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## **A PRELIMINARY REPORT ON THE EFFECTS OF ANTUITRIN (GROWTH HORMONE) ON THE DEVELOPMENT OF FISH EMBRYOS\***

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This is a report of the results of some experiments undertaken in conjunction with an investigation begun several years ago. Most workers in the field of biology recognize that there are two phases involved in the growth and development of a new individual organism. These two phases involve the increase in cell number (mitosis) and the differentiation of these cells into organs or tissues (morphogenesis). The relationships between these two processes have been the subjects of a great deal of investigation. Several years ago the discovery was made that meristematic plant tissues could be treated with the drug colchicine and the process of cell division be so modified as to produce polyploid tissues which exhibited intensified characteristics and other modified morphogenic characters. The effects of this drug, as well as the effects of plant growth hormones such as indole - 3 - acetic acid (auxilin) and of insect larvae such as gall wasps, caused the author to ask this question: What relationship does the rate of cell division have upon or in the process of morphogenesis? Would it be possible to expose a developing embryo to some of these chemicals which definitely influence the process of morphogenesis and determine their effects on the cytological or mitotic processes? Perhaps their morphological effects could be shown to be associated with changes in the mitotic rate or with modifications of the mitotic process.

With this in mind the tropical fish embryo was selected as offering an excellent material for such experimental study. It can be exposed to such chemicals directly and for such lengths of time and at such stages in development as seems suitable and desirable. The development of the embryo can be observed easily and in some forms the internal organogenesis can be traced as the tiny fish are more or less transparent. The rates of cell division vary greatly in different forms and likewise in the rates of differentiation. In some forms the cells divide so fast and the embryos develop with such speed

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that one can observe the process under the microscope in a matter of minutes and hours.

Preliminary studies have been made using auxilin (indole - 3 - acetic acid), thiamine, and colchicine. These have been reported to this society in previous papers. Due to the war and other activities these studies were not completed. They have been revived and are now being continued under the auspices of a grant from the U.S.P.H.S.

In connection with this work an opportunity was provided to secure an experimental sample of "antuitrin growth", an extract of the anterior lobe of the pituitary gland, containing the growth hormone and small amounts of thyrotropic and gonadotropic hormones. This material is prepared by the Parke Davis Co. and is offered to the medical profession for the treatment of "endocrine dwarfism" and similar growth disorders in children. It is given subcutaneously or intramuscularly in doses of 2-5 c.c. and treatment is continued over a period of several months. The substance is standardized in terms of rat units. "A rat unit is defined as the minimal daily amount which, when injected intraperitoneally into a mature female rat, will cause an average daily increase of 1% in body weight over a period of at least ten days." As prepared for biological use, the hormone contains 8 rat growth units per cubic centimeter.

Eggs were collected from various species of tropical fish and were exposed to varying concentrations of "antuitrin growth" for different lengths of time. The results of these preliminary experiments are the subject of this report.

Eggs were obtained from the following species of fish and the observable effects of the hormone were in general the same in all of them: *Brachydanio rerio* (Danio), *Betta splendens* (Betta), *Trichogaster trichopterus* (Blue or Three-spot Gourami), *Macropodus opercularis* (Paradise Fish).

Treatment in general was as follows: The eggs, as soon as laid and fertilized, were removed from the breeding tank and placed in deep Syracuse watch glasses containing 20 c.c. each of distilled water. These glasses were placed in finger bowls for ease in handling and covered with a piece of card to cut down evaporation. In so far as possible the same number of eggs (10-30) was placed in each watch glass. To these glasses were added one, two, three up to 12 drops (minims) of the growth hormone as prepared commercially. In the weaker solutions the embryos were allowed to develop up to hatching. In others the embryos were removed after a period of exposure and allowed to develop in water without the hormone. In still others the embryos were allowed to reach certain stages of morphogenesis before being exposed to the hormone. Control cultures were kept at all times. At certain stages in development, typical specimens were removed and fixed for future sectioning and cytological study. All cultures were kept at the same temperatures (usually about 80° F.).

