Age factor in the induced metamorphosis of thyroidectomized tadpoles

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SUMMARY

Three age-groups of thyroidectomized tadpoles of Rana catesbeiana were treated with a series of the same graded increasing concentrations of thyroxin. Normal froglets were formed from the three groups at the end of the induced metamorphosis. Growth and metamorphosis were compared. It was found that while the growth rate was a function of ageing, the pattern and rate of metamorphosis were not affected by senile processes. Therefore a case of differentiation independent of age was presented.

INTRODUCTION

Thyroidectomized tadpoles were found to be capable of growing to a huge size (Allen, 1918; Hoskins & Hoskins, 1919; Etkin, 1964). They exhibited accelerated and prolonged growth after the onset of metamorphosis of intact controls (Hsü & Yü, 1967). However, thyroidectomy did not prevent them from growing old, for young thyroidectomized tadpoles showed a greater growth rate than old ones and the young adapted themselves better than the aged in adverse conditions (Hsü, Yü & Liang, 1964). Therefore senile processes continue inevitably in thyroidless tadpoles.

Nevertheless, tissue differentiation of these tadpoles stops at the limb-bud stage because metamorphosis never proceeds beyond that limit (Hsü et al. 1964; Hsü & Yü, 1967). This intriguing condition poses two questions yet to be answered: are tissues of the aged thyroidless tadpoles still capable of differentiation if treated with thyroxin? how does the competence of old tissues compare with that of the young when reacting to the hormone? The present study aims to answer these questions by comparing growth and metamorphosis of three age-groups of thyroidless tadpoles induced by thyroxin treatment.

MATERIALS AND METHODS

Larvae of Rana catesbeiana raised from fertilized eggs produced by induced breeding were surgically thyroidectomized at the gill-circulation stage. The successfully thyroidectomized tadpoles appeared normal and remained at

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Table 1. Progress of induced metamorphosis in three age-groups of thyroidless tadpoles treated with thyroxin

<table>
<thead>
<tr>
<th>T4 treatment (months)</th>
<th>T4 conc. (µg/l.)</th>
<th>No. of tadpoles</th>
<th>Average metamorphic stage</th>
<th>Metamorphic pattern (Similar for all groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>C*</td>
</tr>
<tr>
<td>Onset of treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.0–3.0</td>
<td>20</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>3.5</td>
<td>20</td>
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<td>7</td>
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<td>6</td>
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<td>9</td>
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</tr>
<tr>
<td>10–20</td>
<td>10–20</td>
<td>17</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Mortality %</td>
<td></td>
<td>15</td>
<td>38</td>
<td>67</td>
</tr>
</tbody>
</table>

* A, 6-month-old tadpoles; B, 18-month-old tadpoles; C, 30-month-old tadpoles.
† Standard error of the mean.
the hindlimb-bud stage (stage III) after the intact controls had metamorphosed.

Three different age-groups of thyroidless tadpoles were thus maintained: the first group, 6 months old, averaged 1.94 g in body weight and 5.5 cm in total length; the second group, 18 months old, 6.80 g in body weight and 7.4 cm in total length; the third group, 30 months old, 15.80 g in body weight and 11.9 cm in total length. They were treated with a series of the same graded increasing concentrations of L-thyroxin sodium (General Biochemical Inc.) for more than 8 months until they became froglets (Table 1). The initial dose was 2 μg of the hormone per litre of aquarium water. The dosage was increased gradually to a final concentration of 20 μg/l. A fourth group of ten 18-month-old thyroidless tadpoles without thyroxin treatment served as the controls.

All tadpoles were kept in an identical laboratory environment: water temperature at 20 ± 1 °C, same space factor, and 10 h of incandescent illumination each day. The aquarium water was changed every other day and new thyroxin was added. The tadpoles were fed thawed leaves of water-convolvulus.

The pattern of metamorphosis of the tadpoles, their progress in metamorphic stage (Taylor & Kollros, 1946), total length, hind-limb length and body weight were recorded monthly.

RESULTS

Growth

The 18-month-old thyroidless tadpoles without thyroxin treatment grew to a huge size as usual (Hsü et al. 1964; Hsü & Yü, 1967). At the end of the 8th month after the commencement of this study they attained 14.50 g in body weight and 11.5 cm in total length.

However, this pattern of continued growth was not observed in the three groups of thyroxin-treated tadpoles. They grew very slowly, and beginning from the 5th month diminution was seen as indicated in Figs. 1 and 2. Because of the unequal sizes in the different age-groups it was rather difficult to compare their growth rates directly from the growth curves. Therefore the curves of Figs. 1 and 2 were transformed by graphical measurements of the values of the tangents of all points on the curves divided by the body weight or total length. Thus the curves of relative growth rate in Figs. 3 and 4 were obtained by $dW/dt \times 1/W$ or $dL/dt \times 1/L$, where $W$, $L$, and $t$ stand for body weight, total length and unit of time respectively.

Figs. 3 and 4 show a comparable tendency of decreased growth rate in the body weight and total length for all three groups. However, the amount of decrease varied proportionately with the age. The older tadpoles gained their body weight and total length more slowly and lost their growth more quickly than the young ones. Yet the zero growth rate for all groups was found at the same time, after 4 months of hormone treatment.

