Experimental Induction of Tumor-like Lesions of the Notochord of Fish*

BARNET M. LEVY

(Department of Pathology, The University of Texas Dental Branch, Houston, Texas)

SUMMARY

Fish embryos immersed in a solution of β-aminopropionitrile developed tumorous overgrowths of notochordal tissue. The tumors were composed of either the large physaliferous cells of the notochord or a combination of fibroblasts and notochordal cells. Many of the older echordomas were surrounded by bone. There was no evidence of metastasis.

During the course of some investigations of experimental lathyrism in salamanders, we noted and reported that, if the eggs of the salamander Ambystoma punctatum were placed in solutions of the lathyrism factor, the larval forms exhibited tumors of the notochord (2). Since that time, no reports have appeared concerning the production of experimental echordomas in other animals. In our original research we used a crystalline factor extracted from the sweet pea Lathyrus odoratus. Since then the chemical β-aminopropionitrile and other nitriles have been shown to be active lathyrogenic agents. These chemicals were also active tumorigenic substances for the notochord of developing salamanders and other amphibians (1). We wondered whether they would induce similar growths in fish.

MATERIALS AND METHODS

Eight- to 24-hour-old egg clusters of the Japanese Medaka, Oryzias latipes, were placed in finger bowls containing 100 cc. of a 1 or 2 mg. per cent solution of β-aminopropionitrile. Each egg cluster consisted of ten to twenty eggs. Four hundred and twenty-two eggs were used, but 265 embryos died prior to hatching. Embryonic development of the survivors was watched under the dissecting microscope. The developing embryos were removed from the nitrile solution to spring water at or about the time the mandibular arch developed. The time interval varied considerably, depending on the temperature at which the eggs were maintained, but was never longer than 4 days. One hundred and fifty-seven of the animals hatched in spring water. Their development was observed with the dissecting microscope, and they were sacrificed for histologic study at regular intervals. Fish to be studied histologically were fixed in either Bouin's solution or Stockard's fluid. Serial sections were cut at 8 μ on all animals. Eggs of the Anoptichthys jordane, Trichogaster tricopterus, Aequidens portalegreusi, and a mixed breed of goldfish were also treated in this manner with identical results.

RESULTS

The tumors in the various species looked similar. Figure 1 is a photograph of such a tumor-like growth in a Medaka. The egg had been placed in 1 mg. per cent β-aminopropionitrile 24 hours after it was spawned and allowed to remain there for 4 days. The embryo was then hatched in spring water and allowed to remain there for 18 days. The tumor was typical of those produced by this method.

Histologically, the hyperplastic growths were found to have resulted from marked local overgrowths of the notochord or its connective tissue sheath (Figs. 2 and 3). The early stages were usually masses of notochordal tissue which displaced the surrounding muscle tissue and extended to just beneath the epidermis (Fig. 2). The unaffected portion of the notochord maintained its general structure and shape. The tumors were usually rounded. They were not pedunculated but arose from the notochord along a rather broad base continuous with the parenchyma of the notochord. Basophilic or acidophilic material and nonvacuo-
lated cells were seen occasionally in intimate relation with the chordal cell borders. The cells composing this type of notochord overgrowth were, for the most part, identical with the differentiated physaliferous cells of the mature, uninvolved notochord. Smaller fusiform cells without vacuolated cytoplasm were encountered with some frequency, and the little mitotic activity noted seemed to be in these germinal cells.

In older fish the connective tissue which surrounded the normal portions of the notochord appeared greatly thickened and had a basophilic staining ground substance. The tumor-like growths in these older animals had a somewhat different histologic character in that the large physaliferous cells were separated by extremely thick septa, and there were numerous cells filled with an amorphous or finely granular acidophilic staining material. Such tumors were usually surrounded by numerous thin-walled blood vessels engorged by nucleated red blood cells. The tumorous mass was surrounded by bone in many instances. The central area was composed of an acidophilic-staining amorphous substance, in which there was an occasional small osteocyte-like nucleus as well as several pyknotic nuclei. The large physaliferous cells were much smaller in size and fewer in number than those found in tumors of younger fish (Fig. 4).

Although most of the tumors appeared to involve only the notochordal cells, another type was occasionally noted in which the connective tissue sheath as well as the notochord was hyperplastic. Tumors of this type were composed of a mass of fibroblasts which grew between the surrounding muscle cells and extended to the epidermis. Typical physaliferous notochordal cells were seen in the central area of the connective tissue masses (Fig. 5).

DISCUSSION

Apparently, tumor-like overgrowths of the notochord as a response to a lathyrogenic nitrile are confined to lower animal forms, for only in the larval stages of amphibia and fish have such tumefactions been observed. While in the urodele and in the anurians the notochordal sheath was largely absent about the tumors, in fish there were proliferative changes in the connective tissue surrounding the notochord. This change may still be interpreted as a defect in mesenchymal metabolism, but it is reflected in fish as an increase in growth rather than as a failure to grow, as noted in amphibia.

The ecchordomas of fish, like those produced in amphibia, behaved in a benign manner. The tumor masses either thrust aside or compressed the surrounding muscle but rarely invaded it, although in one tumor (Fig. 5) the proliferating fibroblasts grew between muscle bundles.

It is difficult to decide whether these tumor-like overgrowths of tissue represent "true" neoplasms. The lesions are indeed benign. Just where the line between benign neoplasia and hyperplasia should be drawn is often difficult if not impossible to state. We prefer to think of these tumor-like overgrowths as true benign tumors because they develop after a short lag or latent period in the absence of the inciting agent (a nitrile), and they do not regress with age. On the other hand, we do not wish to add $\beta$-aminopropionitrile or aminoacetonitrile to the list of carcinogenic agents until further work on these tumors is completed. The transplantation of chordomas into animals not previously exposed to a nitrile is at present under study and may eventually clarify this point.

ACKNOWLEDGMENTS

The technical assistance of Mrs. Johnnie Goodrich is gratefully acknowledged.

REFERENCES

Fig. 4.—Photomicrograph of tumor of Japanese Medaka 6 months of age. The tumor mass is surrounded by bone. The more central area of the tumor is composed of an amorphous acidophilic staining material. X 240.

Fig. 5.—Photomicrograph of a chordoma in a Japanese Medaka. The tumor fibroblasts (TF) lie between striated muscle bundles (M). The more central area of the tumor (T) contains notochordal cells. X 270.
Experimental Induction of Tumor-like Lesions of the Notochord of Fish

Barnet M. Levy