Thyroid growth and its relationship to metamorphosis in *Rana temporaria*

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INTRODUCTION

In a previous work on pronephric degeneration, larvae of *Rana temporaria* from practically the whole range of the metamorphic cycle were studied (Fox, 1962). Metamorphosis in anurans is dependent upon the presence of a functional thyroid gland (Kollros, 1951; Lynn & Wachowski, 1951; Etkin, 1964), and the same specimens have now been used to investigate thyroid growth and development quantitatively and qualitatively, especially in relation to those major events which occur at the metamorphic climax between stages 49 and 54 (Cambar & Marrot, 1954).

It will be shown that in larvae from 16 mm long (stage 41; see Fox, 1962) until practically the end of the climax (stage > 53), thyroid cells are of stable volume (about 700 μm³), and thyroid enlargement is by cellular proliferation and vesicular expansion. The maximum rate of thyroid growth occurs between 16 mm and 28 mm (stages 41–45).

MATERIAL AND METHODS

Thyroid growth was investigated from stages 29 to 54 inclusive in *Rana temporaria* (the stages were judged by those described for *R. dalmatina*, Cambar & Marrot, 1954). Specimens were serially sectioned transversely at 10 μ and prepared by standard methods. The thyroid of an additional animal of stage 52 (almost identical with the other same-numbered stage) was also measured, and other animals of stages 29 and 54 were used. The rate of development is altered by such factors as crowding, feeding and temperature, and the following information on time/stage relations is consequently only approximately true. At temperatures between 16 and 20 °C stage 26 is achieved by 7 days, stage 30 by 10 days, stage 33 by 15 days, stage 40 by 26 days, stage 42 by 31 days, stage 45 by 42 days, stage 48 by 50 days, stage 49 by 60 days and stage 54 by 70 days.

Quantitative methods were the same as those used for the pronephros (Fox, 1956, 1962). Mean and standard error of the length of 150 thyroid nuclei (obtained from a horizontally serially sectioned specimen at stage 41, 15 mm

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long) were $6.9 \pm 0.13 \mu$, and $7 \mu$ was therefore used to correct the crude thyroid nuclear count from the transverse sections (see Abercrombie, 1946). The nuclear length of thyroid cells therefore is about $64\%$ of that of the pronephros ($11 \mu$).

Transverse sections were projected at a magnification of $\times 440$, the most satisfactory one for use throughout the series.

RESULTS

(a) Qualitative

At stage 29 the incipient thyroid anlage is a median, elongated, anteroposteriorly directed pharyngeal backgrowth, rounded in cross-section and terminating behind at the region of the front of the heart. Thyroid lengths of two specimens were $210 \mu$ and $230 \mu$ respectively. At stage 31 in general the thyroid is similar in length ($180 \mu$) and appearance to that of the previous stage, but somewhat convex-shaped at its hind end with the convexity facing downwards, a prelude to its later division into paired lobes. In both stages the granular tissue includes numerous yolk globules and pigment granules; cell boundaries are hard to distinguish and nuclei are lightly stained. Many of them include recognizable paired nucleoli and, as in all stages, only a few mitotic figures are present. The young thyroid is situated between incipient genio-coracoidei and interhyoidei muscles, and in stage 31, below the front of the medianly fused hyoid cartilages, behind and below the pars reuniens.

In stage 41 paired thyroid lobes (probably situated farther back than in the previous stage) share a common medio-anterior origin ($30 \mu$ long) below the front of the post-copula. Behind, each separate lobe is situated lateral to the post-copula. Incipient follicles, whose walls are of flattened epithelia, have developed (though somewhat obscured by pigment), but there is no colloid within them.

Thyroid lobes of stage 45 terminate anteriorly in common territory below the free hind end of the post-copula, which is joined to the branchial plate $100 \mu$ farther forward. They now comprise large well-developed rounded follicles, whose vacuoles contain conspicuously stained red colloid. A clear gap—which may be an artifact—often exists between the periphery of the colloid and the columnar epithelium of the follicle wall. In stage 47 each lobe is larger than in the previous stage, and is situated medio-ventral to the hyoid; in front each lobe ends freely against the lateral hind end of the post-copula. At the periphery and within the colloid there are chromophobe droplets, but some vesicles are empty.

In stage 49 and succeeding stages the thyroid progressively becomes larger. More droplets are present in the colloid, which now in most cases completely fills the vesicle. The columnar epithelia of the follicles are slightly deeper, and profuse nuclei are located in their outer regions. In many cases follicles have
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become separated during development, and delicate interfollicular blood vessels vascularize the gland.

