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THE GROWTH-PROMOTING EFFECT OF DECOMPOSED AUREOMYCIN ON TADPOLE DEVELOPMENT

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ABSTRACT

Four groups of tadpoles of *Rana plancyi* were immersed respectively in untreated aureomycin solution, NaOH-treated aureomycin preparation, dilute NaOH solution and tap water. The first group of tadpoles showed a lower and the second group a higher rate of growth than the normal controls. The third group had the same growth rate as the normal controls. Thus the untreated aureomycin depressed while the alkalinized and decomposed aureomycin promoted growth of tadpoles. The implications of these results are discussed.

In animal husbandry it is a usual practice to supply a minute amount of aureomycin or terramycin to the basal ration to promote growth. This growth-promoting effect is generally considered a result of antibacterial activity of the antibiotic (1). However the mechanism by which such a benefit is produced has never been convincingly demonstrated.

In tadpoles the effect of aureomycin on development was by no means consistent (2-5). Whereas aureomycin was found to accelerate metamorphosis when fed to tadpoles (4), it produced a retardation of metamorphosis when tadpoles were immersed in the antibiotic bath (3, 5).

This discrepancy was thought to be due to the different methods of administering aureomycin to tadpoles. When tadpoles were fed aureomycin in food, the antibiotic might readily undergo chemical decomposition while passing through the alkaline medium in the intestine particularly if the aquarium temperature was high, since aureomycin is known to be unstable at pH 8.5 and a temperature of 25 C (6). On the other hand by immersing tadpoles in aureomycin bath, the antibiotic presumably could be absorbed as such into the animal body mainly through the skin and mucous membrane and the degree of decomposition would be minimal provided the environmental temperature was low. It would seem, therefore, the possibility exists that the controversial results between feeding and immersing tadpoles with aureomycin could be related to the chemical status of the antibiotic present in the culture medium and that aureomycin *per se* might affect differently from its degraded product(s) the metamorphosis of the tadpoles. The same possibility may intrigue the mode of action of aureomycin in growth-promotion in animal husbandry.

Since tadpoles are poikilothermics, it is possible to minimize aureomycin decomposition by controlling the environmental temperature when rearing them in the antibiotic bath. The present experiment was designed to compare tadpole development by using aureomycin and alkalinized aureomycin in the bath media under controlled temperature conditions.

MATERIALS AND METHODS

Fertilized eggs of *Rana plancyi* from induced breeding were allowed to develop until hatching. 480 selected tadpoles of uniform size at this stage were divided at random into 4 groups of 120. The first group of tadpoles was reared in aquarium containing 5 ppm untreated aureomycin in terms of crystalline chlortetracycline hydro-

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chloride, the second group in aquarium containing alkalinized aureomycin prepared as given below, the third group in 0.0001N NaOH solution and the last group in tap water as controls.

The alkalinized aureomycin was prepared by adding 0.5 ml of 6N NaOH to aureomycin solution containing 150 mg of crystalline chlortetracycline hydrochloride in 99.5 ml of distilled water. After standing 3-5 days at room temperature of 26 C, one ml of this preparation shaken thoroughly was added to 299 ml of tap water. The concentration of aureomycin in this preparation was also 5 ppm in terms of crystalline aureomycin hydrochloride.

All four groups of tadpoles were raised separately in glass aquaria kept in a water bath at the constant temperature of 24 C. The space allotted to each tadpole was 15 ml for the first five weeks and 30 ml for the remaining experimental period.

As chlorine is detrimental to tadpole development and at the same time also hastens aureomycin decomposition, the tap water used in these experiments was always aerated by bubbling with air for 24 hours before use.

The rearing media of the four groups were changed thrice a week. The pH of the four different media was all found to be 7.1. Tadpoles were fed boiled green vegetables.

The four groups of tadpoles were photographed once every two weeks. Total length and body width of each tadpole were measured from enlarged pictures with a scale in milimeter of the same enlargement. During the latter period of the experiment the number of tadpoles reaching stage XX (7) was recorded daily.

