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THE EFFECT OF DOSAGE VARIATION OF AUREOMYCIN ON THE DEVELOPMENT OF TOAD TADPOLES[1]

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ABSTRACT

Aqueous solution of aureomycin in the dose of 15 ppm showed a retarded metamorphic effect on toad tadpoles when drug treatment was begun at hatching stage. The lower doses of 1.5, 0.15 and 0.015 ppm did not exhibit such influence. When tadpoles were immersed in aureomycin solution beginning at stage III, they were not affected at all by any dose tested so far as metamorphosis and growth were concerned. Therefore reaction of toad tadpoles toward aureomycin immersion was more evident in young tadpoles than in older ones.

In 1953, Mustakallio and Telkka fed tadpoles with aureomycin and liver powder, resulting in an increase of body weight of the tadpoles (1). In 1954, they immersed tadpoles in aureomycin solution and found that the same antibiotic retarded metamorphosis (2). In 1960, Hsü fed tadpoles food containing aureomycin and reported an accelerated metamorphosis (3). In 1962, Hsü *et al.* again immersed tadpoles in aureomycin solution and confirmed Mustakallio's finding (4), Thus the same antibiotic produced inconsistent results on tadpole development. Presumably this is due to differences in aureomycin dosage, methods of application and age and species of tadpoles used; hence the occurrence of conflicting results.

The present experiments were designed to use toad tadpoles to verify whether different ages reacted differently to varying doses of aureomycin in the culture medium.

MATERIALS AND METHODS

Fertilized eggs of *Bufo melanosticus* were collected from a pond at the Grass Mountain in the suburb of Taipei. They were used for two experiments. In the first experiment, 210 selected tadpoles of the same size at the hatching stage (5) were divided at random into 5 groups. Each group of 42 tadpoles was raised separately in an aquarium containing 1000 ml of culture medium. Four groups of tadpoles were immersed in 15, 1.5, 0.15 and 0.015 ppm of crystalline chlortetracycline HCl_4 in tap water respectively. The fifth was the control group which was raised in plain tap water.

Another experiment was repeated using older tadpoles of stage III (6) from the same batch of eggs used in the first experiment. For this secod experiment, 198 tadpoles of the same size were divided into 6 groups, 33 animals in each. The concentration of aureomycin bath in these groups was 20, 15, 1.5, 0.15, 0.015 ppm and 0. The last one served as the control group.

The tap water used for the rearing medium was aerated with pump for two days before use. This was done in order to avoid the chlorine effect on tadpole growth and on aureomycin degradation. The medium was changed three times a week. Temperature was kept within a range of 21-24C, so that there was minimal chance of aureomycin destruction due to high temperature (7). The 6 groups of tadpoles were fed boiled green vegetables and kept under identical laboratory condition.

Metamorphic stages of the tadpoles according to Taylor and Kollros (6) were examined once

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or twice a week under a dissecting microscope. All tadpoles were photographed weekly or bi-weekly so that accurate measurement of total length was possible from the enlarged picture. The number of tadpoles reaching stage XX was recorded daily.

RESULTS

No dead tadpoles were found in these experiments.

The results of the first experiment are shown in *Figures 1, 2* and *3*.

Figure 1 indicates evidently that tadpoles immersed at hatching stage in 15 ppm aureomycin showed a significantly lower rate of metamorphic progress than the controls and those immersed in smaller doses of aureomycin solution. The mean difference of metamorphic stage, taken on the 36th day after the onset of the experiment, between tadpoles immersed in 15 ppm aureomycin and controls is 4.5 stages with a P value smaller than 0.01.

