientia (Amphibia) are described. They are: (a) a nephroblastoma, (b) an atypical facial papilloma or melanocarcinoma, and (c) an epithelioma resembling ulcus rodens. The first two tumors occurred in specimens of the South African claw-footed toad (Xenopus laevis D.), the third in a specimen of the common grass frog (Rana temporaria L.).

A NEPHROBLASTOMA IN Xenopus laevis DAUDIN

Neoplasms of various kinds are common in fish, much rarer in reptiles, very rare in amphibians. The first comprehensive review of the tumors described from cold-blooded animals was made by Lucké and Schlumberger (Balls [1] lists 68 relevant titles) in 1948-1949. Cohrs et al. in their textbook Pathology of Laboratory Animals reviewed the literature up to 1962. Finally Balls (1), reporting on six new cases in Xenopus, reviewed the literature up to 1962. The present paper gives a short description of three types of tumors not previously seen in Salientia.

The most common neoplastic tumor in frogs and toads is still the Lucké (7) renal adenocarcinoma which frequently occurs in the American leopard frog Rana pipiens and which has been shown to be transmissible (9). All the other neoplasms seen in amphibians, and particularly in Salientia, are very rare.

Embryonic blastomas—in particular nephroblastomas—are, according to Willis (2), commonly found in the pig, the rabbit, and the domestic fowl. They are less commonly found in hares, rats, and mice. Thus far they have not been listed as occurring in poikilothermal animals. Their classification into renal teratomas and nephroblastomas depends on the type and the degree of differentiation the tissues present. Since they have thus far escaped description in cold-blooded animals, we might be inclined to regard such a tumor as a rarity. In this type of animal, however, such rarity may be more apparent than real. Tropical and subtropical species must be kept in heated cages either, like Xenopus, permanently submerged or, like most terrestrial species, in a warm, moist atmosphere. If any of these animals falls ill, it hides in the most inaccessible corner of the cage; many are, in any case, nocturnal in their habits, and when their death is discovered, decomposition has usually advanced to such a degree as to make further investigation impossible. Much interesting material is lost in this way. Circumstances are a little more promising in laboratories where less attention is paid to the imitation of natural surroundings and more to the health of the animals. However, even those departments which keep large numbers of frogs under constant close supervision have reported few cases of true neoplasms in amphibians.

The tumor here described occurred in a fully grown female specimen of Xenopus laevis D., the South African claw-footed toad (Aglossa), of 120-gm. weight, which had been kept in stock for the performance of pregnancy tests for over a year. The toad, originally imported from Cape Town, South Africa, was kept in a galvanized steel tank together with 59 other females of the same species, and it yielded, during the period of its captivity, correct results when used for these tests. None of the other toads sharing the tank showed any signs of disease, but the specimen in question attracted the attention of the staff because, when it was handled, a large, firm mass could be palpated through the abdominal wall. This toad was neither discolored in any way nor disfigured by hydrops, symptoms which usually herald the start of any serious disorder in Xenopus. It was therefore killed with 2 per cent urethan.

Appearances at post mortem examination.—A view of the abdominal viscerae in situ after removal of the anterior abdominal wall, the right ovary, and the right lobe of the liver, is presented in Fig-
Figure 1. It varies little from that of true renal carcinoma seen before (6), and it seemed at first sight to be almost identical with the material published by Lucké and Schlumberger (14), with the only exception that, in the present case, the tumor was much bulkier than those previously seen. Its retroperitoneal origin was obvious from the fact that the inferior vena cava, the large intestine, and the right oviduct were displaced ventrally. No trace of the right kidney could be found behind the tumor even with the aid of a hand lens. The left kidney was found compressed between the tumor and the left lumbo-dorsal muscles, but it showed no histological abnormality and was, apparently, fully functional. Both the liver and the spleen were found considerably congested and enlarged to twice their normal size. Both ovaries were atrophic and reduced to less than half their normal bulk; but both fat bodies were found very well preserved. These observations are at variance with those usually made in diseased toads where, if the ovaries atrophy, the fat bodies do so as well.

No metastases of the tumor were found anywhere, nor could an invasion of abdominal veins be demonstrated. The tumor seemed to have grown, entirely by expansion, to its final size of 4 X 5 X 3 cm., not invading any other organ but interfering severely with the abdominal circulation and causing severe congestion in the portal circuit.

The neoplastic mass (Fig. 1) was firm and white, with an irregularly bulging surface of the cauliflower type. Cross-section showed a few small darkly stained patches but presented no signs of softening or degeneration. The whole tumor was contained in a thin capsule which, in the right dorso-lumbar region, contained the compressed and thinned-out right kidney.