In stage > 53 large elongated follicles extend either dorso-ventrally or horizontally; others are partially collapsed. Of three specimens at state 54 two possessed thyroids with colloidal droplets. One of them included typical, but independently situated, thyroid follicles (without droplets) medio-ventrally to the paired lobes.

Table 1. Various measurements of thyroid components in different stages of Rana temporaria

From stage 41 onwards measurements of both lobes are combined, and individual cell volume is calculated from the totals of the nuclear populations (corrected) and tissue volume of the whole thyroid.

<table>
<thead>
<tr>
<th>Stage No. (equivalent to R. dalmatina, Cambal &amp; Marrot, 1954)</th>
<th>Total length of larvae (mm)</th>
<th>Total length of thyroid (right and left lobes) (mm)</th>
<th>Total nuclear population of thyroid</th>
<th>Total vol. of the colloid vesicles of the thyroid (mm$^3/10^{-3}$)</th>
<th>Total vol. of the tissue cells of the thyroid (mm$^3/10^{-3}$)</th>
<th>Total overall vol. of the thyroid (cells and vesicles) (mm$^3/10^{-3}$)</th>
<th>Calculated individual cell volume ($\mu^3$)</th>
<th>% volume of colloid vesicles of thyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>9</td>
<td>0.21 (median)</td>
<td>129</td>
<td>—</td>
<td>0.400</td>
<td>0.400</td>
<td>3101</td>
<td>—</td>
</tr>
<tr>
<td>31</td>
<td>11</td>
<td>0.18 (median)</td>
<td>160</td>
<td>—</td>
<td>0.446</td>
<td>0.446</td>
<td>2787</td>
<td>—</td>
</tr>
<tr>
<td>41</td>
<td>16</td>
<td>0.34</td>
<td>508</td>
<td>0.035</td>
<td>0.372</td>
<td>0.407</td>
<td>732</td>
<td>8.6</td>
</tr>
<tr>
<td>45</td>
<td>28</td>
<td>0.55</td>
<td>4016</td>
<td>1.186</td>
<td>2.880</td>
<td>4.066</td>
<td>717</td>
<td>29.2</td>
</tr>
<tr>
<td>47</td>
<td>33</td>
<td>0.89</td>
<td>7450</td>
<td>2.611</td>
<td>5.799</td>
<td>8.410</td>
<td>778</td>
<td>31.0</td>
</tr>
<tr>
<td>49</td>
<td>33</td>
<td>0.68</td>
<td>6992</td>
<td>2.339</td>
<td>4.728</td>
<td>7.067</td>
<td>676</td>
<td>33.1</td>
</tr>
<tr>
<td>50</td>
<td>33</td>
<td>0.68</td>
<td>15710</td>
<td>3.849</td>
<td>12.191</td>
<td>16.040</td>
<td>776</td>
<td>24.0</td>
</tr>
<tr>
<td>51</td>
<td>32</td>
<td>0.72</td>
<td>14900</td>
<td>4.505</td>
<td>11.691</td>
<td>16.196</td>
<td>785</td>
<td>27.8</td>
</tr>
<tr>
<td>52 (1,2)</td>
<td>25.6</td>
<td>0.70</td>
<td>12746</td>
<td>2.888</td>
<td>9.843</td>
<td>12.731</td>
<td>757</td>
<td>22.7</td>
</tr>
<tr>
<td>&gt; 53</td>
<td>14</td>
<td>1.07</td>
<td>23543</td>
<td>6.242</td>
<td>17.647</td>
<td>23.881</td>
<td>749</td>
<td>26.1</td>
</tr>
<tr>
<td>54</td>
<td>12.5</td>
<td>0.75</td>
<td>14738</td>
<td>3.054</td>
<td>6.445</td>
<td>9.499</td>
<td>437</td>
<td>32.1</td>
</tr>
</tbody>
</table>

(b) Quantitative

The data obtained are summarized in Table 1, from which it will be seen that the calculated individual cell volume of the thyroid (volume of tissue/nucleus) of stages 29 and 31 (3100 and 2787 $\mu^3$ respectively) are similar. Thereafter cell volume is about one-quarter of this, and remains practically constant (range 676–842 $\mu^3$) until stages > 53 (Fig. 3). Thyroid cell volume is therefore considerably less than that of the pronephros of amphibians and Neoceratodus (Fox, 1963), and of liver in practically all adult amphibians studied (Szarski & Czopek, 1965). Diploid pronephros and duct cell volumes of young larvae of
Xenopus are about 2400 and 1350 $\mu^3$ respectively, and thyroid cell volume of Rana is about the same as that of the haploid pronephric duct of Xenopus (Fox & Hamilton, 1964). Small cell size throughout the metamorphic cycle and thus a high surface area per unit volume may be of importance in thyroid function.

