Bull. Inst. Zool., Academia Sinica 3: 47-54 (1964)

AGING IN THYROIDLESS TADPOLES[1]

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Received for publication October 1, 1964

ABSTRACT

Normal tadpole life lasts a few months only. During metamorphosis process of differentiation occurs and at the metamorphic climax growth rate reduces drastically. These are considered the expressions of aging. Thyroidless tadpoles, however, keep on growing to a huge size with no sign of differentiation beyond limb bud stage. It seems they are always young. The present study aims to ascertain whether aging exists in thyroidless tadpoles. Two different age groups, 5-month-old and 24-month-old, of thyroidless tadpoles of *Rana catesbeiana* were compared for growth rate of body weight and total length in tap water and in aureomycin solution in a period of more than 30 weeks. The results show that old tadpoles exhibited an inferior growth rate to young ones either under normal or adverse condition. Since decline in growth rate is one of the signs of senile changes, it is proposed that aging propagates inevitably in thyroidless tadpoles.

Among amphibians, anuran tadpoles show a typical form of metamorphosis by a complete transformation from larval form to adult structure. During this process, some organs change histologically or regress completely while new organs develop resulting in froglets which are more differentiated than tadpoles. On the other hand, growth rate of body weight and total length reduce drastically at the metamorphic climax.

Complete extirpation of the thyroid anlage was first shown to prevent metamorphosis by Allen in 1916 (1). Young embryos of *Rana catesbeiana* were thyroidectomized in this laboratory in the spring of 1960. Some of the successfully operated tadpoles are still living now and have gained a huge body size of 101 gm in weight and 21.5 cm in total length during the past 4.5 years as illustrated in *Fig. 1*. Nevertheless, they retain their juvenile form as tadpoles with no sign of differentiation beyond the early limb bud stage. This is in accord with those reported by Allen (2) and Hoskins and Hoskins (3). Therefore permanent dissociation of differentiation and growth in development is obtainable in thyroidless tadpoles.

When differentiation and growth rate are compared between normal and thyroidless tadpoles, there is a striking difference. According to Minot, differentiation and decline in growth rate are the signs of aging (4). Hence senescence occurs in normal tadpole life, while the same process in thyroidless tadpoles is intriguing. In higher vertebrates the role played by thyroid in aging also remains dubious (5).

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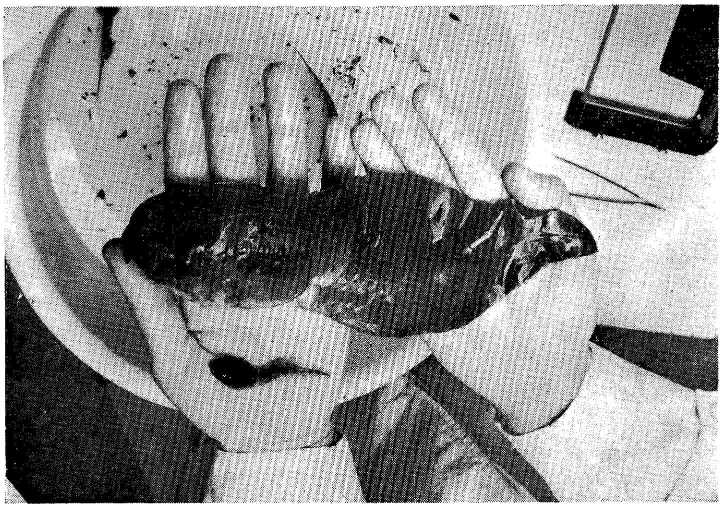

Fig. 1. Comparison between a thyroidless tadpole (upper) and a normal tadpole (lower.)

In a previous study, the retarding effect of aureomycin on the growth of thyroidless tadpoles was shown (6). It is also known that old animals usually adapt themselves to the adverse environment less efficiently than young ones (7). Thus it will be interesting to test if young and old thyroidless tadpoles respond differently to aureomycin treatment.

Therefore the aim of the present experiments is to ascertain whether aging process exists in thyroidless tadpoles by comparing the growth rate of the young and old forms under normal and adverse conditions.