Histology.—Cells in all stages of differentiation, obviously derived from the renal blastema, made up the bulk of the tumor. The stroma consisted of a loose network of fibroblasts embedded in abundant intercellular matrix which accepted periodic acid-Schiff stain as well as Alcian blue and mucicarmine (Fig. 2).

Clear spaces within the stroma were filled with phagocytes containing fatty droplets (Fig. 3). In spite of the extremely scanty vascularization of the tumor no areas of overt necrobiosis were found. All parts of the tumor stained equally well and were obviously of good viability even when they seemed to lie far removed from the nearest capillary vessel.

In the parenchyma every degree of epithelium from the most primitive to the almost completely organized could be seen. The normal epithelial components of an aglossan nephron would be: endothelium lining Bowman's capsule; ciliated cuboidal epithelium lining the neck of the nephron; high-columnar epithelium lining the convoluted tubules; low-columnar epithelium and flask cells lining the collecting ducts (Fig. 6). Of all these cell types only the cuboidal, nonciliated epithelium appears in the sections (Fig. 7, B—D, F, G). Instead of the other cell types two, or perhaps three, new kinds of cells appear which are not found in the normal aglossan mesonephros:

1. Undifferentiated renal blastema, occurring either in loose concentric rings (Fig. 7, A) or in dense conglomerations in the place of glomerular capillary loops (Fig. 7, H).

2. Another kind of undifferentiated tissue made up of large, clear cells with small nuclei and well defined cell walls. These cells are always seen to fill some kind of cavity partly or wholly. These cavities may be abortive Bowman's capsules (Fig. 4, Fig. 7, H).

3. High-columnar epithelium of the "palisade" type. Here the nuclei are long and thin and lie so closely together that no cytoplasm can be seen between them (Fig. 7, C and G). The palisade cells are seen either to form the walls of an abortive tubule or to protrude, papillary fashion, into smaller and larger cystic spaces (Figs. 4, 5, and Fig. 7, G and H).

Among these palisade cells there appear occasionally larger, oval cells whose nuclei do not fill the whole space available, leaving a clear gap of cytoplasm between the nucleus and the cell wall (Fig. 7, D). The numerous cystic cavities, perhaps representing attempts at the formation of Bowman's capsules, can be seen in every field (Figs. 2, 5), and they are lined with all kinds of cuboidal epithelium. Their nuclei, too, vary greatly in shape and size, and the palisade arrangement, though prevalent, is not always present (Fig. 7, E). The two types of epithelial cell most typical of the aglossan kidney, the ciliated columnar and the flask cell (Fig. 6), are not represented in the tumor at all.

The nuclei, on the whole, stained more heavily than normal with basic dyes. Mitotic figures were not seen. The tumor apparently grew slowly from the most primitive to the almost completely organized could be seen. The normal epithelial components of an aglossan nephron would be:
58th day of development (Nieuwkoop and Faber [16]). If one assumes that the development of this nephroblastoma paralleled the growth of the normal organ, its beginnings must lie far back in the very early days of the development of this particular toad.

**AN ATYPICAL FACIAL PAPILLOMA IN Xenopus laevis D.**

An adult female of 110-gm. weight, which had been kept with others of the same species for over 12 months and which during that time had been used several times for pregnancy tests, developed a tumor in the rostral region. The animal had originally been imported from Cape Town; none of the other toads from this batch has shown any similar symptoms. The animal was isolated because it lost color, refused to feed, and seemed to have difficulties in breathing. Closer inspection revealed some swelling and a pink discoloration in the region of the right nostril, the lining of which seemed to be thrown into folds obstructing the entrance (Figs. 8, 10). The condition was not obvious at first, and details could not be ascertained without a hand lens. The toad continued to refuse all food, and after 4 weeks of isolation, during which time the toad became paler and paler, it died.

General pallor, can, in Xenopus, always be taken as a sign of serious illness unless the toad happens to be kept in a vessel with a white bottom. General post mortem dissection revealed no abnormality of the viscera apart from an almost complete atrophy of the ovaries and the fat bodies, signs which may again be taken as typical of a severe systemic illness. After decalcification serial sections were made through the rostral part of the skull.

At low-power magnification these sections revealed the presence of a papillomatous growth at the edge of the nostril (Fig. 10). In the sections the tumor did not seem to obstruct the nostril, but the material must have shrunk considerably during preparation. Of all the skin tumors one may see, papillomas are perhaps the most common, and little attention might have been paid to this one had it not been for the fact that it had, to all appearances, caused the death of the toad and, further, that an unusual relation existed between the dermal tumor and the overlying epidermis. In a typical papilloma which arises from the dermis or the subcutis the epidermis, otherwise unaffected, is thrown into folds which cover the papillae and may show a greater or lesser degree of keratinization on the surface. In this case the epidermis presents a totally different picture. It does indeed cover the polypous mass, yet it no longer presents a homogeneous layer of squamous cells (Fig. 9) but has, in the area covering the polyp, been eroded so as to form numerous pockets of varying depth (Figs. 11, 12). These pockets are sharply defined and in many instances are seen to approach the surface so closely as to leave only one layer of epithelial cells covering them. The normal aglossan epithelium, shown in Figure 9, is seven to ten cells thick. At the surface of the polypous mass the epithelial covering has become completely eroded (Fig. 10, arrow), but this area of ulceration was small.

