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THE ACTION OF AUREOMYCIN ON THE GROWTH OF THYROIDECTOMIZED TADPOLES[1]

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ABSTRACT

Thyroidectomized tadpoles of *Rana catesbeiana* were reared in medium containing 18 and 1.8 ppm chlortetracycline HCl. The aureomycin-treated tadpoles showed a retarded growth rate in relation to total length and body weight when compared with the controls. The heigher dose of aureomycin delayed growth more than the lower one. Aureomycin treatment exerted a lightening effect on pigmention.

The retarded growth and change of pigmentation are considered to be due probably to the influence of aureomycin either on adenohypophysis or on protein synthesis.

Retardation of metamorphosis in *Rana temporaria* with aureomycin (chlortetracycline HCl) treatment was reported by Mustakllio and Telkka in 1954 (1). Hsü confirmed this finding in another 2 species, *Rana limnocharis* and *Rana catesbeiana* and further noted that aureomycin counteracted the action of thyroxin (2).

Aureomycin has been known to interfere with metabolism (3-8), thus indicating that it is a catabolic agent. Its detrimental effect on tadpoles is not, therefore, confined to metamorphosis only but is also concerned with growth.

Hsü showed that aureomycin exerted also a retarded influence on growth of total length in tadpoles (2). However, the effect was not manifested clearly during the metamorphic climax when the tails were being resorbed leading to reduction of body weight. This limitation can be overcome when thyroidless tadpoles are used since they will grow to a huge size and remain as larval form without ever becoming frogs.

For this reason, it is proposed in the present experiments to use thyroidectomized tadpoles to study the effect of aureomycin on growth in terms of the increase of total length and body weight.

MATERIALS AND METHODS

The thyroid primordia of the larvae developed from artificially inseminated eggs of *Rana catesbeiana* were surgically removed at the gill circulation stage (9). The operation was done in the spring. At the end of the autumn of the same year all the control, nonoperated tadpoles metamorphosed while most of the operated animals was still in their tadpole life, retaining gills with hindlegs not more than stage VII of Taylor and Kollros (10).

Out of more than 60 successfully thyroidectomized tadpoles, 30 healthy ones in stage IV with approximately uniform size of total length around 11 cm and

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body weight of about 10 gm were selected. They were divided into 3 groups of 10: the first group was reared in medium containing 1.80 mg of crystalline chlortetracycline hydrochloride (Lederle Laboratory Divisions) per 100 ml of tap water, the second group in medium with 0.18 mg of the same antibiotic per 100 ml of the water, and the third group as the control in tap water only.

The 3 groups of tadpoles were reared in polyethylene bowls, fed boiled green vegetables *ad libitum* and maintained under the same laboratory condition at 19.4±0.1 C with 10 hours of incandescent illumination each day. The medium was changed thrice weekly when new food was given each time.

Total length of the tadpoles was measured once a week on a glass plate laid on a graph paper ruled to mm. Body weight was recorded also weekly to the hundredth of a gram by a torsion balance. The data thus obtained were considered as indication of growth of the tadpoles.

RESULTS

Observations on growth of total length, body weight and hindlegs, and change of pigmentation of the 3 groups of tadpoles were continued for 40 weeks. There was no mortality. The results are as follows:

1. Growth—All the 3 groups of tadpoles, being successfully thyroidectomized with no exogenous thyroxin added, remained unchanged at stage IV through out the experimental period with respect to the progress of metamorphosis. Differences in growth rate of total length and body weight, however, prevailed among tadpoles treated with both doses of aureomycin and the controls as indicated in *Fig. 1.*

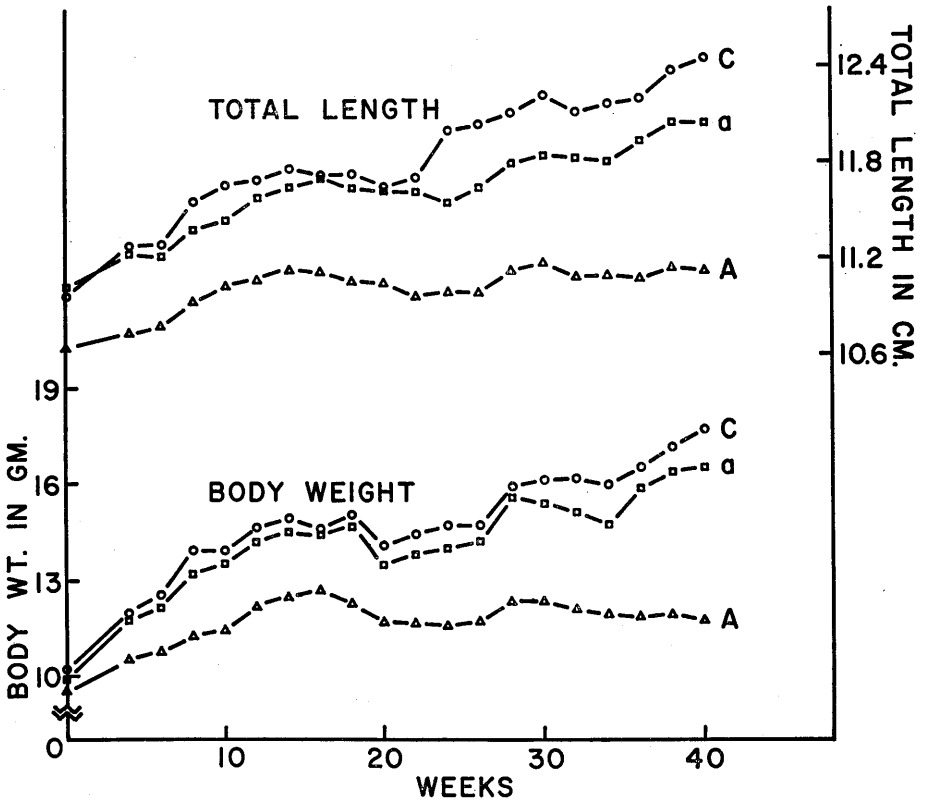

Fig. 1. Growth curves of total length and body weight of the 3 groups of tadpoles. C, a and A represent control tadpoles, tadpoles treated with 1.8 ppm aureomycin and tadpoles treated with 18 ppm aureomycin respectively.