RESULTS

Growth

The growth rate in terms of total length and body width of the 4 groups of tadpoles is shown in *Fig. 1.*

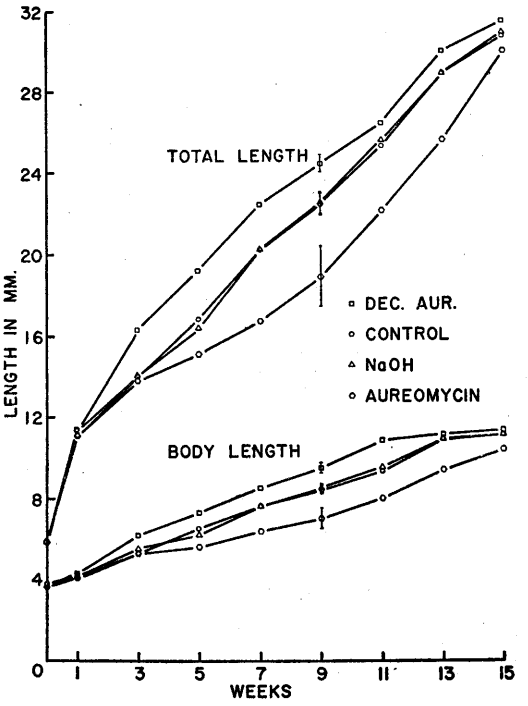

Fig. 1. Growth curves of total length and body width of the 4 groups of tadpoles. Vertical bars represent standard errors.

TABLE I

Statistical differences of total length and body width between tadpoles of different groups 9 weeks after onset of the experiment.

Group	Mean total length±S. E. (mm)	P_1*	P_2	P_3	Mean body width±S. E. (mm)	P_1	P_2	P_3
II†	24.5±0.44				9.5±0.14			
III	22.5±0.51	<0.01			8.5±0.17	<0.01		
IV	22.4±0.54	<0.01	0.90		8.4±0.18	<0.01	0.69	
I	18.9±1.51	<0.01	0.03	0.03	7.0±0.54	<0.01	0.01	0.01

† II: Decomposed aureomycin
III: NaOH
IV: Control
I: Untreated aureomycin

* P_1: Comparison between II and III, IV, I respectively
P_2: Comparison between III and IV, I respectively
P_3: Comparison between IV and I

The curves indicate clearly that the growth rate of tadpoles reared in decomposed aureomycin was greater, while that of tadpoles in untreated aureomycin medium was smaller than the normal and dilute alkali controls. The latter two groups showed a closely similar growth rate.

Table I indicates that tadpoles immersed in decomposed aureomycin had a significantly greater rate of growth than any of the other three groups. The controls were significantly larger than untreated-aureomycin-bathed tadpoles but the difference between the controls and those reared in dilute NaOH was not statically significant. Thus the accelerated growth rate of tadpoles in decomposed aureomycin bath was apparently due to the degraded product(s) of aureomycin only but not the NaOH factor.

Therefore the data show that the action of untreated and decomposed aureomycin on tadpole development was distinctly divergent: untreated aureomycin retarded growth while decomposed aureomycin promoted it.

Metamorphosis

The rate of metamorphosis expressed as the cumulative percentages of tadpoles reaching stage XX is given in *Fig. 2.*

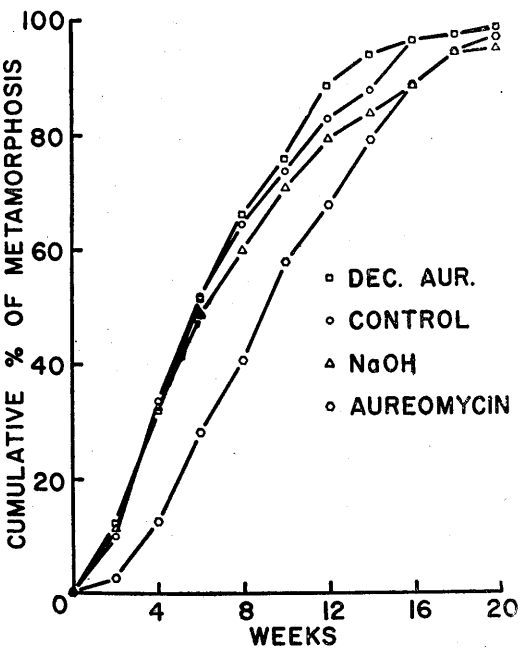

Fig. 2. Cumulative percentage of tadpoles of the 4 groups reaching stage XX.

It is evident that immersion in untreated areomycin bath delayed metamorphosis and that the delay occured chiefly in the earlier part of the process. On the other hand immersion of tadopoles in decomposed aureomycin and dilute NaOH did not affect metamorphosis, when compared with the normal control group.

DISCUSSION

The diametrically opposite effects on tadpole growth, retardation versus acceleration as caused by untreated and decomposed aureomycin respectively, were analysed from the stand point of aureomycin stability.

