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EFFECT OF AUREOMYCIN ON DEVELOPMENT OF FROG TADPOLES AS RELATED TO TIME OF ONSET OF TREATMENT

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ABSTRACT

150 tadpoles of *Rana narina* were divided equally into 5 groups. One group was reared in tap water at stage 0 when hindlimb buds were not discernible; the other 4 groups were reared in 15 ppm aureomycin medium beginning at stage 0, V, X and XV respectively. Metamorphic stages and growth in total length and body width were observed biweekly for 22 weeks. Retardation of metamorphosis and growth of aureomycin-treated tadpoles thus varied with the stage of development at onset of treatment. The earlier treatment was begun, the more pronounced was the effect on growth and metamorphosis. This is in accordance with the general biological principle that younger embryonic organisms are more susceptible to altered environment.

INTRODUCTION

Sanfilippo (1) and Mustakallio and Telkka (2) reported respectively in 1953 and 1954 that metamorphosis was retarded, when frog tadpoles were reared in aureomycin medium. This finding was confirmed by Hsü *et al.* (3) in 1962 using 2 different species of tadpoles, and by Pi and Hsü (4) in 1963 using toad tadpoles. Hsü *et al.* (5, 6) further showed in 1964 and 1965 that when metamorphosis was halted by thyroidectomy, the growth of the operated tadpoles was also retarded by aureomycin, and that growth could be stopped completely if the treatment was continued. In general, therefore, available evidence indicates that development of tadpoles is affected adversely by treatment with aureomycin.

Development is generally of a labile nature, possesing multiple potentialities and thus can lead to different end results according to various conditions. The developing individual may deviate from the norm in different ways depending on age of exposure to an adverse environment. In general, younger embryos are more labile and thus more susceptible to aureomycin treatment. The present experiments were conducted to investigate the extent to which tadpole development can be modified by aureomycin treatment, beginning at different stages of metamorphosis.

MATERIALS AND METHODS

Fertilized eggs were obtained from the induced breeding of a pair of frogs, *Rana narina.* 150 tadpoles of approximately uniform size, with no discernible hindlimb buds (stage 0), were selected for the experiments; 30 were reared in 4000 ml dechlorinated tap water containing 60 mg of crystalline chlortetracycline HCl* (15 ppm) as an experimental group (A1); another 30 were reared in 4000 ml dechlorinated tap water as

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* Aureomycin, kindly supplied by the Cyanamid Taiwan Corporation.

the control group (C). The remaining tadpoles were divided equally into 3 groups (A2, A3 and A4), and each was kept in the same volume of dechlorinated tap water. When the last 3 groups of tadpoles reached stage V, X and XV (7), respectively, they were treated also with 15 ppm of aureomycin. The experimental scheme is shown in TABLE I.

TABLE I

Data pertaining to initial experimental conditions of the 5 groups of tadpoles

Group*	C	A 1	A 2	A 3	A 4
Date (1965)	July 19	July 19	Aug 20	Sept 6	Oct 16
Stage	0±0	0±0	V.3±0.3	IX.3±0.7	XV.3±0.3
Total length (cm±SE)	1.35±0.02	1.35±0.02	2.65±0.04	3.34+0.07	4.88±0.01
Body width (cm±SE)	0.31±0.00	0.31±0.00	0.53±0.01	0.67±0.01	0.72±0.03

* C, control tadpoles; A1, A2, A3, and A4, groups of tadpoles treated with 15 ppm aureomycin at dates indicated.

All groups of tadpoles were fed boiled green vegetables *ad libitum*; the rearing medium was changed 3 times weekly. All tadpoles were maintained at 20±0.8 C. Metamorphic stages were examined biweekly. Total length, from tip of nose to tip of tail, and body width were measured to 0.05 cm from enlarged photographs taken at 2-week interval. The experiments lasted for 22 weeks, at which time all controls had reached metamorphic climax.

RESULTS

I. Metamorphosis

Metamorphosis of experimental groups A1, A2 and A3 was retarded by aureomycin treatment, when compared with the control group, as shown in *Fig. 1*. Tadpoles, treated beginning at stage 0 (Group A1), showed an inhibited metamorphosis and development ceased at stage V.5 whereas all controls reached stage XXII. The difference (XVI.5±1.7 stages) was remarkable. When treatment was started later (Groups A2 and A3), the effect of aureomycin in retarding metamorphosis was less apparent, but the difference in metamorphic rate between control and experimental groups was still evident (TABLE II). In comparing control and group A4, however, there was an insignificant difference. Therefore, the action of aureomycin on metamorphosis was obviously modified by the stage of development at onset of treatment.

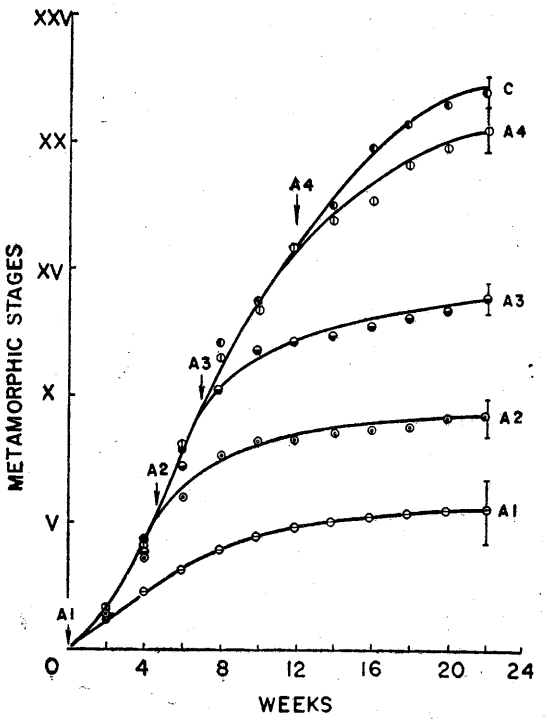

Fig. 1. Effect of aureomycin on attainment of metamorphosis, as related to the stage of onset of treatment. Arrows stand for the beginning of treatment. Vertical bars represent standard errors. Abbreviations as for TABLE I.