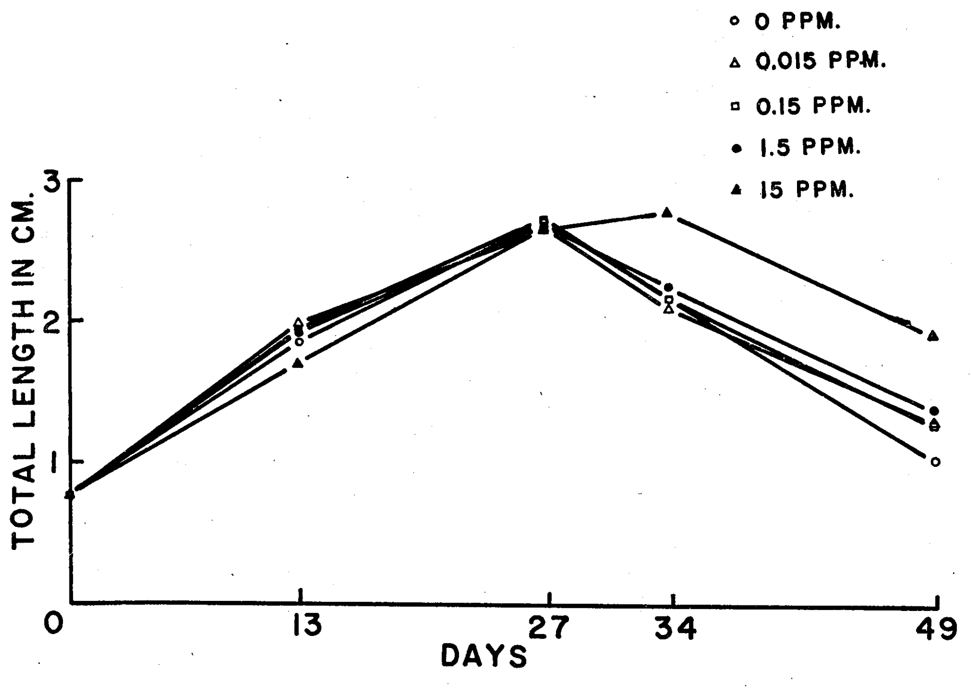

Fig. 1. Growth curves of metamorphic stage of control tadpoles and tadpoles immersed in different doses of aureomycin solution beginning at hatching stage.

It is noted from *Figure 2* that tadpoles treated with the largest dose of aureomycin (15 ppm) increased their total length faster than the control group with a mean difference of 6.3 mm and a P value smaller than 0.01, taken at the 34th day after the onset of the experiment.

Figure 3 shows that, after 60 days of observation from the onset of the experiment, tadpoles immersed in 15 ppm aureomycin had the lowest rate of metamorphic progress among the five groups judged by the cumulative percentage of tadpoles reaching stage XX. This result agrees with that of *Figure 1.*

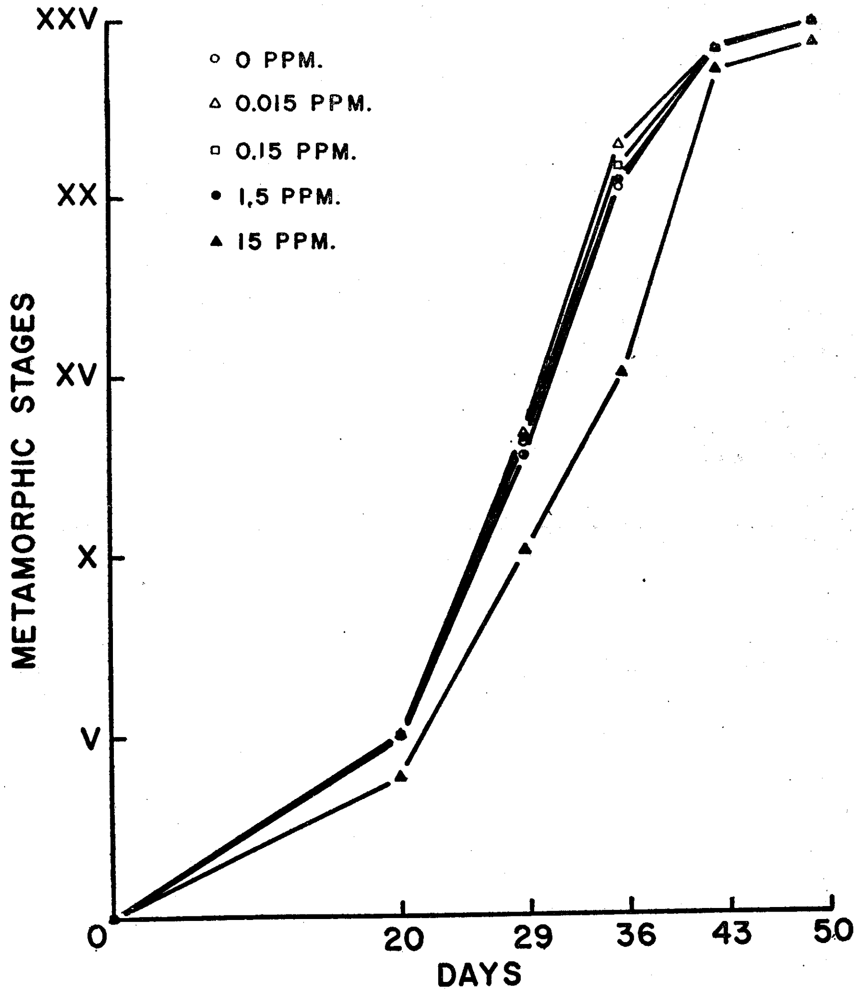

Fig. 2. Growth curves of total length of control tadpoles and tadpoles immersed in different doses of aureomycin solution beginning at hatching stage.