MATERIALS AND METHODS

Larvae of *R. catesbeiana* raised from artificially inseminated eggs were surgically thyroidectomized at the gill circulation stage. Since normal tadpoles of *R. catesbeiana* become froglets within a few months in the subtropical climate of this island, the absence of metamorphosis in due time assures the successful removal of the gland. Such is not the case in the United States where tadpoles of the same species usually require 2 to 3 years to metamorphose. After the normal pals had all metamorphosed, the successfully thyroidectomized tadpoles at stage IV (8) were divided into 2 different age groups: one group of 10 tadpoles at the age of 5 months averaging 6 gm in body weight and 9 cm in total length; and another group of six 24–month-old tadpoles with average body weight of 14 gm and total length of 13 cm. Although experimented in different periods the 2 groups were observed for growth under the same environmental condition with respect to temperature, food, space, light, aquaria and aureomycin dosage.

Each age group was further divided into 2 subgroups: one was reared in an aquarium containing tap water as the normal condition and the other in an aquarium containing 18 ppm crystalline chlortetracycline HCl in tap water as the adverse condition. The aquaria medium allocated to each tadpole was 800 ml through out the observation.

All tadpoles were maintained in a constant-temperature-laboratory at 20 C with 10 hours of incandescent illumination each day. Boiled green vegetables were fed *ad libitum*. The medium was changed 3 times a week when new food was added.

The body weight of each tadpole in the 2 age groups was measured biweekly by a torsion balance to the hundredth of a gram. The total length was also measured biweekly from photographs of the tadpoles. The growth was observed for 39 weeks and 30 weeks on young and old tadpoles respectively.

RESULTS AND DISCUSSION

All tadpoles in the present experiments remained unchanged at stage IV through out the experimentation period. They were thus thyroidless.

The growth curves of body weight and total length of the control and aureomycin-treated thyroidless tadpoles in the 2 age groups were presented in *Figs. 2–5.* It is obvious from the figures that aureomycin distinctly retarded at first and then inhibited the growth of the body weight and total length of tapoles in both age groups. The results check well with that obtained in the previous study (6). However, curves in *Figs. 2–5* can not serve well for the purpose of comparing the growth of 2 different age groups because the body size of the 2 was not comparable. Besides, they can not show clearly the ever changing rate of growth. Therefore curves of relative growth rate are more preferable.

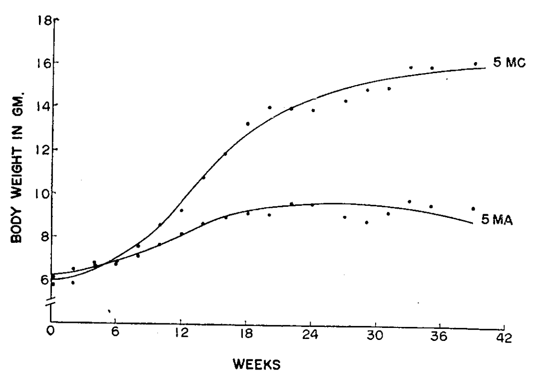

Fig. 2. Growth curves of the body weight of control tadpoles (5 MC) and aureomycin-treated tadpoles (5 MA) beginning at the age of 5 months.

Curves in *Figs. 2–5* were then converted into the desired curves in *Figs. 6, 7, 10* and *11* respectively by graphical measurements of the values of the tangents of all points

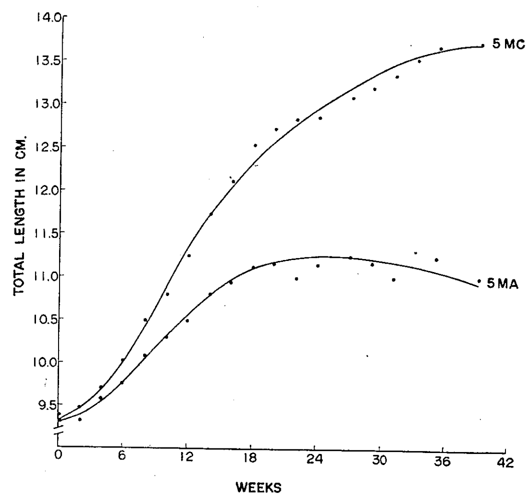

Fig. 3. Growth curves of the total length of control tadpoles (5 MC) and aureomycin-treated tadpoles (5 MA) beginning at the age of 5 months.

