

A preliminary investigation into the effect of thyroid hormones on the metamorphic changes in Meckel's cartilage in *Xenopus laevis*

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INTRODUCTION

Meckel's cartilage in *Xenopus* exhibits marked changes during metamorphosis, both in shape (Shaw, 1982) and histological and ultrastructural appearance (Thomson, 1983, 1986), these changes having been quantified during naturally occurring metamorphic climax (Thomson, 1987).

Since thyroid hormones are known radically to affect the sequence of change during metamorphosis in many amphibia (summarised in Fox, 1983), a preliminary study of possible effects on Meckel's cartilage was carried out. The objectives of this paper are: (a) to establish whether the changes occurring in the cartilage are susceptible to interference by thyroid hormones and (b) to describe the effects of the hormones, relating them to the normal time scale of development of the cartilage (Thomson, 1986, 1987).

MATERIALS AND METHODS

Eggs were obtained by injecting a pair of adult *Xenopus laevis*, anaesthetised with MS222 (tricaine methane sulphonate), with Pregnyl (Xenopus Ltd, Redhill, Surrey). The resulting tadpoles were reared in dechlorinated tap water at 25 °C and fed on nettle powder (D. Napier & Sons, Edinburgh). During development the tadpoles were staged using the external criteria in the Normal Table of *Xenopus laevis* (Nieuwkoop & Faber, 1956), the period of metamorphic climax occupying Stages 57–66. In order to obtain a sample of animals which were developing as nearly as possible at a similar rate, larvae reaching Stage 55NF on the same day were transferred to a separate aquarium.

From this group, ten tadpoles which reached Stage 57 on the morning of the same day were selected. Each was transferred to an individual beaker containing 150 ml of distilled water. To the water in five of these beakers 150 mg of dry thyroid powder (Porcine, Iodine 0.2%, Sigma, Dorset) was added, the animals in the remaining beakers being used as controls. Fox & Irving (1950) have previously shown this concentration (1 mg/1 ml) of hormone to be suitable for inducing specific changes (see Discussion), and to be well tolerated by the tadpoles. Ten animals at Stage 60NF were similarly treated, all the animals being killed by an overdose of MS222 following exposure to the hormones for 48 hours. The remaining tadpoles were allowed to continue normal development, forming the group of animals used in the previously described studies (Thomson, 1986, 1987). The techniques described in these studies

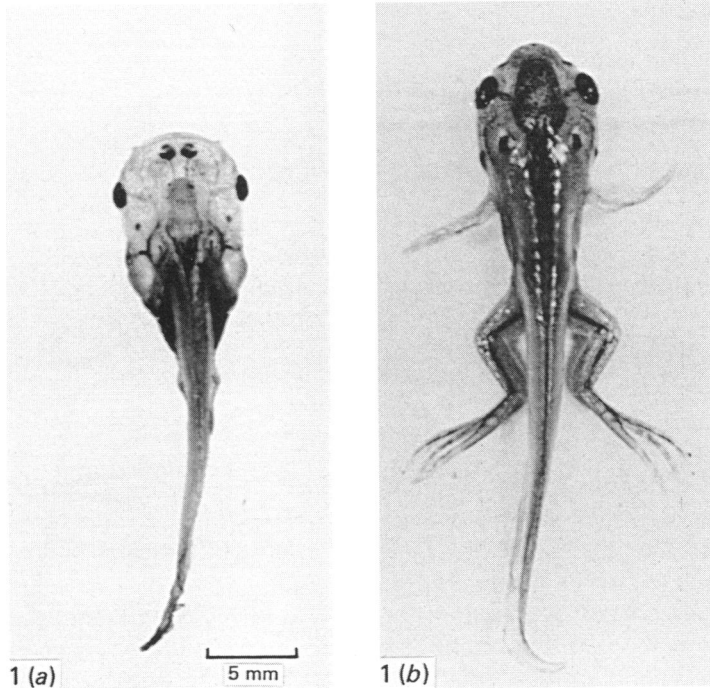


Fig. 1 (a-b). *Xenopus* tadpoles exposed to thyroid hormones for 48 hours at Stage 57NF. (a) Control. (b) Experimental.

were used to examine the cartilage histologically, and to quantify the changes occurring in this tissue, using Student's *t*-test of significance.

RESULTS

Overall head changes

Stage 57NF animals (Fig. 1 a and b)

The rapid shrinkage of the head described by Deanesly & Parkes (1945) is very obvious in the experimental group, as is the precocious development of the front limbs. The development of a prominent 'snout' is also characteristic of animals following thyroid administration, due partly to the greater prominence of the eyes. The lower jaw shape also changes from a shallow curve characteristic of the early stages of metamorphosis to a much deeper U-shape similar to that seen in the later stages in untreated animals.