Figures 4 and *5* of the second experiment show the respective growth curves of total length and metamorphic progress of the control tadpoles and those immersed in different doses of aureomycin solution beginning at stage III. It is noted that when tadpoles were treated at an older stage, there was no difference in either total length or metamorphic stage among the 6 groups.

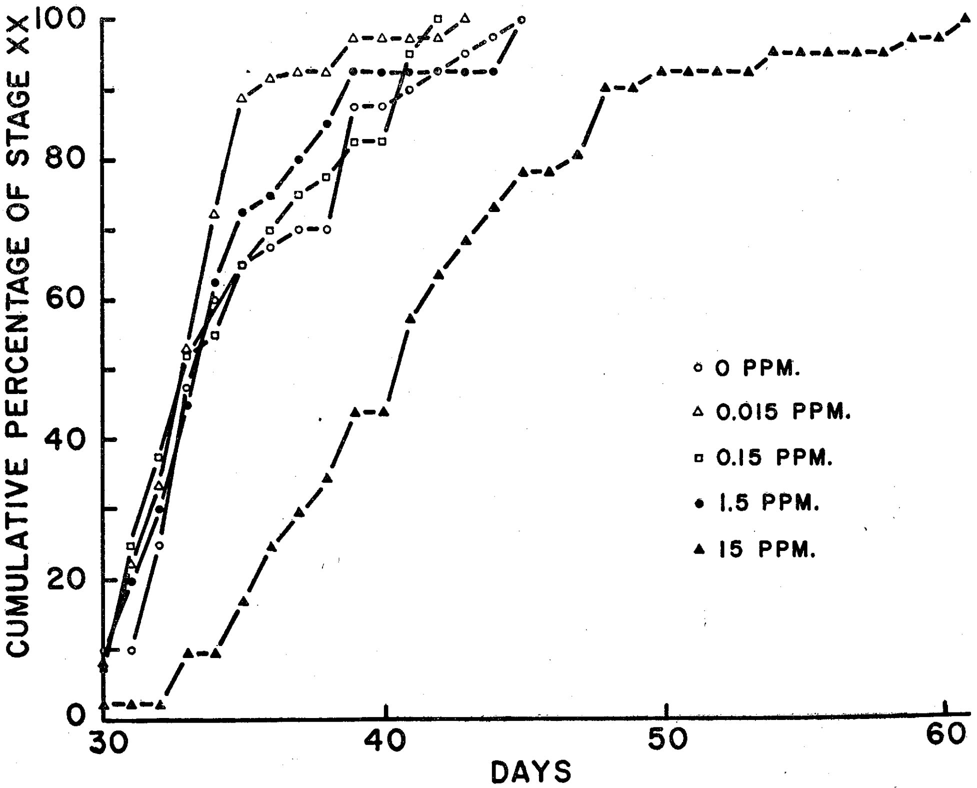

Fig. 3. Cumulative percentage of tadpoles reaching stage XX of the control group and four experimental groups treated with aureomycin beginning at hatching stage.

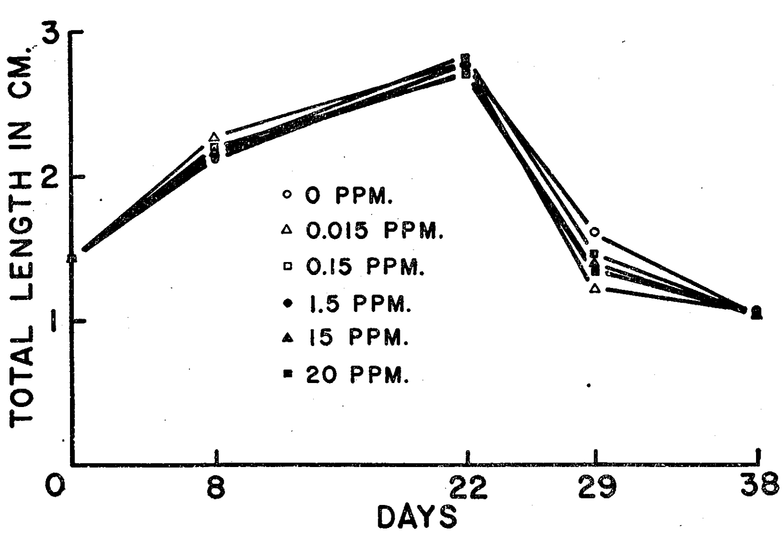

Fig. 4. Growth curves of total length of control tadpoles and tadpoles immersed in different doses of aureomycin solution beginning at stage III.