As total thyroid tissue volume hardly changes between stages 29 and 41, while the nuclear population of the thyroid increases by about four times, this period is predominantly concerned with the attainment of the typical larval thyroid individual cell volume (Table 1, Figs. 1, 2, 6). Thereafter thyroid tissue volume increase is by cell multiplication with stable cell volume.

In stage 54 cell volume of the thyroid is 437 $\mu^3$, which could represent a significant post-climatic reduction. In larval Rana temporaria whose development was inhibited by phenyl-thiourea, thyroid individual cell volume of one control and two experimental animals (stage 49) were 917, 920 and 920 $\mu^3$ respectively.
At stage 54 two control and two experimental animals, which after developmental inhibition were returned to water in which they finally metamorphosed, had thyroid cell volumes of 607, 736, 529 and 630 μ3 respectively. It is possible therefore that cell volume is slightly reduced after metamorphosis, which agrees with the conclusion of Szarski & Czopek (1965), who found post-metamorphic cell shrinkage as a result of decrease of water.

Fig. 3. Regression line of the calculated individual cell volume of the thyroid (ordinate) against numbered larval developmental stages (stages 41–54) inclusive (abscissa).

Fig. 4. Percentage increase of nuclear population of the thyroid (ordinate) against numbered larval developmental stages (stages 29–54 inclusive) (abscissa).

Maximum growth rate of the thyroid (expressed as percentage change) occurs between stages 41 and 45, especially in relation to the thyroid vesicles. Thereafter the graph tends to flatten, and the rise is more gradual (Figs. 4, 5). Maximum percentage decrease in cell volume occurs between stages 31 and 41, owing to cell multiplication with little cytoplasmic growth (Fig. 6).

In R. temporaria the end of premetamorphosis is probably equivalent to stages 41/42 (tiny hind-limb buds visible). In the succeeding prometamorphic stage, or actual start of the metamorphic cycle, there is extensive growth and differentiation of the fore- and externally visible hind-limbs. The metamorphic climax of Etkin (1964) would commence at stage 51 (emergence of both fore-limbs), and ends at stage 54 with the typical frog.

The thyroid data have been expressed graphically as regression lines, whose abscissae are the stage numbers (especially stages 45–54 inclusive, when the
most dramatic changes occur), and ordinates are the component measurements in question (Figs. 1–3).

At stage 47 the pronephric system has reached its maximum size. Thereafter degeneration ensues—to be first recognized at stage 49—and forms part of the programme of late prometamorphosis and climax, under thyroid control

(Fox, 1962, 1963, 1965). According to the regression lines at stage 49, the thyroid nuclear population is about 10800, and vesicular, tissue and overall thyroid volumes are 0.0028, 0.0079 and 0.0109 mm$^3$ respectively. Thus in such a larva of *R. temporaria* with a normally functioning thyroid of this size, pronephric degeneration is initiated.

At stage 50 (left fore-limb emerged) thyroidal nuclear population is about 12500, and vesicular, tissue and overall volumes are 0.0032, 0.0089 and 0.0122 mm$^3$ respectively. At stage 51 these components measure 14180, 0.0036, 0.0099 and 0.0135 mm$^3$.

Perhaps the most striking external climactic change is tail degeneration. In
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stage 52 it is half-degenerated. Thyroid nuclear population is about 15800 and vesicular, tissue and overall thyroid volumes are 0.0040, 0.0109 and 0.0148 mm$^3$ respectively. Finally, with the complete loss of the tail (stage 54), the same components measure 19.160, 0.0045, 0.0129 and 0.0174 mm$^3$.

DISCUSSION

In larvae of _R. temporaria_ the thyroid increases substantially in size from stage 41 onwards. The most rapid rate of thyroid growth occurs between stages 41 and 45, and then growth decreases gradually towards the climax, when the gland reaches its maximum size. These results in general agree with those of previous workers on _R. temporaria_ and _R. pipiens_ (Etkin, 1930; Clements, 1932; D'Angelo & Charipper, 1939; see also Lynn & Wachowski, 1951). In _Pseudacris triseriata_, _Rana palustris_ and _R. catesbeiana_ there is a marked acceleration of growth at the beginning of prometamorphosis, and thyroid enlargement continues to the climax (Etkin, 1936). In anuran tadpoles (_Rana pipiens_) during prometamorphosis thyroid hormone concentration in the blood is extremely low (Etkin, 1964), and the sharp rise which occurs thereafter in prometamorphosis would seem to be related to the period of stable thyroid individual cell volume and intense cellular proliferation, seen in _R. temporaria_ in the present work.