Stage 60NF animals (Fig. 2a and b)

At the end of the experimental period, the control animals had developed to Stage 61NF, and no differences in appearance between them and the experimental animals were apparent.

Light microscopy and cell population changes

Stage 57NF animals

The histological appearance of the cartilage in the experimental animals was markedly different from that of the controls, the lacunae appearing smaller and the

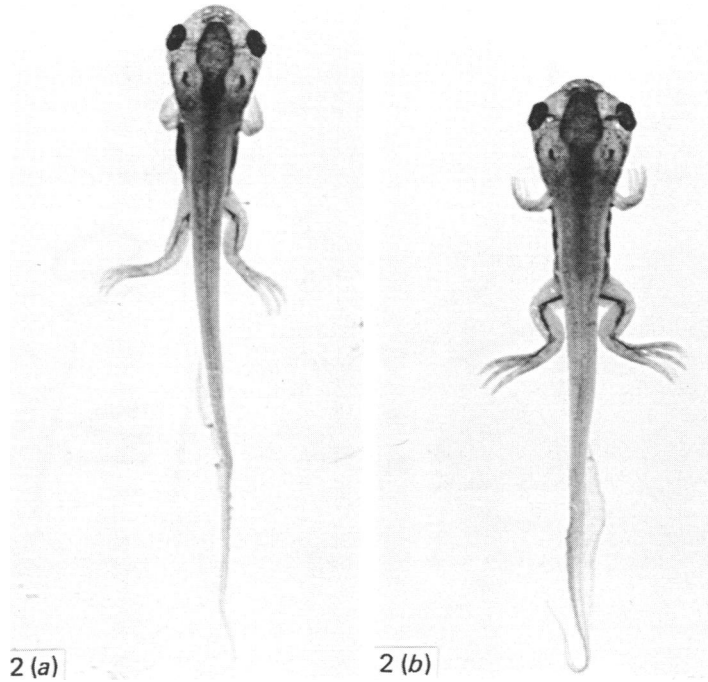


Fig. 2 (a-b). *Xenopus* tadpoles exposed to thyroid hormones for 48 hours at Stage 60NF. (a) Control. (b) Experimental.

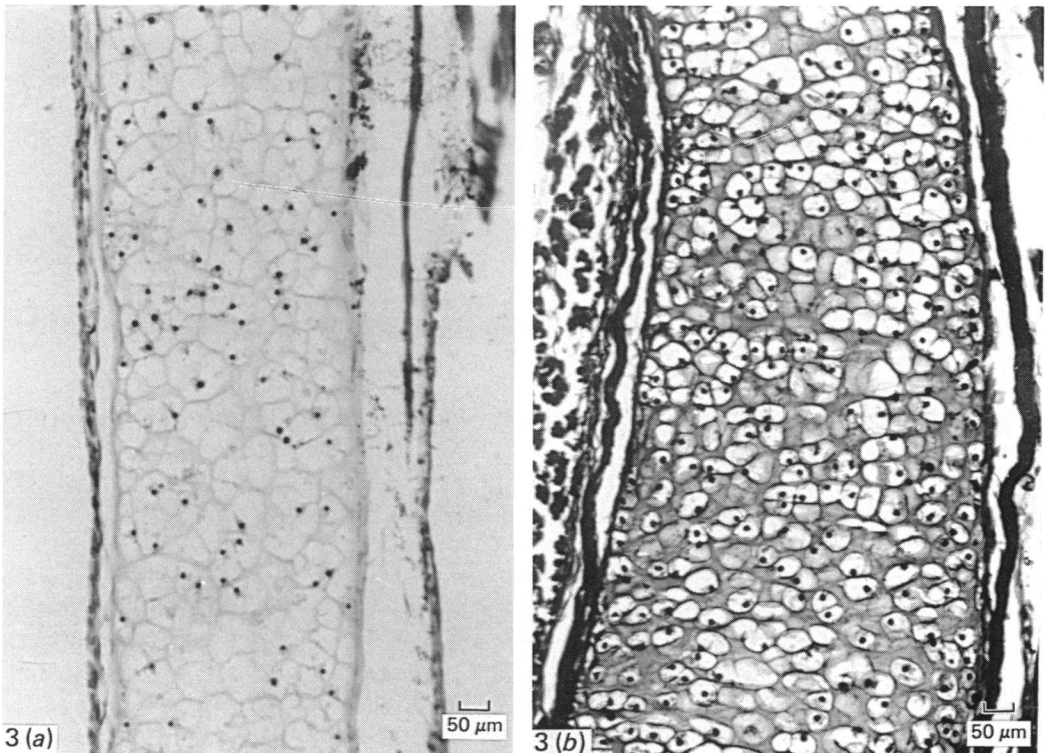


Fig. 3 (a-b). Meckel's cartilage from tadpoles exposed to thyroid hormones for 48 hours at Stage 57NF. (a) Control. (b) Experimental. Heidenhain's haematoxylin.

