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ANTIBACTERIAL ACTIVITY OF ALKALI-DECOMPOSED AND UNTREATED AUREOMYCIN UNDER DIFFERENT EXPERIMENTAL CONDITIONS

TSU-JU YANG[1] AND CHIH-YUN HSÜ[2]

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ABSTRACT

Bacteriological assays of aureomycin after alkaline decomposition indicated a total loss of its antibacterial activity. This finding gives support to our previous suggestion that the growth-promoting effect of aureomycin fed to birds and mammals resulted not from the antibacterial activity of the antibiotic itself but from its degraded product(s) after passing through the alkaline medium of the small intestine at body temperature. Untreated aureomycin in the tadpole medium when incubated for different periods at different temperatures lost certain amount of activity according to temperature, duration of incubation and aureomycin concentration.

In a previous study (1) it was demonstrated that untreated aureomycin depressed while alkali-decomposed aureomycin stimulated tadpole growth. It was then suggested that the growth-promoting effect of aureomycin fed to birds and mammals resulted not from the antibacterial activity of the antibiotic *per se* but from its degraded product(s) after passing through the alkaline medium of the small intestine at body temperature.

Among the tetracyclines, chlortetracycline is most unstable. Its stability in aqueous solution is strictly a function of pH and temperature (2-5).

Pruess stated that chlortetracyline after alkaline degradation exhibited only very slight antibacterial action (6) while Cima and Berti reported a loss of antibiotic activity of chlortetracycline at pH 6-8 and temperature 12-60 C (7). Thus the antibacterial potency of our alkali-decomposed aureomycin should be assessed, if any.

The question as to what extent untreated aureomycin used in the previous experiment retained its bacteriostatic action after standing in the tadpole culture media at different temperatures for 0, 24, 48, 72 hours should also be answered.

The present experiments were therefore designed to investigate the antibacterial activity of alkali-decomposed aureomycin as prepared previously (1) and untreated aureomycin in the tadpole media under different experimental conditions.

MATERIALS AND METHODS

Tadpoles of *Rana catesbeiana* of uniform size at the foot stage (8) were divided into 6 groups of 5. They were reared in polyethylene bowls of 10″ in diameter at different temperatures. Each bowl contained 500 ml of tap water as the medium to which either alkali-decomposed or untreated aureomycin was added. The purpose of using tadpoles in groups 3 and 4 of the present study was to simulate a condition comparable to that of the previous experiment while groups 1, 2, 5 and 6 were designed as a guide for future experiments. The concentration of the antibiotic and the different temperatures used are listed in Table I.

1. Assistant research fellow, Institute of Zoology, Academia Sinica, Taipei, Taiwan.

2. Professor, Department of Biomorphics, National Defense Medical Center, Taipei, Taiwan.

TABLE I
Experimental conditions of the different groups of tadpoles

Group	No. of tadpoles	Aureomycin concentration		Temperature
		Untreated	Decomposed	
1	5	18 ppm	—	30 C
2	5	—	18 ppm	30 C
3	5	5 ppm	—	24 C
4	5	—	5 ppm	24 C
5	5	18 ppm	—	15 C
6	5	—	18 ppm	15 C
7	0	1 ppm standard	—	—

The aureomycin used was crystalline chlortetracycline hydrochloride*, lot No. 48175-101 of the American Cyanamid Company. The alkali-decomposed aureomycin was prepared as before (1). The desired temperatures of 30 C, 24 C and 15 C were obtained by means of specially adjusted incubators. Tadpoles were fed the same boiled green vegetables as used previously. The culture medium of each group of tadpoles was not changed during the observation, nor was extra food given.

