DNA Synthesis and Turnover in the Bullfrog Tadpole during Metamorphosis*

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131I-labeled deoxyuridine (IdUrd) has been used to estimate the turnover of DNA in liver, tail, and hind limb during spontaneous and triiodothyronine-induced metamorphosis. It was found that the total amount of liver DNA remained constant and there was no significant loss of the label from the liver DNA, which would be expected if there was an increase in DNA turnover during metamorphosis. Also, the change in specific activity of liver DNA parallels that of tail DNA during spontaneous metamorphosis. These data suggest that metamorphic transitions in the tadpole liver do not involve significant changes in DNA turnover. It was observed that the incorporation of label into hind limb DNA showed a high variability among individual animals as compared to liver and tail tissue. The data presented suggest that the observed variability is not a random phenomenon but related directly to the rate at which animals will metamorphose.

The regulation of cell replication and differentiation involves many areas of research. The precocious metamorphosis of anurans in response to thyroid hormones is an example (1). It has been shown that the urea cycle enzymes necessary for the transition of ammonia-excreting larvae to urea-excreting adults are synthesized by the liver of thyroid hormone-treated animals in a manner similar to those undergoing spontaneous metamorphosis (2). Reports describing the effects of thyroid hormones on nucleic acids and proteins and on the ultrastructural reorganization in liver cells harbor conflicting assumptions regarding the extent of liver cell synthesis and/or turnover during thyroid hormone-induced metamorphosis and spontaneous metamorphosis (3–7).

It has been assumed that the biochemical changes in the liver of tadpoles undergoing metamorphosis occurred in a "fixed population of cells" (1, 2). This would preclude any significant change in total liver DNA or DNA turnover during metamorphosis. However, there have been several reports which suggested that the metabolism of DNA was also involved in the biochemical changes in the liver during metamorphic transitions. Elias and Sherrick (8) reported a doubling of the liver cell plates during spontaneous metamorphosis. Bennett and Glenn (9) reported a maximal level of nucleolar hypertrophy at stage XX. Campbell et al. (10) reported enhanced mitochondrial DNA synthesis 3 days after triiodothyronine treatment.

Atkinson et al. (3) reported an increase in labeled thymidine incorporation into liver DNA in vivo during spontaneous and triiodothyronine-induced metamorphosis. These observations led Atkinson (11) to postulate that the thyroid hormones induced macromolecular changes in the liver which could not occur in a “fixed population of cells.”

The "fixed cell versus nonfixed cell" controversy is thus a critical impasse to our further understanding of the action of thyroid hormone in tadpole liver. We have tried to approach this question in a different way by using the thymidine analog [131I]IdUrd to estimate the rate of DNA biosynthesis and turnover. It has been shown that after the incorporation of IdUrd into DNA, the iodine moiety (label) remains covalently bound to the DNA until cell death (12–14). When the labeled cell dies, rapid deiodination and excretion limits the reutilization of radioactivity by other cells (15), allowing the estimation of the rate of cell death. Cytological investigations of tadpole liver cells show no evidence of polyplody at any time during thyroid hormone-induced or spontaneous metamorphosis (3, 4, 7). Since liver is a discrete integral tissue, we can assume that the total liver DNA is directly proportional to the number of liver cells. Therefore, the rate of new cell synthesis and/or cell death during metamorphosis can be estimated on the basis of the metabolism of [131I]IdUrd and the total DNA content of the liver.

MATERIALS AND METHODS

Rana catesbeiana tadpoles were purchased from Howe Brothers Minnow Farm, Atlanta, Texas and maintained in outdoor tanks on a diet of collard greens. Animals were fasted for 3 days prior to experimental treatment. The animals used in a given study were selected from a batch shipment on the basis of their similarity of stage in development, general appearance, and weight (±5%). [131I]IdUrd (specific activity 2200 Ci/mmol) was supplied by Dr. K.
DNA Synthesis in Tadpoles

**TABLE I**

Labeling of tadpoles and tissues with tracer \[^{35}S\]dUrd

Twenty-four hours after injection (0.1 &mu;Ci of \[^{35}S\]dUrd), 22 animals of uniform weight and label incorporation were selected. Ten of these were killed immediately (1 day, liver, tail and hind limb data). The remainder were maintained until one animal metamorphosed to stage XXIV (14 days). They were weighed, counted, ranked on the basis of degree of metamorphosis (animals 1 to 12), and killed. Refer to Fig. 1 for photographs of representative animals and the horizontal axis of Fig. 2 for the progression of stage of development.