Table 1. Corrected mean nuclear numbers for cells in Meckel's cartilage following thyroid treatment for 48 hours, and controls

Stage	Control mean	s.d.	Previously established mean	Experimental mean	s.d.
57NF	23608	±840	24536	34018	±1034
60NF	37192	±903	37052	37987	±1250

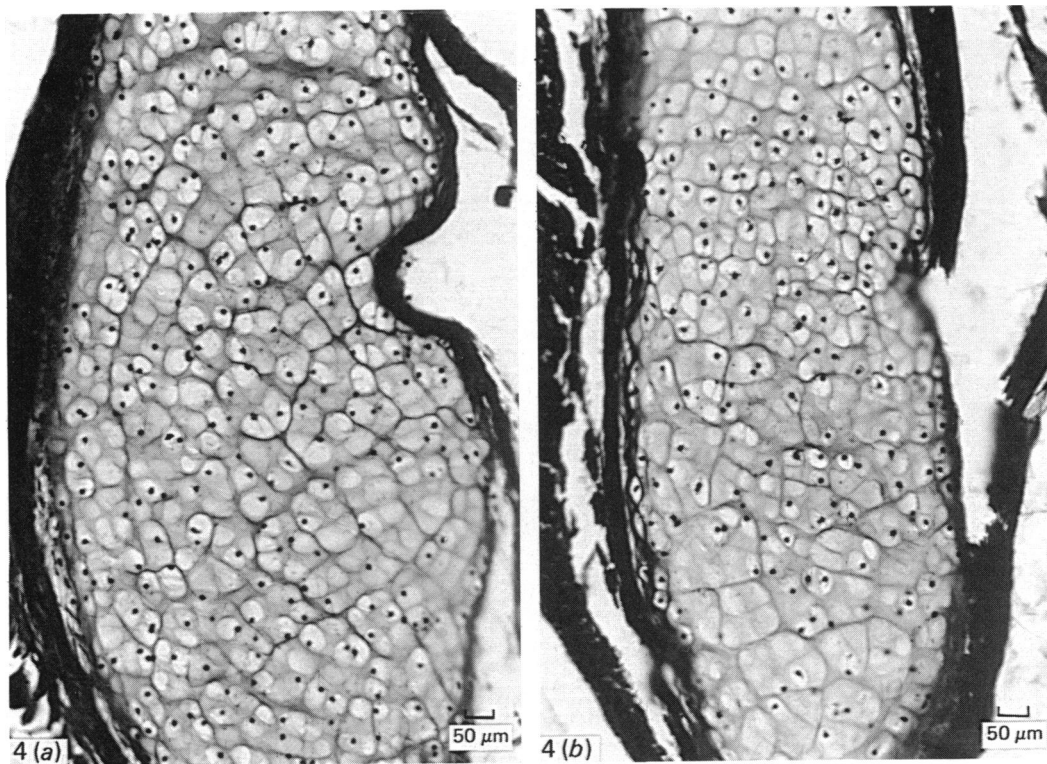


Fig. 4 (a-b). Meckel's cartilage from tadpoles exposed to thyroid hormones for 48 hours at Stage 60NF. (a) Control. (b) Experimental. Heidenhain's haematoxylin.

amount of matrix present appearing much greater, and exhibiting a more intense staining reaction (Fig. 3). Some early ossification has already commenced around the cartilage. The control animals were still at Stage 57NF after the experimental period, tadpoles remaining in this stage for approximately 4 days (Nieuwkoop & Faber, 1956) and therefore the previously described mean nuclear number for Stage 57NF (Thomson, 1987) is included with the present results in Table 1.

The nuclear count for the control animals is within one standard deviation of the Stage 57NF mean (Thomson, 1987), while the experimental mean has risen to a level previously reached at Stage 61NF following development for 7 to 8 days (Thomson, 1987).