The glandular and the connective tissue layers of the dermis, which together form a very tough coat in amphibians, are seen to be completely destroyed in the affected area by the abnormal tissue produced by the tumor. Instead of the regular strands of connective tissue the following features were seen in the place of the dermis: (a) a network of fibroblasts with narrow, elongated nuclei; (b) capillary loops, either single or in convolutions; (c) cells with large, oval, badly staining nuclei (Figs. 11, 12).

The undifferentiated fibroblasts which, in other circumstances, one might have defined as sarcomatous, made up the mass of the tumor, particularly in the deeper part where it adjoined the underlying structures. There was, however, no evidence of any invasion or destruction of neighboring tissues, nor were any metastases found in any part of the body.

The capillary loops were most numerous near the surface. In Figure 11 they are recognized by the nucleated erythrocytes they contain. They formed either single loops or dense convolutions, filling some of the epidermal pockets and being separated from the surface by a one- or two-cell layer of epithelium only. Their presence here probably explains the pink discoloration of the area observed in vivo.

The cells with large, badly staining nuclei were found mainly but not exclusively in the epithelial pockets, and the question arose whether these cells were derived from the disintegrating epithelium or from the eroding tumor. The epithelium overlying the pockets looked as if it were yielding more to pressure than to disintegration. On the other hand, round melanophores, typical of the epidermis of this region could in many instances be seen lying free within the pockets as if they had been dissolved out of the epithelium. Their presence here would, however, be no proof of epidermal disintegration, since the melanophores may have wandered actively into the pockets (Fig. 12).

Remains of dermal glands could be found embedded in the tumor, the glandular tissue appar-
ently resisting the dissolving activity longer than
the rest of the epithelium. Why this action did not
take place on a broad front but confined itself to
the formation of isolated, well defined pockets
could not be explained. It is not easy to explain
the fact that there were two kinds of such pockets,
one filled with capillary loops, the other with the
large, badly staining cells mentioned.

It was not apparent why this trivial-looking
condition should cause the animal to refuse all
food and eventually to die. Breathing was pre-
sumably still unimpaired via the other nostril, and
no damage to any of the major viscera could be
demonstrated.

There remains the question how this tumor
should be classified. Its closest counterpart in
human dermatological pathology may be found in
Dawson's (3) classical treatise on the melanomas.
Under the heading of "Melano-Carcinoma" Daw-
son describes the development of a tumor similar
to the one observed in Xenopus laevis. His draw-
ings show how cells in the basal layer of the epi-
dermis lose their cell bridges, become detached
from one another, and form "cell nests" which are
at first without contact with the dermis. Later this
barrier is broken down, and the cells invade the
dermis through an opposing layer of lymphocytic
infiltration. Pockets of cell nests are formed in the
epidermis, and a comparison of the pictures pre-
sented by the human and the amphibian material
seems possible. However, the analogy breaks down
in the examination of detail. There is no evidence
in our sections to show that in Xenopus the degen-
erative process starts in the epithelium. On the
contrary, it seems to start in the dermis. Also, in
the human material, the epithelial cells starting
the process arose from a nevus, were heavily pigment-
ed, and had hyperchromatic nuclei. All these fea-
tures are absent in the amphibian material. The
excessive vascularity of the invading dermal tissue
is not mentioned by Dawson, while the cellular
polymorphism of the malignant tissue was promi-
nent in both Homo and Xenopus.

If one considers the evolutionary distance be-
tween the two species, complete analogy in the
phenomenology of both tumors is not to be ex-
pected. On the whole, enough similarities remain
to bring this tumor into the group of dermal mel-
ana or melano-carcinoma, a condition frequently
seen in fish, sometimes in reptiles, but rarely in
amphibians. In Xenopus, in particular, sponta-
nenous dermal tumors are extremely rare, and those
that are seen are usually due to infections or to
encysted parasites. Because of the location of the
tumor it seems possible that it may have been ini-
tiated by trauma. Frogs frequently injure the
region of the nostrils in collisions with the walls of
their cages when trying to escape. These injuries
always heal badly, and an accident of this nature
may have started this atypical papilloma.

