The Effects of Chloretone on Developing Fish Embryos[1, 2]

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The purpose of this paper is to determine whether chloretone can be used to inhibit movement of fish embryos within the chorion, and allow for normal morphological development. Chloretone is a known antiseptic, anesthetic, and preservative. Its synonym is trichloro-tert-butyl alcohol. 4.5 grams will saturate 500 c.c. of water at room temperature. The use of chloretone as a movement inhibitor was suggested by Fromme, (1) in which frog embryo movement was inhibited without a subsequent inhibition of morphological development. Moog,(3) on the other hand, showed that anomalies of morphological structure are encountered when using chloretone on frog embryos. It was believed by Mathews and Detwiler,(2) that retardation of Ambystoma embryo development, while in chloretone, was caused by a diminished power of oxidation in the cells. This was confirmed

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by Moog for frog embryos. In this paper, it was found that morphological abnormalities resulted in fish embryos which were subjected to chloretone solutions.

MATERIALS AND METHODS

A saturated solution of Chloretone at 75° F. was diluted with water in the following proportions: 100, 50, 10, 5, 4, 3, 2, 1, and 0 per cent of saturation. 100 c.c. of each solution was poured into 500 c.c. finger bowls. Duplication of the 5, 1, and 0 per cent solutions was prepared and poured into other fingerbowls. Thus two series of solutions were set up; Series "A" being 100, 50, 10, 5, 1 and 0 per cent, and Series "B" being 5, 4, 3, 2, 1, and 0 per cent. Fertilized eggs of the zebra danio, *Brachydanio rerio* (Hamilton), incubated at 75° F. were introduced into the solutions of series "A" at two different development stages: ten eggs each at two hours after fertilization, and ten eggs each at 24 hours after fertilization. Twenty eggs each, cared for under the same conditions, were introduced two hours after fertilization, to the solutions of series "B". The embryos in solutions "A" and "B" were incubated at 75° F. The observations of series "A", were terminated at the end of 166 hours and 50 minutes; all fish still living, being fixed in Bouin's solution, and preserved in 60 per cent isopropyl alcohol. The observations of series "B", 4-0 per cent, were terminated at the end of 120 hours and five minutes, the 5 per cent group terminating at the end of 26 hours and 20 minutes; all fish still living, being treated as in series "A". Preserved materials was filed for future reference.

RESULTS

The results of the observations obtained by this experiment are defined in terms of series "A" and "B". It should be understood, that the two and 24-hour stages first referred to, increased in age as time passed while in their respective solutions, but, for clarity, are referred to throughout by these names.

Series "A". All embryos placed in the 100 per cent saturated solution of chloretone were immobilized five minutes after immersion, and were all dead within 37 minutes.

Immobilization of all embryos in the 50 per cent saturated chloretone solution was accomplished within 20 minutes. The two-hour stage embryos were all dead within three hours and fifteen minutes, the 24-hour stage embryos lasting approximately for seven and one-half hours.

The embryos in the 10 per cent saturated solution of chloretone were also immobilized within 20 minutes. All of the two-hour stages died within five hours. The 24-hour stage embryos did better, showing black pigmentation of the eyes and body, although this pigmentation was late in development and rather sparse. The pericardium was markedly enlarged and the tail showed subnormal development, many times being less than one-third the normal size. The eyes never formed a lens, and the yolk sac began to cloud within 49 hours after immersion. Death ensued; the last of the embryos being dead before 119 hours of immersion.

Embryos, immersed in the 5 per cent saturated solution of chloretone, were immobilized after approximately one and one-half hours. The two-hour stage embryos showed normal external gastrulation. Embryos, both of the two- and 24-hour stages showed profuse but ambornally placed black pigmentation of the body. The pericardium was enlarged, but not as greatly as those in the 10 per cent solution. Eye formation was complete; yellow body pigment was demonstrated, but irregularly placed. The tails were longer, but not at all normal in length. Motion, slow and irregular, was demonstrated by the 24-hour stage embryos, 31 hours after immersion. This movement became more intense during the passage of time and then subsided somewhat. The pectoral fins moved within the chorin continuously

till the end of the experiment, starting about 96 hours after immersion. Several embryos hatched 149 hours after immersion. These were abnormal. The body remained in a coiled position, after hatching, that was not corrected when placed in a 100 per cent solution of water for a period of 17 hours. The embryos moved in the water solution or the original solution by pectoral fin movements with short radial revolutions.

