Observations of Bacterial Antibiotics Which Inhibit Or Prevent the Development of Fish Embryos.[1, 2]

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Several years ago Mr. Robert Ingersol, while working in our laboratory on the embryology of the three-spot gourami, *Trichogaster trichopterus*, (Master's thesis unpublished), observed that the embryos cultured in water from a certain container developed abnormally. With the help of Mr. Ed Alexander, he was able to isolate certain bacteria from the water. These bacteria seemed to be responsible for the abnormal development.

Recently, we observed that when we used, as a culture medium, water that had been stored for several months in a closed container, all the embryos died. When the dead embryos were removed and the water used as a culture for new embryos, there was an 85% kill. It was also observed that these dead embryos did not develop fungus as did those in the control dishes. Agar plate cultures were made from the culture water. Dilutions were made so that individual organisms formed individual colonies. Agar slopes or streaks were then made from each characteristic type of colony. Smears were made and colonies composed of different kinds of organisms were examined and cultured.

Water suspensions of each kind of bacteria were made by adding water to an agar slope culture and shaking it vigorously. These bacterial suspensions were used to incubate embryos of *Brachydanio rerio* (zebra fish). The organisms in those suspensions which seemed to cause the death of the fish embryos were further isolated and tested. A culture of bacteria that was toxic to fish embryos was thus obtained. They were not further identified than that they appeared to belong to the *Bacillus subtilis* group.

After the fish eggs were laid, they were collected and sorted. They were then washed several times with autoclaved tap water. All dishes and the waters used were autoclaved for 30 minutes at 275° F and 15 lbs. pressure. Dishes were kept covered and precautions taken to prevent contamination. Twenty-five embryos in early stages of development were placed in 50 cc of sterile water in each cultural dish. Bacteria of the toxic strain were emulsified in distilled water or autoclaved tap water and the suspensions allowed to stand for varying lengths of time up to five days. Five cc of these bacterial suspensions were then introduced into the test dishes. Check dishes were set up for each experiment. The embryos were cultured 72 hours and the % of mortality was then determined.

In four experiments (200 embryos), the bacteria were allowed to stand in the water for five days before being added to the embryos. The total mortality in the bacterial suspensions was 66% while in the check cultures it was 19%. In one test (50 embryos), where the bacterial suspension was allowed to stand only two days, the mortality for the test culture was 40%, and for the controls 16%.

In two tests (100 embryos), where the bacterial suspension was used fresh, the total mortality in the tests was 24% and in the controls 20%.

In one experiment (50 embryos), the fish were 24 hours old before being exposed to a bacterial suspension that had been allowed to stand for five days. The test mortality was 24% as opposed to a check of 4%. We consider this as evidence of considerable toxicity, as other tests indicate that embryos 24 hours of age are much more resistant than are embryos in cleavage. It also indicated that the toxic substance was increased when the bacterial suspension was allowed to stand undisturbed.

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In other experiments the bacterial suspension was allowed to stand five days and was then autoclaved. In one test (50 embryos), the mortality of the test material was 24% to 28% for the control. In a repeat, the test mortality was 32% to 24% for the check. This would seem to indicate a loss of toxicity due to the autoclaving.

On the basis of these experiments and other observations, it appears evident that the bacterial cultures did increase the mortality among the fish embryos. In those embryos that did survive in the test cultures, there were many more abnormalities than among those in the check dishes. Fungus growth in the test cultures was definitely retarded. Autoclaving the bacterial suspensions before adding them to the test dishes seemed to destroy their toxicity.

Recent observations have indicated that washing all dishes and experimental glassware in a strong solution of chlorox will decrease the normal mortality rate of the fish embryos from 20% to 4%.

In conclusion, it is suggested that certain bacteria growing in water will produce toxins (antibiotics) which are capable of killing young fish embryos. These substances are secreted or dissolve into the water and accumulate when the water is allowed to stand and/or is confined. They are labial in nature and can be destroyed by heat. Heat and chlorox treatment of glassware decreases bacterial contamination and permits a greater number of the embryos to survive.