OBSERVATIONS ON THE EFFECTS OF AUXILIN ON THE DEVELOPMENT AND PHYSIOLOGY OF FISH EMBRYOS

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A year or two ago, the author conceived the idea that the eggs and early embryos of teleost fish would be ideal material on which to experiment with various chemicals, such as colchicine and hormones, in order to determine the relationships between cell division and the processes of differentiation. With this problem in mind, a search was made to find a suitable egg-laying aquarium fish. After some experimentation, the Japanese minnow, Medaka, was selected. In many respects, this fish is ideal for experimental purposes but the author has had difficulty on two scores: these fish are subject to some unknown disease which breaks out in epidemic form and may destroy a whole tank of fish in a few days, and their egg laying habits are somewhat seasonal.

After selecting the fish, a careful study was made of the developmental rate, the effect of different temperatures, and methods of culturing the early embryos and young fish.

The eggs, when laid, remain attached to the mother's vent for several hours. Fertilization occurs within a few moments after oviposition. The eggs are collected by confining the mother and drawing the eggs up into a medicine dropper. Usually ten to twenty eggs are laid in a clutch and the same female may lay a clutch every day for two weeks or more. Following a period of rest, another series of eggs may be laid during the same season.

A clutch of eggs from a single female was divided between two finger bowls, each containing 200 cc. of distilled water. To one was added various quantities of commercial auxilin (indolebutyric acid). Preliminary experiments were made to determine the relative toxicity of the different concentrations of the auxilin. The two cultures, auxilin-treated and control, were allowed to undergo development and after differentiation had progressed to a certain point, the specimens in both cultures were fixed in Smith's fixative preparatory to sectioning. A complete series of embryos was obtained for the early stages of differentiation in each concentration of auxilin. These embryos have been embedded in paraffin and sectioned. They are to be stained and the mitotic index of the eyes of each embryo determined in order to measure the effect of auxilin on the mitotic rate.

This information together with observations on the relative degree of differentiation should give some evidence of the relationship between mitosis and the processes of morphosis.

In preparing this material, several interesting observations were made on the microscopic effects of auxilin on the fish embryos. No embryos hatched from eggs exposed to auxilin, no matter how dilute the

solution might be. A 1% solution of commercial auxilin in distilled water was found to be toxic enough to produce recognizable retardation of development within a few hours. In the early embryos, this seems to be due to a slowing up of the cleavage processes. Cleavage normally requires about 30 minutes at room temperature. Auxilin-treated embryos required 40 minutes. (These are estimates of the time required for each division during the first four cleavages of the egg).

As embryonic development continues, the auxilin seems to affect the formation of the circulatory system first. Red corpuscles do not appear to be formed. The muscular movements of the heart and vessels are weak and ineffectual. As a result of the failure in circulation, disintegration of the tail occurs and death eventually results. Normal embryos with the circulation well developed, when placed in auxilin solutions of approximately 2% concentration, show similar disintegration and a lack of red corpuscles.

Auxilin solutions of as low as 0.05% concentration show some effects on the development in the early stages. The number of somites in the two cultures, auxilin-treated and control, are usually different, the auxilin-treated usually being fewer in the number of somites. The formation of pigment in the eye is slower in the presence of auxilin. When allowed to continue development, the auxilin-treated embryos eventually reach what appears to be the hatching stage but then die. During this time, there accumulates in the urinary bladder an orange red coloration. This colored material in the bladder is characteristic of all auxilin-treated embryos, regardless of the concentration used.

In conclusion, preliminary observations indicate that auxilin is toxic to fish embryos and that it affects the mitotic rate as well as the rate of morphosis; that it causes deterioration of the circulatory system and in some manner affects the functions of the kidneys. Further studies, it is hoped, will reveal the cytological effects.