The stability of aureomycin depends on a number of factors, amongst which the pH and temperature are important. An unbuffered aureomycin HCl solution at pH 2.9 maintained at 4 C showed no measurable loss of activity for 23 days (8). However at pH 8.5 and 25 C it lost 12% of its activity in 30 minutes, 20% in one hour and 40% in two hours (6). At 37 C, aureomycin was unstable even in distilled water and this instability was markedly increased in broth and serum (9).

From unpublished data of this laboratory it was known that 15 ppm aureomycin at pH 7.1 and 24.5±0.27 C stopped growth of *R. narina* tadpoles while elevation of temperature to 30.3±0.7 C similar aureomycin solution caused only retardation of growth of the samd species. Thus aureomycin at a lower temperature exerts a more detrimental effect on growth than at higher temperature, impling that aureomycin at 24 C preserves more of its antibacterial activety than at 30 C. To what extent the untreated aureomycin as used in the present experiment retains its bacteriostatic activity is being studied in this laboratory. When aureomycin was treated with NaOH and then kept at room temperature of 26 C for several days, the antibiotic was apt to deteriorate and lose its activity (10, 11).

The mode of action of aureomycin on bacteria is generally considered to be primarily bacteriostatic. Relatively high concentrations are required for bactericidal activity. This concept is based on the findings that aureomycin interfers with protein synthesis (12, 13) and blocks aerobic phosphorylation (14) and some parts of the Kreb's cycle (15). Likewise, it also retards or completely inhibits the proliferation of cells in tissue culture (16), Therefore it is only natural that untreated aureomycin should retard tadpole growth.

On the other hand the accelerated growth of tadpoles reared in alkalinized aureomycin could be due to the action of decomposed aureo-

mycin. The accelerated metamorphosis obtained in feeding experiment (4) could also be due to this factor, because the aquarium temperature averaged 30.2±0.32 C and the antibiotic was likely decomposed too. However in the present experiment the metamorphic rate of the tadpoles immersed in decomposed aureomycin did not differ from that of the control group. This might be due to difference in the dosage used, 5 ppm in the present experiment in contrast to 100 ppm in the earlier experiment (4).

That decomposed aureomycin accelerated growth and metamorphosis of tadpoles suggests the possibility that the growth-promoting effect of aureomycin in birds and mammals is not due to the antibacterial activity of the antibiotic but is also due to the same mechanism as proposed for the accelerated growth of tadpoles.

In homeothermic animals, the body temperature is always near or above 37 C and pH of the fluid in small intestine is mostly alkaline (17). Under such conditions when aureomycin is administered orally in very minute quantity (usually 25-100 ppm) as food supplement, the antibiotic would have been decomposed, and the bacteriostatic activity left may be either null or negligible. Apparently it is not the original intact aureomycin which promotes animal growth but the effect may be due to decomposed aureomycin.

The mode of action of aureomycin on growth-promotion has never been satisfactorily established. The current theory suggests that the antibiotic would suppress pathogenic bacteria and augment vitamin-B producing microbes in the intestine, thus stimulating growth through a better nutrition (1). However the bacteriological findings were not convincing since sometimes they were contradictory (18).

On the other hand, facts inconsistent with the antibacterial concept of antibiotic promotion of growth were presented by some workers. Hester *et al.* reported that the injection of aureomycin in dairy calves caused an increase in growth but no aureomycin was found in the lumen of the small intestine (19). Nickcll found a stimulation of plant growth in virus tumor in tissue culture, in germinated seeds and in germinated seeds with subsequent growth in soil by the treatment of the antibiotic (20). Barber and co-workers noticed the additive effect on growth and feeding efficiency when aureomycin in combination with diethylstilbestrol and L-thyroxine was fed to growing pigs (21). Grant reported a small but definite increase of the uptake of I^{131} by the thyroid when the antibiotic was fed to rats (22).

Recent evidences at variance with the antibacterial theory of growth-promotion were found in this laboratory. Hsu *et al.* reported that growth rate of germ-free intrauterine embryos of mice was stimulated by injection of aureomycin to the pregnant mother (23). Hsu and Pi found neither acceleration nor retardation in tadpole development when penicillin was supplemented to the food despite of its antibiotic nature (24). Further more the result of accelerated metamorphosis suggested the possiiblity of altering hormonal activity by this antibiotic (4).

All these findings serve to prove that the current theory of antibiotic growth-promotion based on antibacterial action is not likely true. Conversely, the present experiment indicates that the growth-promoting effect of aureomycin may be due to its degraded product(s) rather than intact aureomycin itself.

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