The results were consistent and positive but *not* in the direction of accelerated growth. In each and every case the embryos exposed to "antuitrin growth" showed signs of retardation and abnormal morphogenesis. This was expressed in various ways. Size difference was one very easily observed effect. The treated embryos did not grow as rapidly as the untreated. They were smaller and not nearly as far advanced in their development, as were controls of the same age. The size effects seemed to be in direct proportion to the strength of the solution. Dilute solutions did not retard as much as did more concentrated treatments.

Not only were the embryos retarded in their growth but the formation of organs seemed to be interfered with. The heart and blood cells were not differentiated as in the controls. The heart beat was feeble and the number of red cells was much lower. In the more concentrated solutions, tissue disintegration soon became apparent and death ensued. It is probable that failure to develop in some regions (i.e. tail bud, fin, etc.) was due to the failure of the heart and circulatory system to perform their functions properly.

The death of cells in the more distal and exposed regions occurred first and there was an ulcerated sloughing of tissues which was eventually followed by the death of the embryo. This sloughing, as stated, was probably due to circulatory failure but might also be due to the exposure of these cells to the direct action of the hormone.

We are not prepared to report on the cytological effects of the "antuitrin growth" at the present time. Slides have been prepared by both the smear and section techniques, but time has not permitted the study necessary to draw any conclusions.

Inasmuch as the effects of "antuitrin growth" on developing fish embryos are distinctly different from those expected as a result of the use of this substance in mammalian forms, some comments may be in order. Perhaps the toxic effects may be due to the concentrations used. This seems indicated by the experiments in which varying concentrations of the hormone were used. However, the concentrations used were much more dilute than the tissue cells of the child or rat would be exposed to when injections were made subcutaneously or intramuscularly. The cells of the fish embryo were not exposed to anything like as high a concentration as would be the cells adjacent to the point of injection.

Secondly, the toxic effects of the hormone do not seem to be due to direct action on the exposed cells themselves. Rather the effect is brought about by a disturbance in the process of morphogenesis itself. As stated previously, this seems to be due to a failure of the circulatory system to develop both anatomically and physiologically. It is only as the circulatory system fails to perform that exposed cells show toxic effects.

Thirdly, it is possible that very dilute concentrations of "antuitrin growth" may have a stimulating effect on embryonic development in fish. We have had neither time nor embryos to test this possibility. There is some evidence suggested by other experiments with other hormones that concentrated doses may have effects different from those produced in dilute solutions. Huskins and others have shown that 50 parts per million of indole - 3 - acetic acid is sufficient to retard cell division in plant tissues and modify differentiation so that roots will develop from stems, etc.

In our experiments on auxilin (indole - 3 - acetic acid) conducted several years ago, effects similar to those of "antuitrin growth" were observed. In fish embryos exposed to solutions as strong as 1%  $\pm$  toxicity was detected. The circulatory system failed to develop properly. Toxicity was observed to be closely related to concentration. More concentrated solutions produced more retardation, etc. However, this was to be expected as auxilin appears to retard or slow up mitosis rather than to accelerate it in dilute solutions. Studies on the cytological effects and their relations to dilution will be necessary before these questions can be answered.

#### SUMMARY

1. Fish embryos in stages prior to hatching were exposed to varying concentrations of "antuitrin growth" hormone manufactured by Parke Davis and Co.
2. The embryos exposed to "antuitrin growth" failed to develop normally. Morphogenesis was retarded in all organs and especially in the circulatory system.
3. The toxic effect seemed to be in direct proportion to the concentration of the hormone used.
4. Sloughing of cells and ulceration of external surface occurred in embryos preceding eventual death. This was probably due to interference with circulation more than to direct cellular effect of the hormone.
5. It is hoped through the study of the cytological effects of this and similar hormones to learn something of the relationships between cell division and differentiation.

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