Although this preliminary study has only used a small experimental group, this difference is statistically significant ($P < 0.001$).

Table 2. *Volume fraction of matrix (%) present in Meckel's cartilage following thyroid treatment for 48 hours, with controls*

Stage	Control mean	S.D.	Previously established mean	Experimental mean	S.D.
57NF	16	±0.8	17	47	±2.0
60NF	22.8	±1.0	22	23	±0.74

Stage 60NF animals

The histological appearance of the cartilage in the control and experimental animals was closely similar, the size of the lacunae and the amount of matrix present appearing identical between the animals (Fig. 4). The control animals, on re-staging, had reached Stage 61NF and therefore the mean nuclear number for Stage 61NF (Thomson, 1987) has been included in the Table for comparison.

There is no significant difference between the experimental and the control group.

*Matrix volume fraction changes**Stage 57NF*

The volume fraction of the cartilage occupied by matrix in the control and experimental animals, together with that previously assessed for Stage 57NF (Thompson, 1987) is presented in Table 2, showing a very dramatic increase in the amount of matrix present, corresponding to the observed change in lower jaw shape. The amount of matrix present has risen to a level reached during normal metamorphosis at Stage 64–65NF, following development for 12 to 13 days (Thompson, 1987).

Stage 60NF

The amount of matrix present has not been affected by exposure to the hormones described above, the previously described normal value for Stage 61NF animals has been included in Table 2.

DISCUSSION

Gudernatsch (1914) first demonstrated that thyroid preparations could induce premature metamorphosis in some amphibians and, since his work, many studies of the role of these and other hormones have been carried out (see Fox, 1983 for summary). The studies of Etkin (1935) and Allen (1932) have suggested that the activity of thyroid hormones is maximal near the beginning of active metamorphosis.

Most earlier studies have been concerned with the gross changes produced by interfering with normal thyroid function, though studies by Kalténbach (1953) and Yamaguchi & Yasumasu (1977, 1978) have examined the effects of exposure to thyroid hormones on skeletal development. No previous work, however, has concentrated on the possible effects of such exposure on the cartilaginous components of the skeleton.

The dramatic stimulus to cell division, and especially to matrix synthesis provided by exposure to the hormone at Stage 57NF, associated with a much earlier change in the shape of the cartilage, agrees with Etkin's conclusion (1935) that higher

concentrations of thyroid hormones early in metamorphic climax speed up the metamorphic process.

In this study, the Lag phase essentially disappears (NF Stages 57–60, Thomson, 1987) and the Synthesis phase commences concurrently with the Division phase, whereas in normal metamorphic climax the Division phase is essentially complete before the Synthesis phase commences (Thomson, 1987). Etkin (1935) states that the sequence of metamorphic events is independent of the concentration of thyroid hormone (in *Rana cantabrigensis*), while Deanesly & Parkes (1945) and the results of this study suggest that in *Xenopus* this is not the case, the process being not only accelerated but the sequence of events changed, in that normally the front limbs erupt before the head begins to shrink, this being reversed in the experimental animals.

The findings of this preliminary investigation are also in agreement with the concept that structures in the tadpole have a specific stage in development when they are affected by thyroid hormones (Allen, 1932). In the case of Meckel's cartilage this would appear to occur early in metamorphic climax (or shortly before climax commences), as Jurand (1959) has demonstrated that thyroid hormones play no morphogenetic role until Stage 52–53NF has been reached. This susceptibility of the tissue is apparently lost by Stage 60NF.

Etkin (1935) however showed that the thyroid gland becomes increasingly active during early metamorphosis, and suggests that different organs have different thresholds of response to the action of the hormone. These concepts of a 'time of action' and a 'threshold of action' may in fact simply be different expressions of the same basic idea, as the particular time when a particular tissue responds may be the only point during development when the thyroid hormones are at the correct level, or the receptors in the tissue are functional and too high a hormone level could be as ineffective as too low.

This preliminary study has shown Meckel's cartilage in *Xenopus* during early metamorphosis to be highly sensitive to the action of thyroid hormones, and that this sensitivity is lost shortly after climax has commenced. It is hoped that Meckel's cartilage may prove a useful model for further investigations into the role of the thyroid hormones in the development of the skeletal system during metamorphosis in *Xenopus*.

SUMMARY

The effects of short-term dosage with thyroid powder on the metamorphic changes in Meckel's cartilage in *Xenopus laevis* are described. The Lag phase of development appears to be by-passed, and the usual sequential arrangement of the Division and Synthesis phases is disrupted, the processes of normal metamorphic change being considerably accelerated.

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