| Whole body | Liver | Tail | Hind limb |
|------------|-------|------|-----------|
|            | Weight | % of injected counts | Weight | % of injected counts | Weight | % of injected counts |
| 1 day*     |        |                   |        |                   |        |                   |
| 1          | 6.2    | 34.1              | 0.15   | 0.41              | 1.21   | 1.40              |
| 2          | 8.5    | 32.3              | 0.16   | 0.39              | 1.28   | 1.50              |
| 3          | 6.6    | 31.8              | 0.15   | 0.38              | 0.63   | 0.90              |
| 4          | 5.2    | 32.4              | 0.18   | 0.36              | 0.68   | 0.76              |
| 5          | 4.9    | 30.1              | 0.13   | 0.38              | 0.51   | 0.86              |
| 6          | 6.0    | 32.0              | 0.16   | 0.42              | 0.42   | 0.60              |
| 7          | 5.6    | 31.1              | 0.20   | 0.33              | 0.39   | 0.45              |
| 8          | 4.7    | 28.7              | 0.19   | 0.34              | 0.25   | 0.24              |
| 9          | 4.9    | 29.9              | 0.17   | 0.31              | 0.15   | 0.21              |
| 10         | 4.6    | 26.1              | 0.17   | 0.32              | 0.17   | 0.22              |
| 11         | 5.3    | 28.4              | 0.20   | 0.31              | 0.06   | 0.11              |
| 12         | 4.6    | 25.0              | 0.21   | 0.33              | -c     | 0.51              |

| 14 days    |        |                   |        |                   |        |                   |

* One day whole body data includes all animals selected for the study, 1 day after injection.

† The values expressed for the 1-day animals include mean and S.D. for 10 determinators.

Methods and media were as described. The half-life of \[^{35}S\]dUrd was determined to be 60 days.

**RESULTS**

\[^{35}S\]dUrd Incorporation—The radioactivity in the acid-insoluble material was associated with the nucleic acid fraction and was acid-precipitable after alkaline hydrolysis. More than 90% was solubilized by acid hydrolysis (10 N HClO₄, 100° 1 hour), as well as by enzymatic digestion by DNase I and snake venom phosphodiesterase (Worthington Biochemical Corp.). Labeling of the acid-insoluble fraction (DNA) occurs during the first 6 hours after injection. After 24 hours, essentially all the label in the tissues studied was associated with the acid-insoluble material. These investigations suggest that the metabolism of dUrd by tadpoles was essentially as that reported for other systems (13, 14).

Spontaneous Metamorphosis—The rate of spontaneous metamorphosis was clearly not identical, (Table I, Fig. 1), allowing the analysis of 12 animals in various stages of development (XVI to XXIV) whose DNA had been “prelabeled.” We observed essentially no change during metamorphosis in the specific activity of liver DNA (Fig. 2) or in total liver DNA (cpm/specific activity from Table I and Fig. 2). The amount of label in the tail diminished as shown in Table I. The observation that the specific activity remains constant (Fig. 2) is consistent with the assumption that the DNA was uniformly labeled with dUrd and, in the case of tail DNA, subsequently degraded as metamorphosis progressed.

The quantitative removal of the tail, necessary for an accurate estimate of total tail DNA, becomes more difficult in advanced stages. However, the estimation of the specific activity of the tail DNA was not dependent on exact quantitative removal of total tail. Therefore, we believe that the specific activity determinations represent a high degree of reliability, although the estimation of total tail DNA would necessitate a much larger group of experimental animals.

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A. Kent, unpublished observations.

G. Hofer (13), synthesized by a modification of the procedure described by Hughes et al. (14) and suspended in Earle's balanced salt solution (10 µCi/ml). Tadpoles were injected intraperitoneally with 0.1 µCi of \[^{35}S\]dUrd. Radioactivity was counted using a well scintillation counter (Nuclear Chicago). All data have been corrected for isotopic decay.

One day whole body data includes all animals selected for the study, 1 day after injection.

The values expressed for the 1-day animals include mean and S.D. for 10 determinators.

Insufficient tissue for measurement.

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**TABLE II**

Labeling of tadpoles and tissues with tracer \[^{35}S\]dUrd

Twenty-four hours after injection (0.1 &mu;Ci of \[^{35}S\]dUrd), 22 animals of uniform weight and label incorporation were selected. Ten of these were killed immediately (1 day, liver, tail and hind limb data). The remainder were maintained until one animal metamorphosed to stage XXIV (14 days). They were weighed, counted, ranked on the basis of degree of metamorphosis (animals 1 to 12), and killed. Refer to Fig. 1 for photographs of representative animals and the horizontal axis of Fig. 2 for the progression of stage of development.