In _R. pipiens_, from prometamorphosis to climax, the concentration of thyroid hormone in the blood rises about 70 times (Etkin, 1964). Iodine binding increases from prometamorphosis (late Shumway stage 25, hind-limb buds present; probably equivalent to stage 39 in _R. temporaria_) to climax by 40 times (Kaye, 1961), though in _Xenopus_ from 2 to 6 weeks (beginning of climax) it increases by only 20 times (Saxen _et al._ 1957). In a comparable period in _R. temporaria_ (stage 41 to the highest recorded tissue volume at stage > 53) thyroid tissue volume increases by nearly 50 times. At similar stages of the cycle in _Bufo bufo_ thyroidal epithelial volume increases by 26 times (Morita, 1932). _Pseudacris_ showed similar and _R. palustris_ and _R. catesbeiana_ smaller increases in epithelial volume (Etkin, 1936). Thus in various genera increases in thyroid tissue volume and iodine utilization, through prometamorphosis to climax, differ. Likewise thyroid size is variable, for in a number of different larval anurans, the largest thyroids are found in the largest genera (Morita, 1932; Etkin, 1936). Furthermore, as it is well known that in different amphibian genera similar tissues may show different degrees of responsiveness to thyroid hormone (Lynn & Wachowski, 1951), it may well be that the circulatory concentration of thyroid hormone in different genera at similar stages is also variable. However, in most anurans, as in _R. pipiens_, presumably there is a marked and rapid increase in circulatory concentration of thyroid hormone during the metamorphic cycle. This no doubt coincides with an increasing potential hormonal output of the thyroid, which probably reaches its peak when the gland is at maximum size during climax.
Peripheral colloidal chromophobe droplets are first seen in stage 47. At the height of the climax droplets are at their largest and most plentiful. Presumably there is a relationship between the follicular histological appearance and the high climactic thyroidal activity. However, follicle histology, and consideration of the measurements of the percentage volume of the colloid vesicles of the thyroid, do not support the results of previous workers on R. temporaria and R. pipiens, who described colloid shrinkage at climax (Clements, 1932; D'Angelo & Charipper, 1939). Vesicles are not collapsed and colloid is present at all stages in larvae of R. temporaria, though during growth, follicles become more closely packed together. The colloid vesicle percentage volume is fairly constant at about 30% from stage 45 to the end of climax, and if there had been a pronounced collapse of follicles and marked loss of colloid, then this measurement would be expected to fall noticeably. The results support those of Etkin (1936), that there is no specific stage of colloidal evacuation.

During larval development the output of thyroid hormone presumably rises steadily, as the thyroid enlarges under the influence of adenhypophyseal TSH. At prometamorphosis and climax, the circulatory thyroid hormone concentration is maintained through a thyrostatic mechanism or ‘feed back’ system, operating on the pituitary and hypothalamus, via the thyroxine level (see Etkin, 1964). If the control mechanism merely effects the release of limited amounts of hormone from the colloid into the circulation, to maintain or raise the circulatory concentration, and if thyroid hormone is synthesized continuously to replace the deficit from the vesicles, then this would account for the failure to observe pronounced vesicular contraction at climax.

In Eleutherodactylus where there is no marked stage of colloid storage or release, it is assumed that hormone is released from the gland as quickly as it is formed, in order to elicit the ‘telescoping’ of larval stages, which is characteristic of its direct embryonic development (Lynn & Wachowski, 1951; Lynn & Peadon, 1955).

SUMMARY

1. Thyroid growth of larvae of Rana temporaria was investigated in relation to specific stages (29–54 inclusive; see Cambar & Marrot, 1954) and events in the metamorphic cycle.

2. Growth is by cellular multiplication and vesicular enlargement, and from stage 41 (16 mm larva) to almost the end of metamorphosis cell volume is constant.

3. Maximum rate of growth in vesicular, tissue and overall volumes occurs between stages 41 and 45 (16 and 28 mm) respectively.
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RESUMÉ

La croissance de la thyroïde et ses rapports avec la métamorphose chez Rana temporaria

1. On a examiné la croissance de la thyroïde de larves de *Rana temporaria* en rapport avec les stades spécifiques (29 à 54 inclus; voir Cambar et Marrot, 1954) et les événements du cycle métamorphotique.

2. La croissance a lieu par multiplication cellulaire et élargissement des vésicules, et le volume cellulaire est constant depuis le stade 41 (larve de 16 mm), presque jusqu'à la fin de la métamorphose.

3. Le taux de croissance volumétrique maximale pour les vésicules, le tissu et l'ensemble, est atteint entre les stades 41 et 45 (16 et 28 mm) respectivement.

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REFERENCES


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