0, 24, 48 and 72 hours after immersing tadpoles in the culture media at different temperatures, 50 ml of the culture medium in each bowl was taken and centrifuged at 3,000 rpm for 30 min in the International PR-2 refrigerated centrifuge. The supernatant was then sterilized through UF sintered glass filters. Two samples were taken from each filtrate and assayed simultaneously by the broth dilution method of Herrell and Heilman for assaying aureomycin in body fluids (9). *Sarcina lutea* PCI No. 1001** was used in the place of *Bacillus cereus* No. 5. The bacterial concentration of the inoculum was adjusted by Klett-Summerson photoelectric colorimeter to the approximation of $4\text{–}8 \times 10^5$ cells per ml, and was also checked by cell count made on agar plate. Should the results of the 2 methods of counting fail to agree with each other, the experiment was repeated. Difco's thioglycollate medium was used as to minimize the deterioration of aureomycin as recommended by Herrell and Heilman. Separate pipet was used in each dilution.

The same aureomycin was used for the standard assay. Each μg of the aureomycin had 0.97 μg equivalent potency of the FDA standard chlortetracycline.

The highest dilution of the sample from the tadpole cultures inhibiting bacterial growth (end point) multiplied by the end point concentration of the standard gives the concentration of aureomycin in the sample. The discrepancy between FDA and our standard was negligible. Thus the concentration was expressed as such and no further conversion to FDA standard was made.

The tadpoles were not further studied for growth since no accurate measurements of bodily changes were possible in such a short observation.

RESULTS

1. Assay of alkali-decomposed aureomycin.

Table II indicates clearly that no detectable antibacterial activity was found in alkali-decomposed aureomycin culture under any condition listed. Further observation showed that bacteria even survived in the stock preparation of alkali-decomposed aureomycin of 1,500 ppm aureomycin-equivalent.

2. Assay of untreated aureomycin.

Table III shows that aureomycin in tadpole media deteriorated in all the experimental conditions as incubation went on. The deterioration varied according to temperature and aureomycin concentration.

Results from Group 1, 18 ppm at 30 C, indicated a sharp decrease in aureomycin concentration after 48 hours of incubation and a very low concentration after 72 hours while samples from Group 5, 18 ppm at 15 C, still showed quite a high concentration even after 72 hours of incubation. When aureomycin concentration was lowered to 5 ppm as indicated by Group 3, there contained almost no demonstrable active aureo-

* Kindly supplied by the Cyanamid International, Pearl River, N. Y., U. S. A.

** Kindly supplied by Dr. C. S. Yang of the National Taiwan University, Medical College, Taipei, Taiwan.

TABLE II

Antibacterial activity of alkali-decomposed aureomycin under various experimental conditions

Group	Temperature	Time tested, hr			
		0	24	48	72
		Aureomycin concentration, μg per ml (ppm)			
2. 18 ppm	30C	0.0	0.0	0.0	0.0
4. 5 ppm	24C	0.0	0.0	0.0	0.0
6. 18 ppm	15C	0.0	0.0	0.0	0.0
7. 1 ppm standard	—	0.96	—	—	—

TABLE III

The remaining antibacterial activity of untreated aureomycin under various experimental conditions

Group	Temperature	Time tested, hr			
		0	24	48	72
		Aureomycin concentration, range μg per ml (ppm)			
1. 18 ppm	30C	7.68-3.84	3.84-1.92	0.48-0.24	0.24-0.12
3. 5 ppm	24C	3.84	0.96-0.48	0.24	0.12-0.0
5. 18 ppm	15C	7.68-3.84	3.84-1.92	3.84-1.92	3.84-1.92
7. 1 ppm standard	—	0.96	—	—	—

mycin after 72 hours of incubation although some remained after 48 hours.

The loss of aureomycin activity in Groups 1, 3 and 5 at 0 hour was considerable when compared with that of the standard. This might result from the 2 hours of processing before titration at room temperature around 20 C and other unknown factors.

DISCUSSION

The effect of antibiotics as growth stimulants in birds and mammals has been known for more than 10 years (10). The current theory suggests that the growth-promotion is due to the antibiotic action upon intestinal microflora, causing either suppression of pathogenic bacteria or enhancement of vitamin-B producing microbes with a result of better nutrition (11-13). The bacteriological findings were, however, not convincing since they were contradictory in some cases (14). Furthermore, evidences at variance with the antibacterial concept of promotion of growth were found both by other workers (15-17) and in this laboratory (18, 19).

