# RIBONUCLEIC ACID SYNTHESIS DURING THYROXIN-INDUCED METAMORPHOSIS IN BULLFROGS

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Developmental changes in hemoglobin synthesis have been extensively studied in the bullfrog, Rana catesbeiana. Moss and Ingram (1965) have examined the in vitro synthesis of hemoglobin by circulating red blood cells during the administration of thyroxin to tadpoles, and have reported an initial marked decline in hemoglobin synthesis followed by the production of hemoglobin characteristic of the adult bullfrog. Radioautographic studies of the red blood cells provided evidence that the increased rate of protein synthesis was associated with a cell type which differed from the mature red blood cells in size and shape. It has been further concluded that these new cells are, in fact, synthesizing frog hemoglobin (De Witt, 1968).

The differentiation of a new erythrocyte line may well be accompanied by large changes in RNA synthesis. These studies represent initial attempts to examine the production of RNA by blood cells during progressive stages of thyroxin-induced metamorphosis in the bullfrog.

Rana catesbeiana tadpoles were purchased from the Connecticut Valley Biological Supply Company, Southampton, Massachusetts, and kept in aerated tap water containing $2.5 \times 10^{-8}$ M thyroxin. After various periods

of thyroxin treatment, five tadpoles were injected intraperitoneally with 20 uc of uridine-6-$^{3}$H (New England Nuclear Corporation) dissolved in amphibian Ringer's solution. Twenty-four hours following injection, blood cells were collected in a small volume of cold heparinized Ringer's and washed once in the same solution. Total RNA was extracted at 4° C by the method of Brown and Littna (1964) with the following modifications. 0.1 ml of packed cells was suspended in 4.0 ml of 0.1 M sodium acetate buffer, pH 5.0, containing 4 ug/ml of polyvinyl sulfate, and lysed rapidly by repeated freezing and thawing in methanol-ice. Sodium lauryl sulfate was added to a final concentration of 0.5%, and the solution was shaken at 4° C for 12 minutes with an equal volume of redistilled, water-saturated phenol. DNase treatment was for 7 minutes at 20° C with electrophoretically-purified DNase (Worthington Biochemical Corporation). The final precipitate was dissolved in 1.0 ml of 0.01 M sodium acetate containing 1 ug/ml polyvinyl sulfate, and concentration was determined spectrophotometrically at 260 mu.

Approximately 50 ug of RNA was layered over a linear 5-20% sucrose gradient containing 1 ug/ml polyvinyl sulfate and centrifuged for 7 hours at 30,000 rpm in the SW50 rotor of the Beckman Model L preparative ultracentrifuge. Five-drop fractions were collected by puncturing the bottom of the tube. Each fraction was diluted with 1.0 ml of 0.01 M Tris-HCl, pH 7.2, and following determination of optical density at 260 mu, carrier herring sperm DNA was added and the nucleic acids were precipitated with 0.3 ml of cold 50% trichloroacetic acid. The precipitates were collected on Millipore filters (23 mm diameter, 0.45 u pore size) and washed twice with cold 5% trichloroacetic acid. The dried filters were placed in

