Adult haemoglobin in developmentally retarded tadpoles of *Xenopus laevis*

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**SUMMARY**

Tadpoles of *Xenopus laevis* reared in water containing 0.01% propylthiouracil continue to grow but fail to develop or metamorphose. The haemoglobin of such tadpoles has been extracted in buffer, converted to a cyanmet form, and run on polyacrylamide gels. The developmentally retarded tadpoles are found to possess adult-type haemoglobin rather than the tadpole type which normally characterizes their developmental stage.

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**INTRODUCTION**

Amphibian tadpoles retained in water containing propylthiouracil continue to grow but fail to develop or metamorphose. We have examined the haemoglobins of such tadpoles by polyacrylamide gel electrophoresis, and find that they possess almost entirely adult-type haemoglobin. Untreated tadpoles of the same stage in development possess a haemoglobin pattern characteristic of tadpoles, while normal metamorphosed animals of the same chronological age possess an adult-type pattern (Maclean & Jurd, 1971a). Our present finding complements the observation that anaemic adult *Xenopus* synthesize some tadpole-type haemoglobin (Maclean & Jurd, 1971a) and that neotenous adult *Ambystoma* have an adult rather than a tadpole haemoglobin pattern (Maclean & Jurd, 1971b).

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**MATERIALS AND METHODS**

*Animals*

Normal adult *Xenopus laevis* were bought from a dealer following direct importation from South Africa. Tadpoles were reared in tanks and fed on nettle powder and Complan (Glaxo, England) and staged according to the normal tables of Nieuwkoop & Faber (1967).

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Chemical agents

Except where otherwise specified, all reagents were supplied by British Drug Houses Ltd, Poole, Dorset.

Rearing of developmentally retarded tadpoles

Ten larvae at stage 52 (Nieuwkoop & Faber, 1967) (mean wet weight 0.13 ± 0.1 g, mean total length 2.0 ± 0.17 cm) were immersed in 101 of a solution of 0.01 % (w/v) propylthiouracil (Sigma, London) in tap water. The immersion fluid was changed at intervals of 2 weeks.

Increases in wet weight, total length, and stage number were compared with those of controls (mean wet weight 0.11 ± 0.10 g, mean total length 1.70 ± 0.13 cm) in tap water.

Preparation of haemoglobins

Both adults and tadpoles were anaesthetized in MS 222 (Sandoz, Ltd, London), the adults having 0.5 ml of blood removed by ventricular puncture, the tadpoles being bled by incising the hearts of the anaesthetized tadpoles while they were immersed in Ringer solution. The blood cells were washed twice by centrifugation in 50 ml of Rugh's Amphibian Ringer Solution (Rugh, 1962), then washed once in a tris (hydroxymethyl)-methylamine (Tris)/diamino-ethane-tetra-acetic acid (EDTA)/boric acid buffer at pH 8.6, also containing 0.01 % dithiothreitol (Calbiochem Ltd, Los Angeles, U.S.A.) (Maclean & Jurd, 1971b). The final pellet of cells was resuspended in this TEB buffer to give a final concentration of approximately 100 × 10^6 cells per ml. The TEB buffer was then modified by the addition of 0.2 vols. of 2 % K_3Fe(CN)_6, 0.5 % KCN, 0.1 % NaHCO_3 in order to convert the haemoglobins to the stable cyanmet form. The cells were then lysed by the addition of 0.1 mg per ml of saponin and, after separation of the cell debris by centrifugation at 8000 g for 10 min, the clear red supernatant was removed and stored at 4 °C.

Electrophoresis of haemoglobins

Electrophoresis through polyacrylamide gels was carried out with a Shandon SAE 2731 disc electrophoresis apparatus. Gels were prepared using 20-62 ml of TEB buffer, 2-4 ml glycerol, 0-03 ml TeMeD (Tetramethyl-1,2-diamino-ethane), acrylamide 2-50 g, and Bis acrylamide 0-05 g, and were polymerized by the addition of 1 ml of 3.5 % ammonium persulphate (freshly prepared). Gels were prerun at 4 °C for 1 h using 2-5 mA per gel tube. Samples of haemoglobin solutions were placed on gels with a hypodermic syringe, following the addition of 0.1 g per ml of sucrose to the samples in order to increase the density of the sample solution and ensure its clean placement over the gel.
Fig. 1. Two tadpoles of *Xenopus laevis* at the same stage of development (stage 54). The one on the left is a control animal of about 1 month reared in tap water, while that on the right is a much older animal (about 18 months) reared since stage 52 in propythiouracil. The compound has permitted continued growth but has retarded development beyond a larval stage.