AN EPITHELIOMA RESEMBLING ULCUS
RODENS IN Rana temporaria

From the point of view of comparative pathol-
ogy this tumor has some interesting features. It
occurred in a common grass frog caught as an
adult frog in the south of England by a naturalist
and subsequently kept in a cage as a pet. Since it
was fully grown at the time of capture and since
the owner kept the frog 4 years, it must have
been about 6 years old when the first signs of dis-
ease became apparent. This, for a frog, must be
regarded as equivalent with old age.

The growth started in the right maxillary region
between the bone and the orbit, where it gradually
reached a diameter of 8 mm. Although the right
eye does not look damaged in sections, the frog
became gradually blind in this eye. This may have
been owing to the gradual encroachment of the
tumor on the cornea (Figs. 13, 15). As long as the
frog could see with the left eye, it continued to
feed. When eventually it stopped feeding after an
illness of 6 weeks it was, to all appearances, com-
pletely blind. Sections through the left half of the
skull show not only a massive retroretinal hemor-
rhage filling the whole intraocular space but also
an extreme congestion and distension of all the
veins in that part of the skull. This condition was
probably due to venous thrombosis caused by the
advancing tumor.

Fig. 1.—Xenopus laevis. Adult female with nephroblasto-
toma. Ventral view of intestines after removal of the right ovary
and the right lobe of the liver. Note the displacement of the
inferior vena cava, the right oviduct, and the large intestine.
Note also the preservation of both fat bodies. d, oviduct;
fr right, fl left, fat body; i, large intestine; o, ovary; s, spleen;
t, tumor; v, ventricle; v, vena cava inferior.
Fig. 2.—Nephroblastoma in *Xenopus laevis*. Survey picture of central part of the tumor. X40.

Fig. 3.—as Figure 2. Phagocytes filled with Sudan-stained droplets. Frozen section.

Fig. 4.—as Figure 2. Attempts at the formation of uriniferous tubules.

Fig. 5.—as Figure 2. Attempts at glomerulus formation.

Fig. 6.—*Xenopus laevis*. Section through the normal kidney showing flask cells surrounding the collecting tubules. Periodic acid-Schiff reaction.
FIG. 7.—as Figure 2. Details of renal blastoma.
Fig. 8.—*Xenopus laevis*, adult female with tumor of the right nostril. Slightly enlarged.

Fig. 9.—*Xenopus laevis*. Normal epidermis. Compare with Figs. 11 and 12.

Fig. 10.—*Xenopus laevis*. Transverse section through the region of the right nostril showing facial papilloma. Eroded surface arrowed. X15.

Fig. 11.—As Figure 10. Epidermal pocket filled with capillaries, recognizable by the presence of the nucleated erythrocytes they contain.

Fig. 12.—As Figure 10. Epidermal pocket formed by the advancing tumor. Note presence of spherical melanophores inside and outside the pocket.

Fig. 13.—*Rana temporaria*. Facial epithelioma invading the cornea.

Fig. 14.—As Figure 13. Well differentiated epithelial cells from one of the advancing columns of the tumor. Notice presence of cell bridges.
Fig. 15.—Rana temporaria, adult female. Transverse section through the right maxillary region at the level of the eye, showing an eroding epithelioma below the eye. c, cornea; e, ulcerating epithelioma; h, Harderian gland, invaded by the infiltrating tumor; i, iris; l, lens; n, nictitating membrane; o, orbital cartilage; r, retina. The region marked X is shown at higher magnification in Figure 13.
Sections through the tumor itself show all the features of an ulcerating epithelioma. The base of the ulcerated area, which was small, consists of a mass of typical epithelial cells spreading out, in the form of thick strands toward the maxillary process, the Harderian gland, the ocular muscles, and the cornea. In some of the most advanced strands, found between the ocular muscles, the features of the epithelial cells were so well preserved that even the intercellular bridges could be seen (Fig. 14). Mitotic figures were seen, but they were not very frequent.

The laminar structure of the cornea did not allow the tumor to advance in the shape of thick strands as elsewhere. It had to split up into sheets which, at the advancing point, are only one cell thick (Fig. 13).

No metastases were discovered in any other part of the frog. The growth of the tumor was followed for 6 weeks, at the end of which time the frog was killed because it had become completely blind. Although malignant, the tumor was growing slowly and did not, apparently, interfere with the general health of the frog.

In their natural surroundings frogs have so many enemies that few of them survive to reach old age, and no descriptions of senile epitheliomas or rodent ulcers among either free-living or captive frogs have so far been published. Clinically and histologically the tumor here described can hardly be distinguished from a rodent ulcer, and it would be of great interest to know whether this tumor would appear more frequently if a population of frogs were allowed to live to the end of their natural life span.

ACKNOWLEDGMENTS

I wish to thank Dr. E. Cotchin of the Royal Veterinary College, London, for his kind assistance in the evaluation of these tumors.

REFERENCES

Three Different Types of Tumors in Salientia

E. Elkan


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/23/10_Part_1/1641

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.