THE EFFECT OF THE DRUG COLCHICINE ON THE EARLY LIFE OF FISH

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ABSTRACT

The alkaloid drug colchicine is a mitotic inhibitor. The results of this study show that colchicine influence the normal functioning of the mitotic process in *Sarotherodon galilaeus, Sarotherodon melanotheron* and the hybrid *S. galilaeus,* X *S. melanotheron* leading to the production of unusual chromosomal events such as anaphase bridges, laggards and polyploid cells. These unusual events could have serious genetic implications in the area of variability of the chromosome number.

The use of colchicine also produces results with consistent karyotypes and better morphology as well as providing detailed information on the behaviour of the chromosome of the early life of fish. The knowledge of such information will be of great use in cytotaxonomy, fish breeding and in studying the effects of sub-lethal levels of water pollutants on fish.

INTRODUCTION

A large array of chemicals are known to have effects on chromosomes. Rotenone commonly used as a fish and insect poison has been employed by Meisner and Sorensen (1966) for inhibiting the mitotic spindle in chinese hamster cells in culture. Others like colchicine, colcemid and hydroxyquioline are strictly mitotic poisons, producing a general blockage of mitosis or a destruction of the spindle. Diepoxides, mustards and some purine derivatives are radiomimetic. Kihlman (1951, 1952) has reported that some of these chemicals induce chromosome stickiness without breakage, some chromosome breakage without stickiness and some both phenomena.

Colchicine being a very water - soluble chemical may have difficulty penetrating the lipophilic yolk found in fish eggs and larvae. To overcome this problem, Baski and Means (1981) have recommended longer exposure and higher concentration of colchicine as necessary for the diffusion of the chemical into cells.

Colchicine and other related chemicals are so commonly used in chromosome studies that many cytogeneticists have not seen chromosomes that have not been treated with any chemical.

This paper therefore describes the behaviour of chromosomes treated or untreated with colchicine.

MATERIALS AND METHODS

Sarotherodon galilaeus broods were obtained from the Oyo State Ministry of Agriculture fish farm. Agodi Ibadan. *S. melanotheron* were obtained from Oba Dam at the University of Ibadan. They were maintained at 27°C and injected with 10010/100g body weight of Human Chorionic Gonadotropin (HCG) to induce spawning and spermiation. Eggs of the spawned

female fish were stripped into fertilization bowls and milt of spermiated males were collected in vials. Fertilization was effected by mixing the eggs and milt and then incubated.

8 - 12 hours old embryos were collected and fixed in freshly prepared 1:3 acetic ethanol solution. 40-hour old embryos and one-day old hatched larvae were exposed to 0.10% colchicine for various lengths of ranging from 3 to 25 hours. Embryos and larvae that served as controls were not pre-treated with colchicine. The treated and the untreated embryos and larvae were fixed in freshly prepared 1:3 acetic ethanol solution. Slides were prepared by employing the method described by Kligerman and Bloom (1977). The slides were stained in formic acid, lactic acid, propionic acid, orcein stain (F.L.P. orcein) for 30 minutes. Various stages of mitosis were examined at a magnification of x 400 using a binocular microscope.

Photographs of mitotic stages were taken at a magnification of x 1000 with a Leitz phase contrast microscope equipped with a 5mm camera and Ilford Pan F film.

RESULTS

Thread - like prophases, metaphases, anaphases and telophases were observed in the 12-hour old and 40 hour old embryos that were fixed directly without colchicine pretreatment. Regular and irregular cells were obtained in each mitotic stage. For instance, whereas clumping of chromosomes featured prominently at prophase and metaphase, irregular events at anaphase included single and double chromatin bridges as well as laggards. Unequal distribution of chromosomes were observed in some telophase cells.

In the 40-hour embryos and the larvae that were pretreated with colchicine, the effectiveness of colchicine ininbiting spindle formation was time dependent. A mixture of prophase, metaphase, anaphase and telophase cells were obtained in embryos and larvae exposed to colchicine for about 3 hours. In addition, a high harvest of condensed metaphases was recorded. Unusual events observed in this trial included polyploid prophase and metaphase cells.

Embryos and larvae that were exposed to between 10 hours and 25 hours in colchicine produced cells that were all at metaphase stage. No cell was seen at prophase, anaphase and telophase stages. This suggest that the chemical worked optimally when embryos were exposed to the chemical for longer period of time.

DISCUSSION

The study has confirmed the earlier report of many cytogeneticists that colchicine arrests cell division at metaphase. While the present study determined the optimum time of colchicine treatment on eggs and larvae to be greater than 10 hours, this does not agree with the findings of some other authors like Kligerman and Bloom (1977) and Nijjhar et al (1983) who reported that the optimum time range to obtain all metaphase cells at 0.01% colchicine treatment was 3 - 6 hours. Possible explanation for the 10 hours exposure of eggs and larvae to colchicine treatment has been provided by Baksi and Means (1988) to be the lipophilic nature of the egg yolk thus making longer exposure of the eggs to colchicine treatment absolutely necessary.

For the first time, the behaviour of chromosomes at other stages of mitosis is being described. None of the anaphase bridges persisted to the end of telophase. The breakage of chromosomes would definitely lead to serious genetic variability particularly in the area of chromosome number. To date, there is no agreement on the actual chromosome number of many of the species of cichlids (Jalabert *et al* 1971) and Kornifield et al (1979). The observance

of polypoid cells in this study agrees with findings of Smith and Lemoine (1979) who induced polyploid in the Brook Trout with colchicine. The consequence of these unusual events may be greater than variableness of the chromosome number. Swanson (1957) has noted that gross upsets in somatic divisions would lead to death of the affected cells and eventual to deaths of the organism. Swanson further reported that it was only when somatic disturbances are relatively innocous in their effects, or are expressed only infrequently or in a non-vital portion of the body that the organism can survive.

The genotoxicity effects of other chemical agents on fish could be determined using this cytogenetic approach. However, the effect of colchicine on the reproductive condition can only be determined if the larvae are grown to maturity. Then it will be seen whether the sporadic polyploid cells and other unusual chromosome events will produce sterility in the fish or not.

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