Losing all its antibiotic potency as shown in Table II alkali-decomposed aureomycin of this study acted in the same way as that found by Cima and Berti. Thus the growth-promoting effect on tadpoles could not be possibly due to its antibacterial action. This direct bacteriological finding, therefore, supports the view that promotion of growth by feeding minute quantity of aureomycin together with the basal ration to domestic animals is not due to its antibiotic activity but to the result of the decomposed products(s) of aureomycin while passing through the small intestine.

Aside from the temperature factor rendering untreated aureomycin to lose part of its activity, other factors such as the metabolic products of the tadpoles, unavoidable change of food, increase of pH of the medium and the 2 hours of processing of sample at room temperature before titration might also contribute to the deterioration of aureomycin activity. Thus the antibacterial activity in every case listed in Table III showed certain amount of loss. However, in Groups 1 and 5 there still remained some potency equivalent to 0.24-0.12 and 3.84-1.92 μg per ml respectively at the end of 72 hours of incubation.

The experimental conditions of Group 3 were

identical with those of the previous experiment (1) in aureomycin concentration (5 ppm) and temperature (24 C), but in the previous experiment there were 3 changes of medium including renewal of the antibiotic whereas in Group 3 there was no change of medium and no renewal of the antibiotic. Taking the present data of Group 3 into consideration, it is conceivable that tadpoles of the previous experiment were in an almost non-antibacterial environment only on the last day of the third change of the medium in a week while on the remaining 6 days of the week they were always in a medium with antibacterial activity of at least 0.24 μg per ml of water (*Fig. 1*), and yet the the actual concentration could be higher because of the loss of potency during the 2 hours of processing of the test sample in the present study. Under such antibiotic condition growth of tadpole was impaired (1). So when aureomycin was fed to animals for the nutritional purpose the growth would be hampered likewise if the antibiotic were not decomposed.

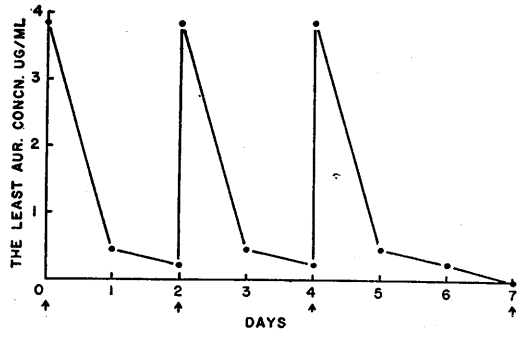

Fig. 1

The antibacterical activity of untreated aureomycin in the tadpole medium. Arrows indicate changes of medium and renewal of the antibiotic.

However, microflora of the tadpole intestine were not so sensitive to aureomycin as the tadpole itself. For a preliminary observation showed that the intestinal microbes of the tadpoles survived well in the medium of 18 ppm aureomycin and the growth was suppressed significantly in a medium of 100 ppm. Thus aureomycin, while still inhibitory to tadpole growth, can not suppress the growth of intestinal microflora of the tadpole.

The opposite effects of immersing and feeding tadpoles with aureomycin on development (20-22) could be explained in view of the present bacteriological findings. Tadpoles while immersing in aureomycin medium get very little of the antibiotic into the GI tract directly by taking in the soaked vegetables. For tadpoles, like freshwater fishes (23, 24), normally do not drink water through the GI tract but they take in water osmotically in lieu of hypertonicity of the body fluid with respect to the surrounding water. Therefore they would be subjected to the action of intact aureomycin. On the other hand when aureomycin was fed to tadpoles, the antibiotic would be decomposed by the intestinal fluid and the high surrounding temperature and lose all its antibacterial activity. Thus it could be the non-antibiotic property of the decomposed aureomycin which stimulated development.

Experiment reported elsewhere demonstrated that alkali-decomposed aureomycin could be separated into a soluble and a nonsoluble portion and that it was the latter which stimulated growth (26). According to Waller and Hutchings, aureomycin could be degraded by alkali into a series of products (27-30). It will be interesting to test each of these products for tadpole growth when and if the samples of the alkaline degraded products of aureomycin requested from the Cyanamid International reach us.

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