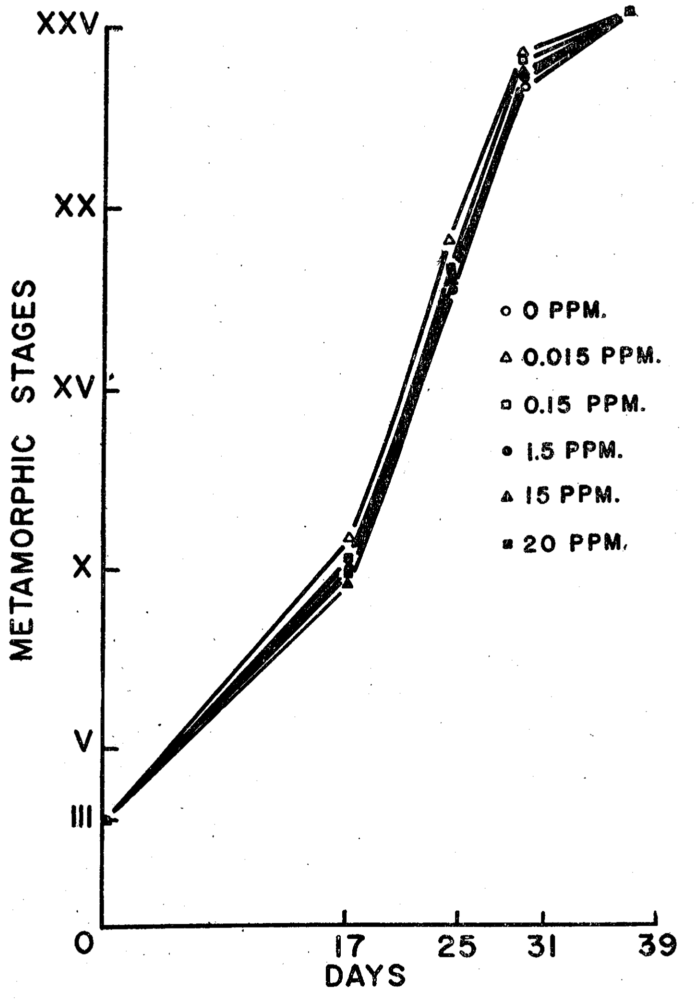

Fig. 5. Growth curves of metamorphic stage of control tadpoles and tadpoles immersed in different doses of aureomycin solution beginning at stage III.

DISCUSSION

In the present experiments no death occurred among the control and aureomycin treated tadpoles whereas in a previous study the mean mortality rate for *Rana catesbeiana* and *Rana limnochairs* were 3.6% and 6.7% respectively (4). This could be interpreted to be due to species difference.

Tadpoles of *Rana catesbeiana* and *Bufo melanosticus* look very tough and strong while those of *Rana limnocharis* are delicate and tender. Toad tadpoles could withstand aureomycin treatment well and survived even when the drug treatment was started as early as the hatching stage. It appears that toad tadpoles could tolerate adverse condition better than frog tadpoles.

The species difference between toad and frog tadpoles manifested again in the reaction of development toward aureomycin immersion. When tadpoles of *Rana catesbeiana* and *Rana limnocharis* were immersed in 15 ppm of aureomycin beginning at stage III (hindleg bud stage), they showed retarded metamorphosis (4). But when toad tadpoles in the present experiment were bathed in the same concentration of aureomycin solution at the same stage, the antibiotic did not exert any retarded influence on metamorphosis.

However, when toad tadpoles were treated with the same method but at an earlier developmental stage (hatching stage), the effect of retarded metamorphosis showed up both in the progress of metamorphic stage (*Fig. 2*) and in the cumulative percentage of metamorphosis (*Fig. 3*). It shows then that the sensitive period of tadpole development to aureomycin treatment is different in frogs and toads.

The retarded metamorphosis produced in the present experiments was thought due to hormonal disturbance because the growth of total length of tadpoles was accelerated instead of retarded, as shown in *Fig. 1*. It is generally considered that delayed metamorphosis usually brings forth an increase of body size.

The suggestion of hormonal disturbance by aureomycin treatment is further supported by the result of a previous experiment that exogenous thyroxin conteracted the action of aureomycin on retarded metamorphosis (4).

The effect of different doses of aureomycin on toad development is very interesting. When young tadpoles at hatching stage were treated with aureomycin bath of various concentration, it was only the highest dose of 15 ppm which caused a retarded effect on metamorphosis while lower doses of 1.5, 0.15, and 0.015ppm did not. When older tadpoles of stage III were treated likewisely, even a dose of 20 ppm did not show any retarded result. Therefore the minimal dosage of aureomycin to retard development of toad tadpoles was 15 ppm for hatching stage and above 20 ppm for stage III under the present experimental condition. It is only natural that the effective minimal dosage was different for different developmental stages, because younger embryos are often more labile and thus more sensitive to environmental change than the older ones. However the minimal dosage could be variable according to temperature and pH value of the culture medium, since these two conditions are the important factors influencing stability of aureomycin (7).

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