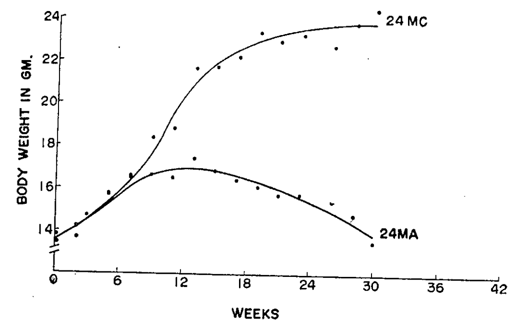

Fig. 4. Growth curves of the body weight of control tadpoles (24 MC) and aureomycin-treated tadpoles (24 MA) beginning at the age of 24 months.

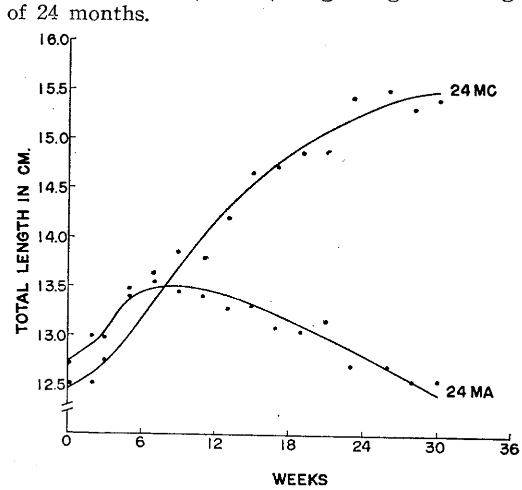

Fig. 5. Growth curves of the total length of control tadpoles (24 MC) and aureomycin-treated tadpoles (24 MA) beginning at the age of 24 months.

on the curves divided by body weight or total length. In other words, the curves of relative growth rate were obtained by $\frac{dW}{dt} \times \frac{1}{W}$ or $\frac{dL}{dt} \times \frac{1}{L}$ where W, L, and t stand for body weight, total length and unit of time respectively.

The shape of a curve with the rate of growth plotted against time is generally determined by 2 opposing factors: a growth-acceleralating force originating within the organism to expand itself indefinitely and a growth-limiting force from the environment as well as the organism to counteract the former. The environmental pressure may be the shortage of food, of O_2 or of space; temperature drop; seasonal variation or other detrimental factors in the ambient water; while the intrinsic factor is aging process. The expansion drive to grow on the one hand and the environmental insalts assisted by aging process on the other hand are constantly harassing each other as the organism lives on, resulting in rising and declining phases of growth. So, with the passage of time, when the restricting force overcomes the expansion force growth is brought gradually to a standstill.

The curves in *Fig. 6* demonstrate fairly well the rise and fall of the growth rate of body weight in 2 age groups. The rising segments show that old tadpoles as well as young ones responded to a new and better environment by gaining gradually the growth rate when they had been transfered from a crowded common pool to the present aquaria; but the 2 age groups showed different magnitudes in growth rate.

The aquaria medium of the new environment was changed thrice a week with an allocation of 800 ml per tadpole and food was taken *ad libitum*. So the supply of O_2 and food and the removal of waste are thought not to be inadequate. Other considerations are that the space was kept constant through out the experimentation and that the same space was allowed for both old and young tadpoles. However, as the tadpoles were devoid of thyroid, their metabolic rate was low and they appeared very sluggish. Thus the space factor seems not seriously significant in the present experiments. However, to what extent the decline of growth rate of each age group in *Fig. 6* was due to environmental assault and to what extent due to aging process seem perplexed.

When growth rate in both rising and declining phases is compared between the 2 age groups (*Fig. 6*), it is clear that young tadpoles always grew faster in body weight

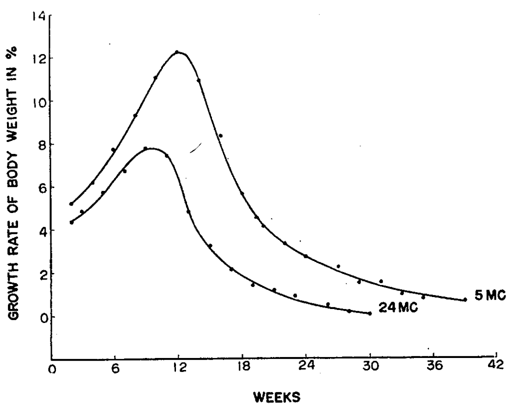

Fig. 6. Curves of the relative growth rate of the body weight of tadpoles beginning at the ages of 5 months (5 MC) and 24 months (24 MC) under normal condition.

