

OKLAHOMA JUNIOR ACADEMY

SELECTED PAPERS

Effects of 742 Solution on *Brachydanio rerio* Embryos¹

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A vast amount of research is being done with various new chemical compounds in medicine. Often a chemical is found which appears to cure an experimental animal of some illness; however, before this chemical can be declared a cure and used for humans, much research must be done to make certain that no harmful effects will be produced by its use. It was believed that a chemical referred to as 742 (Oklahoma State University code number) had medicinal properties. Before it could be considered for medicinal purposes, biological assays of its effectiveness and potential danger were made in several laboratories over the nation, one of which is a laboratory under the supervision of Dr. Roy W. Jones at Oklahoma State University. The experimentation related to this study represents only a part of the total work done with the material. The chemical 742 used in this research was furnished by the Lasdon Foundation of Colorado Springs in solution in propyl glycol. Its chemical structure is Estra - 1, 3, 5 (10), 16 - tetraen - 3 - ol.

PROCEDURE

Experiments were set up over a period of two months. The same general procedure was used throughout; only the concentrations of the solutions varied.

The eggs were collected daily, washed thoroughly, and placed in the incubator at 28 C (the same temperature as the water in the fish tanks) for a 24-hour observation period. During this time, the fertile eggs began a period of rapid cell growth and took on the appearance of fish embryos. The infertile eggs had opaque yolks and soon disintegrated. The healthy, fertile eggs were washed and placed ten in a dish. Each dish contained 50 ml of solution: the controls, 50 ml of aerated water and the test plates, 50 ml of 742 solution. The concentration of the 742 was varied as follows: 0.50 parts, 0.75 parts, 0.80 parts and 1.00 part 742 solution to 1 million parts aerated water.

At the end of the first 24 hours the *B. rerio* embryos were removed from their chorions and thereafter examined every 24 hours until they reached 72 hours of age, remaining in the incubator constantly at 28 C, other than during the actual time of observation.

Measurements of each embryo were made from head to anus and anus to tail by using an ocular micrometer. In addition to the careful compilation of measurements, observations were also recorded at the same intervals as to physical changes in each embryo, thus permitting an accurate determination of the effects of the various concentrations of 742 solution and its side effects.

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A quite frequent observation was the presence of a definite swelling or enlargement at the tip of the tail. Other anomalies were a definite bloating around the cardiac region in many specimens (as high as 80% in the higher concentrations).

To preserve the embryos so that the tumors resulting from the 742 solution could be carefully observed, the *B. rerio* embryos were fixed by the Tellyesnechky method, stained with Harris' hematoxylin, and imbedded in paraffin in order that cross and sagittal sections could be made on a microtome.

These sections were permanently mounted on slides.

RESULTS

It was found that 742 solution definitely caused tumors. The tests made at various concentrations of 742 solution resulted in the following findings:

Parts per million 742 solution	Percentage of embryos with tumors
0.00	0.00%
0.50	42.9%
0.75	57.1%
0.80	57.5%
1.00	88.3%

DISCUSSION

It was necessary to remove the embryo from its chorion so it could be measured; this proved a difficult and delicate process. Consequently many specimens were destroyed. Although there were ten specimens in each test plate, the percentage of affected embryos could not always be based on ten specimens since one or more of them was sometimes destroyed in the process of removing it from its chorion. The results shown are based on the total number of embryos remaining at the end of the test. The embryos were examined under a microscope, and some specimens may have been very close to a tumorous condition but not counted as such.

Other results were noticed during these tests; for example, some were lacking pigment, and bending of the tails frequently preceded the definite formation of a tumor on the tip of the tail. In some of the plates, when the solution was 0.50 ppm., occasionally an embryo could be found which showed no ill effects; however, in the stronger solution, all embryos were affected in some manner, though not necessarily with tumors. It is reasonable to assume that the affected embryos would have developed tumors if the experiment was allowed to continue over a period more than 72 hours. This was not felt to be necessary since it was observed that tumors were being developed by the 742 solution.

CONCLUSIONS

From this experiment it can be seen that 742 causes tumors on *B. rerio* embryos. Further research could be done on the effect of 742 other than the causing of tumors. The bloating of the cardiac and stomach regions was particularly interesting and could be a project for further research.

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