| Whole body | Liver | Tail | Hind limb |
|------------|-------|------|-----------|
|            | Weight | % of injected counts | Weight | % of injected counts | Weight | % of injected counts |
| 1 day*     |        |                   |        |                   |        |                   |
| 1          | 6.2    | 34.1              | 0.15   | 0.41              | 1.21   | 1.40              |
| 2          | 8.5    | 32.3              | 0.16   | 0.39              | 1.28   | 1.50              |
| 3          | 6.6    | 31.8              | 0.15   | 0.38              | 0.63   | 0.90              |
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| 7          | 5.6    | 31.1              | 0.20   | 0.33              | 0.39   | 0.45              |
| 8          | 4.7    | 28.7              | 0.19   | 0.34              | 0.25   | 0.24              |
| 9          | 4.9    | 29.9              | 0.17   | 0.31              | 0.15   | 0.21              |
| 10         | 4.6    | 26.1              | 0.17   | 0.32              | 0.17   | 0.22              |
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| 12         | 4.6    | 25.0              | 0.21   | 0.33              | -c     | 0.51              |

| 14 days    |        |                   |        |                   |        |                   |

* One day whole body data includes all animals selected for the study, 1 day after injection.

† The values expressed for the 1-day animals include mean and S.D. for 10 determinators.

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A. Kent, unpublished observations.
It is interesting to note (Table I) that the specific activity of the hind limbs decreases sharply (animals 1 through 5) and then remains essentially constant (animals 5 through 12). The sharp decrease was predictable due to the increase in total DNA. However, there is also an overall increase in hind limb DNA from animal 5 through animal 12 with no predicted decrease in specific activity (Fig. 2, Table I). Furthermore, as shown in Table I, there was an increase in labeling of the hind limbs as a function of stage of development. This may appear to be inconsistent with the contention that, due to chemical breakdown of the analog in vivo, label cannot be incorporated into DNA after the initial labeling period. However, it appears that the apparent "labeling" of the hind limbs during the 14-day period shown in Table I actually reflects increased incorporation of \([^{3}H]dUrd\) during the first few hours of labeling into limb tissue of animals which will metamorphose more rapidly. This would account for the leveling off of the specific activity of hind limb DNA. This interpretation is also suggested by the labeling of hind limbs during the first 24-hour period (Table I) where the percentage of injected counts per min (3.1 ± 1.4) covers a range of values similar to the range shown for the 14-day animals (animals 1 to 12, Table I).

**DISCUSSION**

It is paradoxical that, while a great deal is known about the biochemical adaptations of liver tissue during spontaneous and thyroid hormone-induced metamorphosis, there is little evi-
ence to indicate whether these transitions occur in a fixed population of preexisting cells or in cells synthesized during the onset of metamorphosis. We have shown that there is essentially no change in total liver DNA during spontaneous metamorphosis. Furthermore, these observations as well as others (6, 7, 19) clearly demonstrate that thyroid hormone-induced metamorphosis did not result in a significant change in the content of liver DNA. Thus the question of fixed or nonfixed liver cells may ultimately be decided on the basis of turnover rates. Is the premetamorphic DNA of the liver replaced (turned over) during spontaneous metamorphosis or thyroid hormone-induced metamorphosis? Our observations clearly indicate that neither spontaneous nor triiodothyronine-induced metamorphosis have a significant effect on the turnover of liver DNA.

In view of the data presented, we believe that the increased incorporation rate of labeled precursors into liver DNA of metamorphosing animals (3) reflects a negligible increment in content and/or turnover of liver DNA. However, the data reported here are not incompatible with the suggestion that the rate of mitochondrial DNA synthesis increases during metamorphosis (3, 10), since mitochondrial DNA accounts for such a small fraction of tadpole liver DNA. It should be kept in mind, that the rate of one (or more) of the processes involved in the physical transportation and metabolic transformations necessary for the incorporation of intraperitoneally (or intravenously) injected presumptive precursors into liver cell DNA may vary during metamorphosis, resulting in alterations of the specific activity of precursor pools and, consequently, artificial incorporation kinetics.

The use of the Rana catesbeiana tadpoles in studies involving spontaneous metamorphosis has one inherent disadvantage. It is difficult to obtain a group of animals which is undergoing spontaneous metamorphosis at a uniform rate because of the long larval period. An interesting point in this regard is the apparent increase of labeled DNA in the hind limbs as a function of stage of metamorphosis (see “Spontaneous Metamorphosis”). It appears that this effect is the result of an increased rate of hind limb DNA synthesis by those tadpoles, identical in all other measurable respects to others in the study, which will metamorphose more rapidly. Since the incorporation of label into the tail is unaffected, the ratio of hind limb counts per min/tail counts per min (1 day after injection of $^{[3]H}dUrd$) may provide an estimation of the propensity to metamorphose. It is possible that $^{[3]H}dUrd$ injection and subsequent quantitation of the $\gamma$ label located in the hind limbs (with lead shielding the tadpole body) of the live animal may prove to be a valuable tool for detecting the onset of spontaneous metamorphosis well before the development of other characterized physiological and morphological criteria.

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