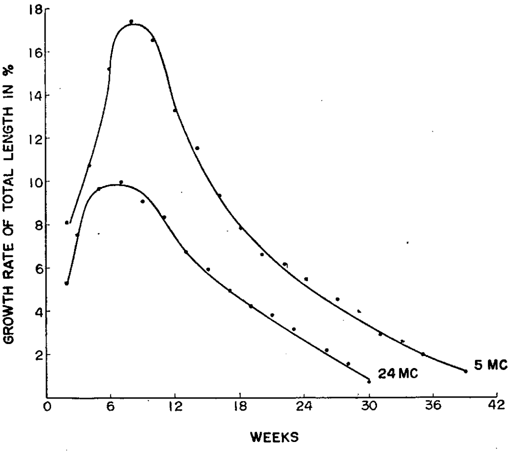

Fig. 7. Curves of the relative growth rate of the total length of tadpoles beginning at the ages of 5 months (5 MC) and 24 months (24 MC) under normal condition.

than the old ones. *Fig. 7* which represents the growth rate of total length shows the similar trend expressed either chronologically or between groups.

Minot's generalization that aging is associated with decline in growth rate was confirmed by Brody's critical examination in domestic animals and men (9). The theory is adopted here to denote the existence of senescence in thyroidless tadpoles.

In birds and mammals growth is distinctly limited at certain stage of the life cycle, while the aquatic cold-blooded animals such as fishes grow continuously with the rate becoming slower but never ending when approaching old ages (10). The present data on growth of tadpoles under normal condition lend weight to the suggestion that the belated, poikilothermic tadpoles showed a comparable tendency of growth as that of fishes.

The growth of the body length in fishes generally follows the inverse exponential function of $L_t = L_\infty(1-e^{-K})$ where L_t is the length at age t, L_∞ is the asymptotic or maximal length and K a constant, the curvature of the growth curve (11). According to Beverton and Halt the equation may be converted to a linear function of $L_{t+1} = L_\infty(1-e^{-K}) + L_t e^{-K}$. Based on this equation and the present data of the growth of the total length of the 2 age groups under normal condition, approximately straight lines were plotted with total length at age $t+1$ against that at age t as shown in *Fig. 8* where t stands for a unit of 2 weeks. The slope of the line thus drawn approximates the value of e^{-K} which was the growth rate, and the intersection of the line with the bisector gives an estimate of L_∞.

Fig. 8 shows that growth rate of total length in young and old tadpoles were 0.93% and 0.82% respectively, and that the estimated asymptotic length of the 2

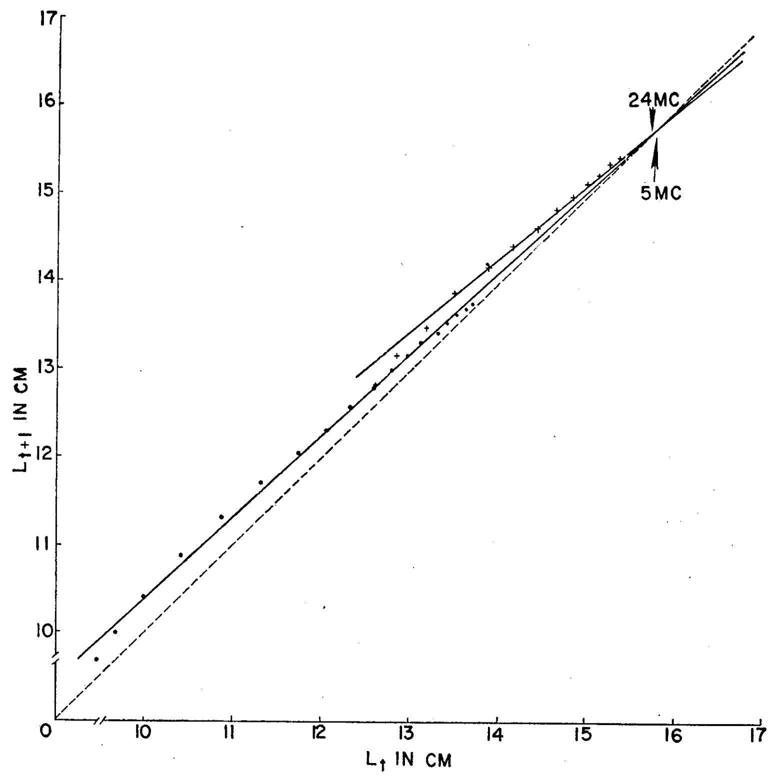

Fig. 8. Growth rate of the total length of tadpoles beginning at the ages of 5 months (5 MC) and 24 months (24 MC) under normal condition.