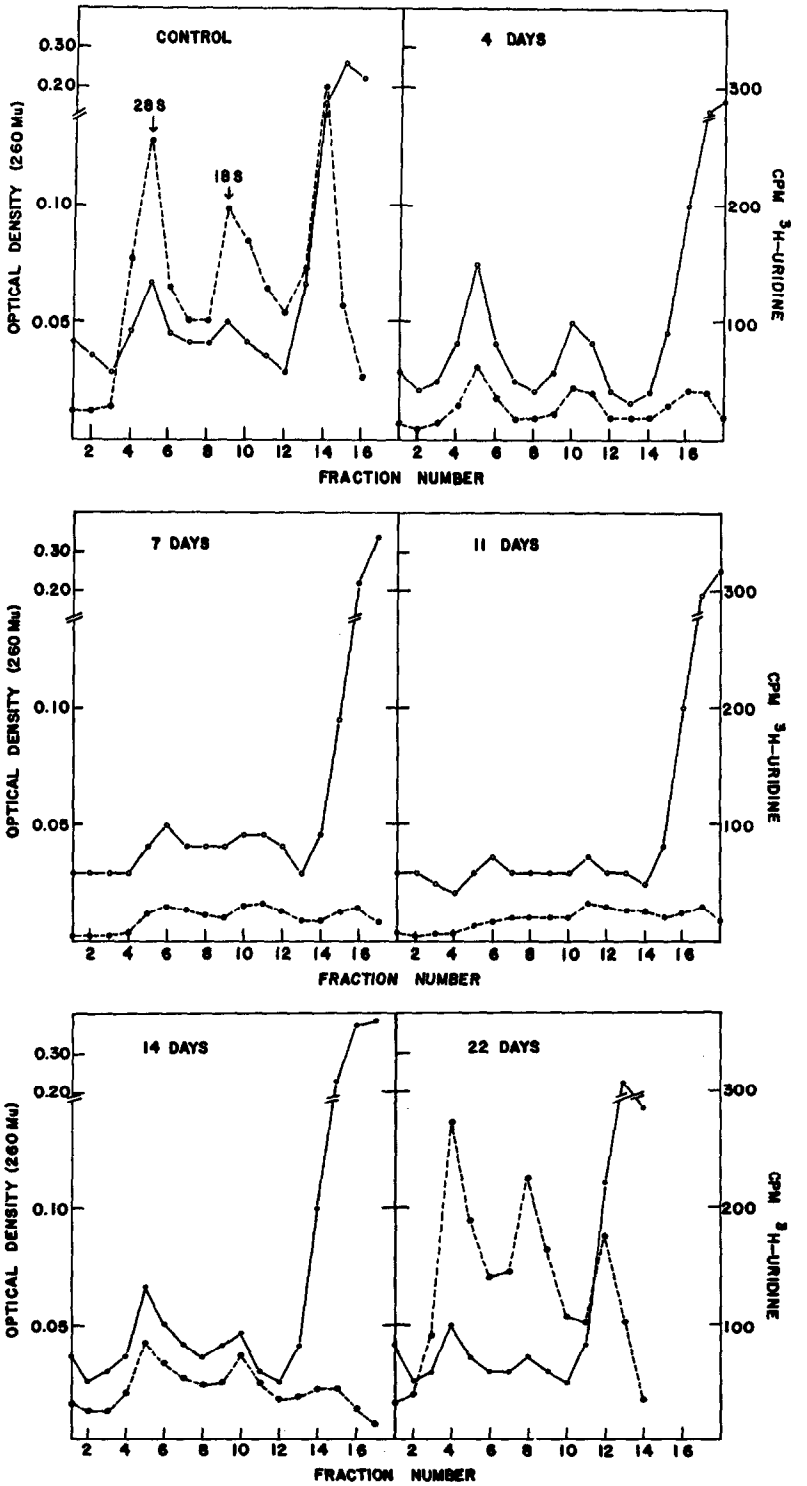


Figure 1. Sucrose gradient sedimentation patterns of RNA extracted from blood cells of Rana catesbeiana tadpoles after various periods of thyroxin administration. 50 ug of RNA were layered on 5-20% sucrose gradients in all cases except for the 22-day sample, in which only 30 ug of RNA were used. Optical density (solid lines) and counts per minute of $^{3}$H-uridine (broken lines) are plotted versus fraction number. Note the change of scale on the optical density axis.

scintillation vials containing 10 ml of purified toluene, 1.0 mg POPOP (1, 4-bis-2-(5-phenyloxazolyl)benzene and 40 mg PPO (2, 5-diphenyloxazole) and counted on a Packard Tri-Carb liquid scintillation spectrometer.

Total RNA extracted from blood cells of tadpoles following various periods of thyroxin treatment was characterized by sucrose gradient centrifugation, as shown in Figure 1. Sedimentation coefficients were determined with reference to Escherichia coli RNA extracted by the same procedure. The recovery of acid-precipitable counts applied to the sucrose gradients was greater than 90%.

Table 1

| Days of thyroxin treatment | Specific activity of RNA (cpm/ug) | | Hind leg/tail |
|---|---|---|---|
| | 28S peak | 18S peak | |
| Control | 77.8 | 78.4 | 0 |
| 4 | 23.0 | 17.2 | 0.01 |
| 7 | 11.6 | 14.2 | 0.03 |
| 11 | 9.2 | 17.1 | 0.04 |
| 14 | 25.5 | 32.0 | 0.11 |
| 22 | 109.2 | 112.0 | 0.23 |

Specific activity of 28S and 18S ribosomal RNA extracted from blood cells of tadpoles following various lengths of thyroxin treatment, as described in the text. Hind leg to tail ratios are used as a measure of gross morphological development.

RNA synthesis, as measured by the incorporation of $^{3}$H-uridine, declines rapidly during the initial eleven days of thyroxin administration. This period is also characterized by an apparent breakdown of ribosomal

RNA with subsequent loss of discrete ribosomal RNA peaks by eleven days. The decline is followed by a marked increase in RNA synthesis in later days, as well as a reappearance of discrete optical density peaks corresponding to ribosomal RNA. The course of RNA metabolism is illustrated by the change in the specific activities of the 28S and 18S ribosomal peaks during thyroxin treatment (Table 1).

Repeated attempts to extract RNA from tadpole blood cells using a hot phenol extraction procedure at either pH 5.2 or 7.2 yielded small amounts of RNA which failed to produce discrete optical density peaks on sucrose gradients. Breakdown of RNA was avoided in the experiments reported here by extraction with phenol at $4^{o}$ C and by the use of polyvinyl sulfate to inhibit RNase activity (Fellig and Wiley, 1959). The large amounts of ultraviolet-absorbing material observed at the top of the sucrose gradients are due to low molecular weight nucleic acid components carried through the procedure, and have been eliminated by other workers using Sephadex gel filtration (Homma and Graham, 1963). The absence of large quantities of labelled material in these low molecular weight peaks demonstrates that they are not the result of breakdown of the newly-synthesized, higher molecular weight RNA.

The decline and subsequent increase in RNA synthesis we have observed in blood cells of tadpoles during the course of thyroxin treatment parallels a similar change in hemoglobin synthesis reported by Moss and Ingram (1965). They postulated that thyroxin exerts both repressive and inductive regulatory action on hemoglobin synthesis by halting production of the old cell line synthesizing tadpole hemoglobin and stimulating the proliferation of a new cell line which synthesizes frog hemoglobin.

Changes in RNA synthesis of the type we have observed support their postulated mechanism of thyroxin action.

The effects of thyroxin on polyribosome distribution and base composition of RNA in tadpoles are currently being studied, and will be reported elsewhere.

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