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FURTHER STUDIES ON ALKALI-DECOMPOSED AUREOMYCIN ON TADPOLE GROWTH

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ABSTRACT

The alkali-decomposed aureomycin was separated into precipitate and supernatant portions. By the differential test of the 2 portions on tadpoles of *Rana plancyi*, it was found that the precipitate stimulated while the supernatant retarded tadpole growth.

It was shown in a previous report that alkali-decomposed aureomycin (1, 2) stimulated while untreated aureomycin retarded tadpole development (3). The present report deals with studies on tadpole growth as affected by the soluble and precipitate portions of the alkali-decomposed aureomycin.

MATERIALS AND METHODS

Fertilized eggs were obtained from induced breeding of *Rana plancyi*. 480 tadpoles of the same size at hatching stage were divided at random into 4 groups of 120. They were reared in separate aquaria, each containing 4,000 ml of the following culture media respectively.

1) 1 ml of the decomposed aureomycin mixture, thoroughly shaken, per 100 ml of tap water.
2) 1 ml of the supernatant per 100 ml of tap water.
3) 1 ml of the precipitate per 100 ml of tap water, shaken thoroughly.
4) Plain tap water as the control group.

The decomposed aureomycin mixture was prepared by adding 0.75 ml of 6 N NaOH to 149.25 ml of distilled water containing 1 g of crystalline chlortetracycline HCl. While standing 3 days at room temperture, the preparation changed its color from yellow to dark brown. A bright colored precipitate gradually appeared and a bluish purple fluorescence developed.

The precipitate was separated from the supernatant by filtering through a filter paper. After washing three times with distilled water the precipitate was dried in an oven at 30 C for 3 days. Finally greyish white powder was obtained, weighing approximately 700 mg.

The 4 groups of tadpoles were fed boiled green vegetables and kept at room temperature averaging 31.1±0.44 C. The culture media were changed thrice weekly.

All tadpoles were photographed weekly so that accurate measurement of total length and and body length was possible from the enlarged pictures.

RESULTS

The increase of total length and body length of the 4 groups of tadpoles is shown in *Fig. 1*, indicating that the growth rate of the tadpoles in the precipitate and mixture groups was greater than those reared in the control and supernatant groups, the latter being smaller than the controls.

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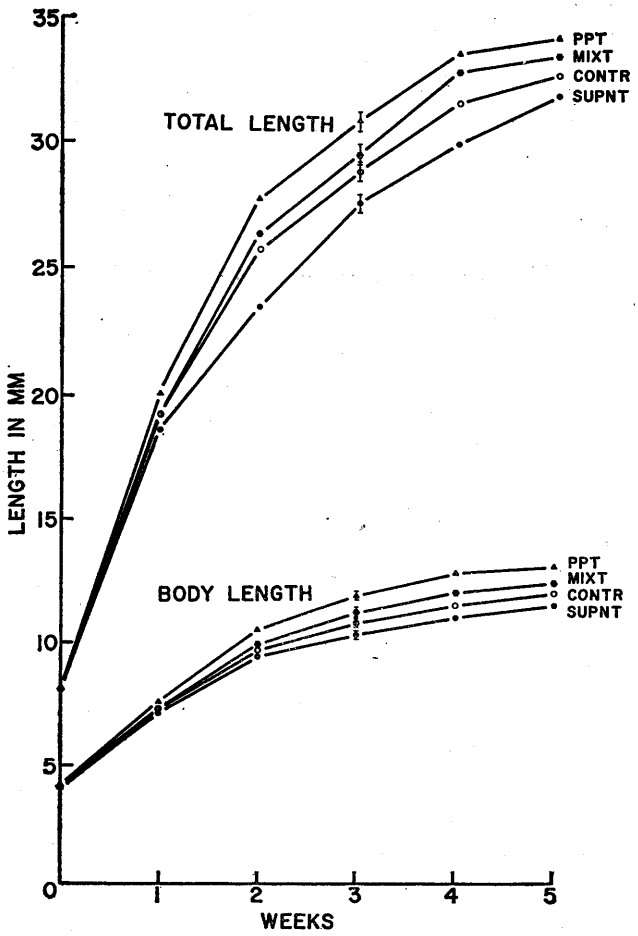

Fig. 1. Growth curves of total length and body length of the 4 groups of tadpoles. Vertical bars represent standard errors. Abbreviations are the same as those of TABLE I.

The significance of the mean differences of total length and body length of tadpoles between groups is presented in TABLE I.

The table shows that the growth rate of tadpoles in precipitate was definitely greater than any of the other 3 groups and that the tadpoles in supernatant were the smallest of all. However, the mean difference of growth rate between control and mixture groups was not significant although the latter was the larger one.

It is thus evident that, by the differential test of the different portions of alkali-decomposed aureomycin, the precipitate portion was responsible for stimulating and the supernatant responsible for retarding tadpole growth.