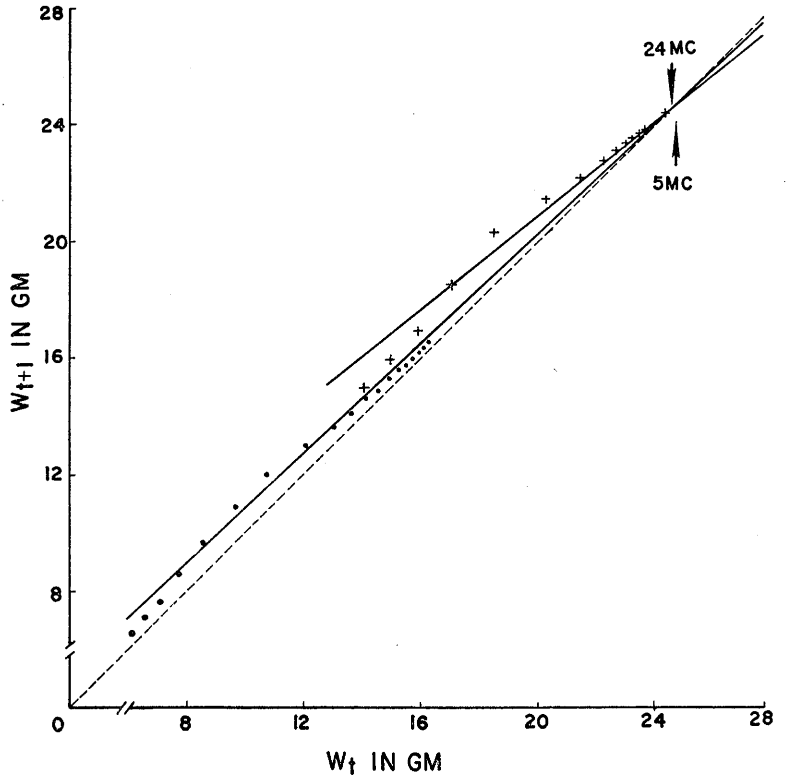

Fig. 9. Growth rate of the body weight of tadpoles beginning at the ages of 5 months (5 MC) and 24 months (24 MC) under normal condition.

age groups are practically the same (L_∞ of the young is 15.5cm; of the old, 15.6cm). The results indicate that young ones were labile, still possessing a considerable potential to grow; whereas the reserve power of the old was restricted. The difference is interpreted as one of the expressions of senile changes.

Likewise, a linear relationship was obtained with the body weight of tadpoles at age $t+1$ against that at age t as shown in *Fig. 9.* The results indicate again the superior growth rate of the young to that of the old (0.95% vs 0.80%) and the approximation of the asymptotic body weights of the 2 groups (24.6 gm for the young and 24.8 gm for the old).

The estimated asymptotic total length and body wight for the thyroidless tadpoles are confined only to the present experimental condition; for when the environment has been improved by circulating the medium with filtered and aerated water 5 hours daily, measurements of the 4.5-years-old tadpole mentioned in the beginning of the paper are 4 times in body weight and 1.4 times in total length the present estimated values (101.0 gm against 24.8 gm and 21.5 cm against 15.6 cm). This shows clearly the influence of environment on growth. However, there is a limit. The 4.5-years-old tadpole measures 101.0 gm and 21.5 cm while a 3.5-years-old one presents a body size of 99.7 gm and 20.6 cm. So during a period of 4.5 years, the tadpole has grown from a minute egg to a mass of 100 gm, but a year's difference makes the older tadpole gain 1.3 gm and 0.9 cm only—showing the exhaustion of growth rate with the passage of time in spite of the improved environment.