Embryos in a 1 per cent solution and those in water only (controls) exhibited similar development. Embryos in the 1 per cent solution showed little or no retardation of motion. Development of these embryos was in most cases slightly slower than in the control group, but the difference was very slight. Gastrulation was normal and so was formation and placement of black and gold pigment. Eye formation was normal. Hatching of the 24-hour stage embryos in the 1 per cent solution began 31 hours after immersion, while those in the control solution began 25 hours after immersion. All had hatched at the completion of the experiment. Swimming motion of embryos in the 1 per cent solution was the same as that of the controls.

Series "B". After immersion of the embryos in a 5 per cent solution of chloretone for 22 hours, 11 of the 20 were dead. The embryos had undergone complete gastrulation, and two embryos had formed tails. Although motion was impeded, later embryonic development was variable and abnormal. Black pigmentation was late in development and poorly dispersed; the pericardia were enlarged; the tails of most of the embryos were abnormally short and blunt; and the eyes varied in the degree of formation and pigmentation. One embryo developed acephalically. In general, development of these embryos was much retarded in comparison with all other groups of this series.

Embryos in the 4 per cent solution were immobile at the time of tail development, following a seemingly normal gastrulation. Black pigment was profuse, but variable in placement and the gold pigmentation almost normal. Eye development was complete; motion within the chorion, in the solution, began 46 hours after immersion; the pericardia were abnormally large. No hatching took place before the end of the experiment.

Those embryos in the 3 per cent solution of chloretone were immobilized after the development of the tail. Eye and pigment development was normal, but slower than in the 2, 1, or 0 per cent solution and the pericardia were slightly enlarged. Twitching motion of the head was observed approximately 42 hours after immersion. This motion, in time, became stronger, extending to the rest of the body. The pectoral fins moved constantly. Hatching started about 81 hours after immersion. The resulting embryos were malformed, exhibiting a semicircular curvature of the spine and consequent abnormal movement in the solution.

Embryos in the 2 and 1, per cent solutions showed slightly abnormal development while within the chorion. Those in the 2 per cent solution were somewhat inhibited in movement at the beginning of the tail formation. Hatched embryos of the 1 per cent and control solutions were the same as in series "A". The newly-hatched embryos in the 2 per cent solution showed variability of malformations, from those of the 3 per cent solution to the controls. One particular deformity of interest that seemed prevalent was a downward curvature of the head about 35° from the horizontal.

DISCUSSION

On the basis of the results obtained, it seems practical to assume that chloretone not only inhibited chronological development, but also produced morphological changes. Mathews and Detwiler,(2) believed that retardation of development appeared to be the result of a general lowering of the growth potentials by chloretone, caused by diminished power of oxidation in the cells. Fromme,(1) in his work on frog eggs, in order to avoid this retardation, used solutions of 0.025 to 0.3 per cent (by weight) and found only neg-

ligible differences of development between experimentals and controls in that series. On the other hand, Moog,(3) found that after the onset of neuralization in the same species in a 0.09 per cent (by weight) solution of chloretone, the effects became progressively more severe, and virtually all increase of respiration up to the heart-beat stage was obliterated. Abnormalities appear as the upper 50 per cent of the oxygen consumption was inacitvated. As the lower 50 per cent was inactivated, development was arrested and necrosis ensued, at a rate proportional to the extent of lowering of the oxygen rate.

According to the results obtained in this study, it seems that these results coincide with those obtained by Moog, and not as was found by Fromme, since the specimens did not develop normal structures or show only negligible differences of development.

It seems evident, that from the results obtained, the older the embryo is before it is placed into a concentrated solution of chloretone, the greater is its range of toleration for this compound. Thus by employing the reasoning of Moog in this situation, there is some sort of direct correlation of the ability to consume oxygen in the solution of chloretone with the particular developmental age of the embryo; or there is a correlation of a lesser oxygen demand of the embryo as it gets older developmentally than there is when it is younger.

In any case, it can be definitely established that chloretone cannot inhibit embryonic movement without a subsequent retardation as well as change in morphological development. It is the opinion of the writer that these changes are brought about by a similar mechanism to that which acts upon frog eggs.

CONCLUSION

It can be seen by these results, that chloretone has an inhibitory effect upon the movement of fish embryos, when used in comparatively high concentrations. The bad feature of this is, that it interferes with embryonic metabolic activities, thus resulting in morphological abnormalities of varying intensities, and in death. In very low concentrations no visible effect is seen regarding the inhibition of movement, and thus, this drug is an impractical one to use as a movement inhibitor.

LITERATURE CITED

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