TABLE II

Comparison of metamorphic stages attained by control and aureomycin-treated tadpoles

Group*	Initial stage	Final stage	Difference	P
C	0±0**	XXII.0±0.7	—	—
A 1	0±0	V.5±1.6	XVI.5±1.6	0.001
A 2	V.3±0.3	IX.3±0.8	XII.7±0.8	0.001
A 3	IX.3±0.7	XIV.0±0.7	VIII.0±0.7	0.001
A 4	XV.3±0.3	XX.5±1.0	1.5±1.0	0.23

* Abbreviations as for TABLE I

** Mean stage±SE

II. Growth

Pari passu with the retardation of metamorphosis, growth of the aureomycin-treated tadpoles was inhibited. *Fig. 2* shows growth in total length of each of the 5 groups of tadpoles. The controls reached the maximal total length at the 12th week and then decreased in length suddenly because of resorption of tails. Group A4 progressed with the same rate before treatment as that of the controls but could not maintain this rate after aureomycin treatment was begun. Metamorphosis in Group A4 was but slightly retarded by the treatment (*Fig. 1*), and thus resorption of tails did not take place as fast as in the controls.

Tadpoles of Groups A1, A2 and A3 did not attain a full total length equal to that of controls but developed slowly, and the rate of growth in total length also depended on time of onset of treatment.

TABLE III gives a comparison of body width of the 5 groups of tadpoles. It showed the same effect as for growth in total length.

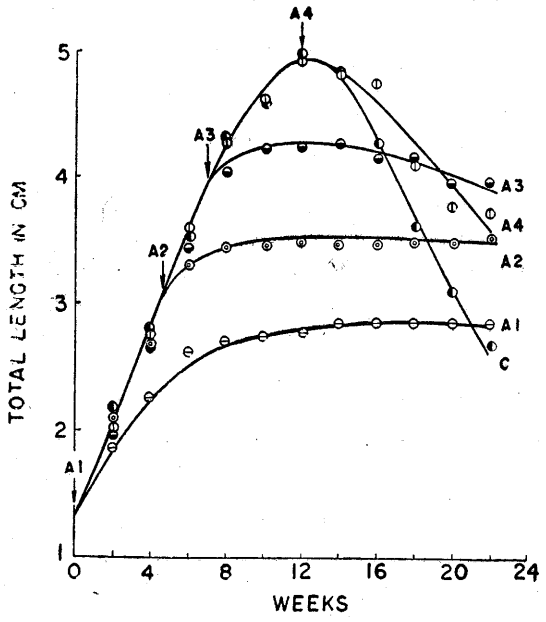

Fig. 2. Effect of aureomycin on growth of total length, as related to length at time of onset of treatment. Abbreviations as for TABLE I

TABLE III

Comparison of body width of the control and aureomycin-treated tadpoles

Group*	Initial width	Final width	Difference	P
C	0.31±0.0**	0.92±0.02	—	—
A 1	0.31±0.0	0.63±0.02	0.29±0.03	0.001
A 2	0.53±0.01	0.70±0.02	0.22±0.03	0.001
A 3	0.67±0.01	0.78±0.07	0.14±0.03	0.001
A 4	0.72±0.03	0.87±0.03	0.05±0.04	0.16

* Abbreviations as for TABLE I

** Mean width±SE (cm)

DISCUSSION

The present results are in accordance with the general biological principle that younger embryonic organisms are more susceptible to adverse environment than older ones. They also confirm our previous work on the effect of aureomycin on development of toad tadpoles: treatment beginning at hatching stage caused retarded metamorphosis, whereas treatment at stage III had no such effect (4).

Under the conditions of this study, aureomycin treatment started at stage 0 caused a high mortality (60%). Tadpoles treated beginning at stage XV appeared to be as healthy as the controls (TABLE IV).

TABLE IV

Mortality of the control and aureomycin-treated tadpoles

Group*	C	A 1	A 2	A 3	A 4
Initial No.	30	30	30	30	30
Final No.	30	12	23	23	30
Mortality %	0	60	23.3	23.3	0

* Abbreviations as for TABLE I

The indifference of the tadpoles in Group A4 to aureomycin treatment could be due to inadequacy of the dosage used or to length of treatment. They might have suffered a comparable or delayed developmental arrest, if aureomycin had been given in a dose higher than 15 ppm, or for a longer period of time.

It is of interest to note that treatment with the same antibiotic in approximately the same dose on thyroidectomized tadpoles (8) gave a paradoxically different result regarding age factor in that aureomycin exerted a more pronounced effect on growth of 24-month-old than on 5-month-old animals. Thyroidless tadpoles will probably never metamorphose unless exogenous thyroxin is given. Intact tadpoles of *Rana catesbeiana* usually become froglets at the age of 4 months under the subtropical climate of this island. So, as life span of tadpoles of this species is concerned, those at the age of 24 months may be considered senile; whereas the age of 5 months, the prime of life. Generally, aged organisms are fragile and their reaction to adverse condition is as susceptible as that of the very young. Therefore, aureomycin would exert a comparable detrimental effect on both ends of the life span of tadpoles.

The present results of retardation of metamorphosis and growth in tadpoles did not differ essentially from those obtained by other workers (1, 2) and from our studies (3, 4, 5, 6 and 8). Therefore, the possible mechanism of action of aureomycin on tadpoles discussed previously (5) holds true for the present results.

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