Fig. 1 also shows that differences in total length and body weight between control tadpoles and those treated with the larger dose of aureomycin were greater than those between the controls and experimental tadpoles treated with the smaller dose of aureomycin, revealing a dose effect which could be also obviously expressed in terms of percentage increment during the period of experimentation as indicated in TABLES I and II.

TABLE I

Percentage increment of total length of the three groups of tadpoles

Aureomycin dosage mg/100 ml water	Initial mean total length in cm±SE	Final mean total length in cm±SE	Mean difference in cm±SE	P value	% increment
0	10.99±0.459	12.48±0.258	1.49±0.526	0.01<0.001	13.6
0.18	11.04±0.295	12.07±0.161	1.03±0.336	0.01<0.001	9.3
1.80	10.65±0.105	11.14±0.218	0.49±0.242	0.04>0.05	4.6

TABLE II

Percentage increment of body weight of the three groups of tadpoles

Aureomycin dosage mg/100 ml water	Initial mean body wt in gm±SE	Final mean body wt in gm±SE	Mean difference in gm±SE	P value	% increment
0	10.22±1.553	17.76±1.214	7.54±1.966	0.0001	73.7
0.18	9.97±0.885	16.56±0.301	6.59±0.937	0.00001	66.1
1.80	9.52±1.045	11.78±0.902	2.26±1.380	0.05	23.7

Therefore it is evident that the action of aureomycin on development of thyroidectomized tadpoles was to retard growth. This was obviously more so affected with a larger dose.

2. Pigmentation—The 3 groups of tadpoles were reared in white polyethylene bowls with an incandescent light source. Under such background the control tadpoles appeared, of course, lighter in color than those in dark background. However, pigmentation of the aureomycin treated tadpoles was still lighter. Hence, the experimental tadpoles appeared silvery white as if hypophysectomized while the controls looked dark (*Fig. 2*). Under microscopic examination *in vivo*, the melanin granules of the pigment cells on the dorsum of the albino tadpoles were concentrated and those of the controls were comparatively dispersed.

Fig. 2. Pigmentation of the thyroidectomized tadpoles: *left*, aureomycin-treated and *right*, untreated.

DISCUSSION

The retarded growth of the aureomycin-treated thyroidectomized tadpoles in this experiment was comparable to that obtained with intact tadpoles (2) except that the action of the antibiotic was more obvious in the operated animals. Thyroidectomy, therefore, did not interfere with aureomycin action on growth. Apparently retardation of growth by the antibiotic treatment was not mediated through the thyroid gland.

However, the retarded growth may be attributable to other endocrine factor. Growth of the frog tadpoles was first shown to depend on growth hormone of the pars anterior of the pituitary by Smith in 1916 (11). He also demonstrated in another ablation experiment the control of pigmentation by the pars intermedia (12). On account of the double results of retarded growth and lightening of pigmentation in aureomycin-treated tadpoles of the present experiment, it is, therefore, possible that these changes were due to the disturbing effect of aureomycin on the normal function of the adenohypophysis of the master gland.

The influence of aureomycin on endocrine activity is not unknown. In intact tadpoles Hsü found aureomycin counteracted the action of thyroxin (2). Unpublished data in this laboratory indicate a hypofunction of the thyroid, treated with aureomycin.

The retarded growth of the aureomycin-treated tadpoles may be considered from another point of view, the antibacterial nature of the antibiotic. At the molecular level aureomycin was shown to interfere with protein synthesis (3, 4 and 5), to block aerobic phosphorelation (7) and to disturb some part of the Krebs cycle (8). Franklin reported in 1963 that aureomycin reduced the biosynthesis of ribosomal protein of both rat liver and *E. coli in vitro* with a concentration as low as 4 μM equivalent to 1.9 ppm (5). He further claimed that the reduction of protein synthesis was due to the fact that the drug prevented the amino acid of the transfer-RNA-amino acid complex from transfering to the ribosomal protein. It seems that the adverse effect of the antibiotic on protein synthesis is general since it occurs in both mammalian and bacterial cells.

In the present experiment the dose of aureomycin was 18 ppm, approximately 10 times higher than that of Franklin's lowest solution. Hence, it is justifiable to postulate that aureomycin would also reduce protein synthesis in the tadpole cells, resulting in a stunted growth.

In addition to the retarded growth of tadpoles obtained in this experiment, the antibiotic played the same detrimental effects on other organisms. Aureomycin was found to retard or inhibit cell proliferation in tissue culture (13) and in protozoa (14). Bevelander and his associates reported that tetracycline, another antibiotic of the same family to which aureomycin belongs, caused inhibition of development in larval sand dollars (15), in chick embryos (16), in teeth of rats (17) and in infants and new born rats (18).

All these adverse effects of the tetracycline family were found in accord with the present result. They were obtained when the antibiotics were applied parenterally in a considerable dose with very few chances of being decomposed by high temperature and alkalinity. However, it is noted that when aureomycin was administered in small quantity orally to domestic animals the result was, on the contrary, a promotion of growth. This effect is considered to be due to entirely different action of aureomycin under the decomposed status and was, therefore, discussed elsewhere (19-21).

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