Sample size was 0.02 ml of haemolysate per gel tube. Electrophoresis with 2.5 mA per tube was continued for 1 h at 4 °C, after which the samples were rapidly removed and photographed in the tubes.

**RESULTS**

**Growth and development of the tadpoles**

After 23 days the control tadpoles had advanced to stage 57 while the experimentals were only at stage 54. There was, however, no significant difference at this time in the mean total length and mean wet weight of the controls (4.08 ± 0.24 cm, 0.38 gm) and experimentals (4.32 ± 0.2 cm, 0.37 ± 0.10 g). Following this, the controls advanced into metamorphic climax (stages 58–66). This period is characterized by a decrease and eventual cessation of growth accompanied by drastic morphological change including tail regression. The stage number of the experimentals remained unchanged at 54 but total length and wet weight increased until, after 18 months, the mean total length was 9.11 ± 0.36 cm and mean wet weight was 2.06 ± 0.17 g (see Fig. 1).
Fig. 2. Polyacrylamide gel electrophoresis of haemoglobin from normal adult *Xenopus* (left), stage-54 tadpoles (centre), and giant stage-54 tadpole (right). Gels are unstained and have been photographed within the glass running tubes.

**Haemoglobin electrophoretic patterns**

Adult *Xenopus* possess one main haemoglobin, A₁, which is followed in electrophoresis by a second very faint band, the haemoglobin A₂. Stage-54 tadpoles, which lack forelegs and have only small hind legs, have four distinct haemoglobins T₁, T₂, T₃ and T₄, of which T₂ is probably the same as A₁, while T₃, the strongest tadpole band, migrates faster than A₁ (Maclean & Jurd, 1971a). As seen in Fig. 2, the haemoglobin of the 'giant' developmentally retarded tadpole although at stage 54 of development, is almost entirely the main adult haemoglobin A₁. A trace of the main tadpole haemoglobin T₃ is discernible in this sample in Fig. 2 also, and the presence of this faint band was confirmed by later staining of the gels with orthodianisidine (O'Brien, 1961).

Fig. 3 shows a comparison of the haemolysates of two 'giant' tadpoles with the haemoglobin of a normal adult, and both tadpoles are seen to possess almost exclusively the adult-type haemoglobin.
Adult haemoglobin in developmentally retarded tadpoles of Xenopus

DISCUSSION

Despite the fact that the Xenopus 'giant' tadpole, developmentally retarded by propylthiouracil, conforms in gross morphology to a normal tadpole in all but size, its red cells contain the haemoglobin of the adult Xenopus. This seems to imply that the haemoglobin switch from tadpole-type to adult-type haemoglobin (Jurd & Maclean, 1970; Maclean & Jurd, 1971a), which normally occurs at about metamorphosis, is not closely coupled to the other hormonally controlled aspects of development. It may be that the haemoglobin transition in Xenopus is determined more by chronological age, or size, or some other independent factor, rather than the hormonal control by thyroxine which is apparently antagonized by treatment with propylthiouracil. Moss & Ingram (1968) have treated tadpoles of the bullfrog Rana catesbiana with thyroxine and find that the hormone has the effect of suppressing the synthesis of the tadpole-type haemoglobin and stimulating the production of the adult-type haemoglobin. Since, in the observations and experiments of Moss & Ingram, the cessation of synthesis of the larval haemoglobin preceded the appearance of the adult haemoglobin, the effect of thyroxine may be simply to suppress the continued synthesis of the larval haemoglobin. Actual production of the
adult-type molecule may perhaps be triggered by many factors, one of which might be compensation for the lack of the tadpole haemoglobin. Our present findings complement the earlier observations that anaemic adult *Xenopus* synthesize some tadpole type haemoglobin (Maclean & Jurd, 1971a) and that neotenous adult *Ambystoma* (Axolotl) have an adult, rather than a tadpole, haemoglobin pattern (Maclean & Jurd, 1971b).

REFERENCES


*(Received 1 September 1975)*