The effect of age on growth was further observed in the ratio of hind-limb
length over body length (Fig. 5). It was obvious that the three groups of metamorphosing tadpoles had their own ratios, the magnitude of which was inversely proportional to their ages.

The results indicate that, after thyroxin treatment, the pattern of growth was comparable for the three groups but that the rates of growth were different due to an effect of age.

Metamorphosis

The control group of thyroidless tadpoles remained at stage III throughout the experimental period, while the three thyroxin-treated groups showed normal advances and the same time scale in metamorphosis, resulting in perfectly normal froglets as shown in Table 1 and Fig. 6. The results indicate that no discrepancy of tissue response to thyroxin was observed in spite of age differences.

This study therefore shows differences of the effect of age on growth and differentiation during induced metamorphosis of thyroidless tadpoles. Growth rate was slowed down while differentiation was not affected by senile processes.
Age and induced metamorphosis in thyroidectomized tadpoles

Fig. 3. Relative growth rate of body weight in three age-groups of thyroidless tadpoles after thyroxin treatment.

Fig. 4. Relative growth rate of total length in three age-groups of thyroidless tadpoles after thyroxin treatment.

Fig. 5. Changes in the ratios of hind-limb length (H.L.L.) to body length (B.L.) in three age-groups of thyroidless tadpoles after thyroxin treatment.
Fig. 6. Advances of metamorphosis in three age-groups of thyroidless tadpoles after thyroxin treatment (6-month-old on the left, 30-month-old on the right and 18-month-old in between). Scale markings in both cm and in. for A–E.

(A) Before treatment
(B) After 4 months of treatment, 4-5 μg/l.
(C) After 6 months of treatment, 6-5 μg/l.
(D) After 7½ months of treatment, 8 μg/l.
(E) After 8 months of treatment, 10 μg/l.
DISCUSSION

The pattern of the relative growth rate of the induced metamorphosing tadpoles in this study was different from that of normal thyroidless ones in our previous report (Hsu & Yü, 1967). In the present case the growth rate was decreasing throughout metamorphosis, reaching negative values, whereas in the previous study the growth rate was found to increase at first and then to decrease, but it never reached a negative value. The difference was presumably due to the occurrence of metamorphosis in the present case. Growth and differentiation are always antagonistic; they seldom occur simultaneously at the maximum rate. While differentiation is interrupted and growth dominates in thyroidless tadpoles, the opposite is true in metamorphosing tadpoles.

However, there was a common phenomenon in the two cases: the effect of ageing on growth. Age had an effect not only on normal growth (Hsu et al., 1964) but also on diminution.

On the other hand the ability of tissues to differentiate was the same in all groups; the young and the old metamorphosed alike: there was no effect of age on differentiation.

The ability to differentiate resides in the activation of tissue genes which control the synthesis of specific proteins. The genetic information is, in a way, immortal; it is always there and correct unless depleted or mutated. During development, genes are switched on and off. In thyroidless tadpoles the genes for metamorphosis are not activated because of lack of thyroxin. Whenever the cells are stimulated with the right inducer, the hormone, differentiation is resumed. This study therefore presents a case of differentiation independent of age.

Our finding on delayed differentiation agrees with the result of Prahlad & DeLanney (1965). They found that all age-groups of the genetically thyroxin-deficient Mexican Axolotl (Siredon mexicanum), ranging from 3 to 571 days old, showed the same capacity to metamorphose when treated with T₃ or T₄. In the case of Drosophila melanogaster, Hadorn (1965, 1966) and Schubiger & Hadorn (1968) demonstrated that imaginal discs of genitalia and leg, cultivated for years without differentiation in the abdomens of adults, were able to form normal adult structures when transplanted back into metamorphosing hosts. Therefore it appears that the retention of the capacity to differentiate until triggered by the right stimulus is common to all animals.

It was Etkin (1935) who first attempted to induce thyroidless tadpoles of Rana cantabrigensis into metamorphosis with thyroxin administered in the ambient water. By gradually increasing the hormone concentration from 1 μg to 1 mg per litre he obtained approximately normal froglets. However, his experiment was terminated by death of the animals. Later, the same author reported that thyroidectomized or hypophysectomized tadpoles of Rana pipiens were able to metamorphose normally with a patterned sequence of increasing
thyroxin concentrations (Etkin, 1964) although he did not mention the exact dosage.

The final concentration of thyroxin in this study was 20 µg/l. at 20 °C, which was far below that of Etkin’s for normal tadpoles. The discrepancy was due mainly to the duration of hormone treatment. Etkin’s administration of thyroxin was an acute treatment whereas ours was a chronic one.

The thyroidless froglets obtained in this study were not only normal morphologically but also physiologically. The 30-month-old group grew and attained an average weight of 10.5 g with an increment of 34.5% over the initial body weight in a period of 2 months after the end of the induced metamorphosis.

In this experiment, some tadpoles could not stand the thyroxin treatment and succumbed during the induced metamorphosis. As shown in Table 1, the mortality rate of the three groups varied according to their age. This was another manifestation of senile processes.

REFERENCES


(Manuscript received 25 June 1970)