DISCUSSION

The effects of the precipitate and supernatant portions of alkali-decomposed aureomycin on tadpole growth were diametrically opposite: acceleration versus retardation. The net effect of the 2 should approximate the result of the decomposed aureomycin mixture. From *Fig. 1* it is noted that the mean total length of tadpoles reared in mixture group was approaching closely to the difference between the mean total length of the precipitate and supernatant groups, and so was the mean body length. Therefore the experimental result agreed with the expected.

The difference of the mean total length (mean body length) between the mixture and control groups in the present experiment was not as significant as previously reported (3). This might be attributed to differences in dosage and temperature. In the present experiment aureomycin used in the decomposed mixture preparation was 67 ppm whereas in the previous experiment it was only 5 ppm. A higher concentration of

TABLE I

Statistical comparison of total length and body length of tadpoles among the 4 groups

Group	Mean total length±S.E., mm	*P_1	P_2	P_3
†Ppt.	30.7±0.39			
Mixt.	29.3±0.43	0.01		
Contr.	28.7±0.37	<0.01	0.27	
Supnt.	27.4±0.36	<0.01	<0.01	0.01
Group	Mean body length±S.E., mm	*P_1	P_2	P_3
†Ppt.	11.7±0.19			
Mixt.	11.0±0.22	0.01		
Contr.	10.6±0.20	<0.01	0.16	
Supnt.	10.1±0.17	<0.01	<0.01	0.05

†Ppt.: precipitate.
Mixt.: decomposed aureomycin mixture.
Contr.: control.
Supnt.: supernatant.
*P_1: comparison between precipitate group and mixture, control, supernatant groups respectively.
P_2: comparison between mixture group and control, supernatant groups respectively.
P_3: comparison between control and supernatant groups.

aureomycin might be conceivably imcompletely decomposed, with the possibility that some intact aureomycin would still exert its retardation effect, thus resulting in a less significant difference between the mixture and control groups in the present experiment.

In order to stabalize aureomycin solution in the aquaria, tadpoles in the previous experiment were kept at 24 C (3). In the present experiment since aureomycin was already decomposed, tadpoles were reared at room temperature averaging 31±0.44 C. Under lower temperature tadpoles developed more slowly, and thus had a longer embryonic period being subjected to the action of the drug. Therefore the growth-promoting effect of the decomposed aureomycin mixture was more obvious than that of the present experiment.

Solubility test showed that the precipitate was amphoteric and sparingly soluble in water. Microscopically it consisted of at least 3 kinds of crystals: narrow prism, bipyramidal and tabular forms, together with some amorphous particles. Melting point test indicated a rather large range between 210 and 250 C. Moreover according to Waller and Hutchings there is a series of degraded products of aureomycin by alkaline treatment (4-7). Therefore the precipitate obtained from the present experiment might be a mixture of compounds probably containing some intact aureomycin too.

Aside from its retardation effect on tadpole growth the nature of the flourescent supernatant portion of the alkali-decomposed aureomycin is not known at present.

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REFERENCES

1. PRUESS, L. M. 1954. Tetracyclines (chlortetracycline). *Encyclopedia of Chem. Tech.* **13:** 777.
2. CIMA, L. and T. BERTI. 1955. Relation between chemical transformation and antibacterial activity of tetracyclines. *Boll. its. sieroterap. Milan.* **34:** 186-191. In *Chem. Abst.* **49:** 14171 h.
3. HSÜ, C. Y., H. M. LIANG and C. L. CHU. 1963. The growth-promoting effect of decomposed aureomycin on tadpole development. *Bull. Inst. Zool., Academia Sinica* **2:** 1-6.
4. WALLER, C. W., B. L. HUTCHINGS, C. F. WOLF, R. W. BROSCHARD, A. A. GOLDMAN and J. H. WILLIAMS. 1952. Degradation of aureomycin III—3,4-dihydroxy-2,5-dioxocyclopentane-1-carboxamide. *J. Am. Chem. Soc.* **74:** 4978.

5. WALLER, C. W., B. L. HUTCHINGS, A. A. GOLDMAN, C. F. WOLF, R. W. BROSCHARD and J. H. WILLIAMS. 1952. Degradation of aureomycin IV—Desdimethylamino-aureomycinic acid. *Ibid.* **74:** 4979.
6. HUTCHINGS, B. L., C. W. WALLER, R. W. BROSCHARD, C. F. WOLF, A. A. GOLDMAN and J. H. WILLIAMS. 1952. Degradation of aureomycin V—Aureomycinic acid. *Ibid.* **74:** 4980.
7. WALLFR, C. W., B. L. HUTCHINGS, C. F. WOLF, A. A. GOLDMAN, R. W. BROSCHARD and J. H. WILLIAMS. 1952. Degradation of aureomycin VI—Isoaureomycin and aureomycin. *Ibid.* **74:** 4981.