The expression of aging process in terms of susceptibility to injurious agents on the kidney and liver and on the organism as a whole in dogs was revealed by MacNider (7). He reported that young animals appeared to be more resistant and to possess a larger capacity in stabilizing a fundamental equilibrium in the organism than the aged. Therefore, as age progresses adaptation of the organism to the changing environment becomes less efficient.

The result of the treatment of aureomycin on the growth rate of the body weight of the 2 age groups is shown in *Fig. 10.* Old tadpoles outgrew young ones in the first 6 weeks. But from then on the growth rate of the 2 deviated greatly from each other, with the result that the young kept on gaining weight whereas the aged was declining. About in the 13th week, the young reached a maximal rate and the aged dropped to a standstill. Since then both had shown a declining rate with a negative value at the end indicating actual reduction of body weight—the old ones being reduced more. The same tendency is observed in *Fig. 11* where growth rate of total length is concerned.

It is evident that the antibiotic exerted a great pressure on the growth in both age groups; however, aureomycin played its detrimental effect more severely on older tadpoles as shown in TABLE I. The results were interpreted to mean old tadpoles withstood the antibiotic assault less adaptively than young ones. Thus aging process is shown to occur in thyroidectomized tadpoles under adverse conditions, too.

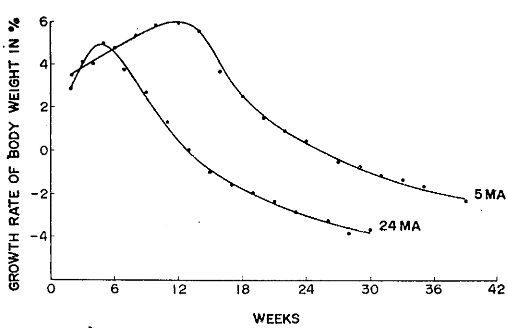

Fig. 10. Curves of the relative growth rate of the body weight of the aureomycin-treated tadpoles beginning at the ages of 5 months (5 MA) and 24 months (24 MA).

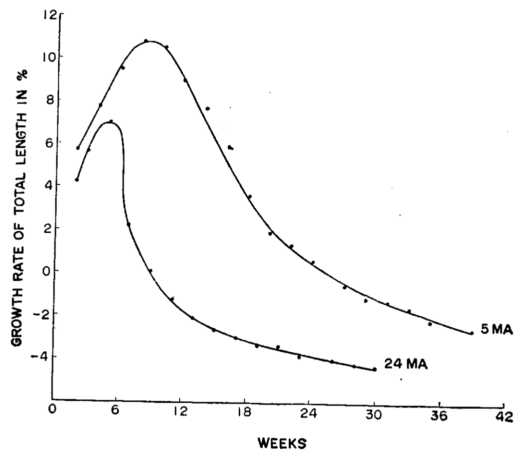

Fig. 11. Curves of the relative growth rate of the total length of the aureomycin-treated tadpoles beginning at the ages of 5 months (5 MA) and 24 months (24M).

TABLE I

The effect of age on growth rate of aureomycin-treated tadpoles
(based on ***Figs. 10 and 11****)*

Parameters	Growth	5-month-old tadpoles	24-month-old tadpoles
Time, max rate	Body weight	12th week	5th week
	Total length	9th week	5th week
Time, zero rate	Body weight	25th week	13th week
	Total length	25th week	9th week
Maximal rate	Body weight	6.0%	5.0%
	Total length	10.8%	7.0%
Rate at 30th week	Body weight	—1.0%	—3.9%
	Total length	—1.2%	—4.4%

The present study as well as earlier works indicates an acceleralated and prolonged growth in thyroidless tadpoles. According to Brody, the maximal growth rate occurs at the prime of an animal's life (9). Then thyroidectomy is thought capable to prolong youthful period in tadpoles. Nevertheless, the operation did not prevent tadpoles from growing old. Therefore it is proposed that senile process propagates inevitably in thyroidless tadpoles.

Acknowledgements The authors wish to thank Mr. Yuan-po Kou, associate professor of engineer methematics in Chung Yuan Institute of Technology and Science for his valuable suggestion in presenting the curves. Appreciation is also due to the Taiwan Cyanamid Corporation for the kind supply of crystalline chlortetracycline hydrochloride.

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