BIOCHEMISTRY OF AMPHIBIAN METAMORPHOSIS

II. ARGINASE ACTIVITY*

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The metamorphosis of Amphibia may be regarded as an important phase of biochemical evolution (1). While considerable attention has been given to morphological changes during this process (2, 3), much less effort has been directed towards the biochemistry of amphibian metamorphosis. Some of the early data have been summarized by Needham (4). More recent work has been reported by Melnic (5), Nowinski (6), and Riggs (7).

Munro (8) observed an increase in urea production in the course of metamorphosis of Rana temporaria tadpoles with an increase in arginase activity. The enzyme arginase has been well characterized for several other species. Experimentation was begun in 1952 to extend the observations of Munro and to correlate changes in enzyme activity with morphological changes. Prior to the completion of these studies, Munro published his second paper in this field regarding nitrogen metabolism in induced metamorphosis (9).

Agents other than thyroid preparations affect liver arginase in other species (10–13). They were studied with and without thyroxine in an attempt to separate morphological and enzymatic changes. Some of these agents have been found to enhance thyroxine- and triiodothyronine-induced metamorphosis (14). These observations may aid in the clarification of the mode of action of thyroxine on liver arginase in tadpoles, particularly in view of Barth's report that the metabolic response to thyroxine precedes the morphological response (15).

Methods

Treatment of Animals—The Anurae used in this study, Bufo terrestris and Rana hecksheri, Wright, were collected from natural habitats in north-

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ERN FLORIDA. THE TADPOLES WERE MAINTAINED IN TAP WATER IN THE LABORATORY PRIOR TO USE AND FED AT REGULAR INTERVALS, AND THE WATER WAS CHANGED 12 HOURS AFTER FEEDING.

PRIOR TO BEING USED, THE ANIMALS WERE FASTED, 24 HOURS FOR THE BUFO TADPOLES AND 48 HOURS FOR THE RANA SPECIES. THESE PERIODS WERE USED TO ESTABLISH A BASE-LINE LEVEL OF ARGINASE ACTIVITY. AFTER THIS PERIOD, THE ANIMALS WERE EMPLOYED AS CONTROLS OR TREATED AS INDICATED IN TABLE I. THEY WERE MAINTAINED AT 29° ± 0.5° IN AN INCUBATOR DURING ALL EXPERIMENTS.

**TABLE I**

***Methods and Periods of Administration of Metabolic Agents to Anurae***

<table>
<thead>
<tr>
<th>Species</th>
<th>Hormone</th>
<th>Method of administration</th>
<th>Concentration or dose per day</th>
<th>Period of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B. terrestris</strong></td>
<td>Thyroxine*</td>
<td>Immersion</td>
<td>2 × 10⁻⁷ M</td>
<td>24-75 hrs.</td>
</tr>
<tr>
<td></td>
<td>Triiodothyronine†</td>
<td>&quot;</td>
<td>2 × 10⁻⁷ &quot;</td>
<td>24-75 days</td>
</tr>
<tr>
<td><strong>R. hecksheri, Wright</strong></td>
<td>Thyroxine</td>
<td>Injection</td>
<td>0.5 ml. 1 × 10⁻⁴ M</td>
<td>1-21 days</td>
</tr>
<tr>
<td></td>
<td>Triiodothyronine</td>
<td>&quot;</td>
<td>0.5 &quot; 1 × 10⁻⁴ &quot;</td>
<td>1-7 days</td>
</tr>
<tr>
<td></td>
<td>Growth hormone‡</td>
<td>&quot;</td>
<td>1 rat unit</td>
<td>1- 5 days</td>
</tr>
<tr>
<td></td>
<td>Hydrocortisone§</td>
<td>&quot;</td>
<td>0.05 ml. 1 × 10⁻⁴ M</td>
<td>1- 4 days</td>
</tr>
<tr>
<td></td>
<td>Insulin</td>
<td></td>
<td></td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>2,4-Dinitrophenol</td>
<td>Immersion</td>
<td>1 × 10⁻⁴ M</td>
<td>1- 4 days</td>
</tr>
<tr>
<td></td>
<td>Epinephrine‡</td>
<td>Injection</td>
<td>0.05 ml. 1 × 10⁻⁴ M</td>
<td>1- 4 days</td>
</tr>
</tbody>
</table>

* L-Thyroxine contributed by the Glaxo Laboratories.
† Triiodothyronine contributed by the Smith, Kline and French Laboratories.
‡ Gift of Armour and Company for this study. Rat unit defined by Li et al. (16).
§ Hydrocortisone contributed by The Upjohn Company.
‖ Pharmacopoeia of the United States, thirteenth revision, Easton, 265 (1947).
¶ Recrystallized, Eastman Kodak Company.

The ratio of the hind limb length to the tail length was chosen as the morphological index. This index is the reciprocal of the allometric index suggested by Roth (17). Measurements were made prior to and at intervals during the course of treatment. Upon conclusion of treatment, these measurements were repeated and the livers removed. The livers of the Bufo tadpoles were removed under magnification to exclude extraneous tissue.

**Arginase Activity**

*B. terrestris*—Owing to the small size of the livers in this species, twelve animals were used for each experiment and the livers pooled to provide...
an adequate amount of tissue. A 2 per cent homogenate was activated with cobaltous ion at 50° ± 0.5° according to Hunter and Downs (18). The assay procedure of Archibald and Van Slyke (19, 20) was employed, and the activity was determined at 37° ± 0.1°. An amendment to include a constant amount of acid for the controls was made in this procedure.

R. hecksheri, Wright—Individual livers were lyophilized prior to assay. A 0.2 per cent solution of the dry tissue was made, and activation and enzyme assays were carried out as for Bufo.

Free Amino Acids

Ethanolic extracts of the liver, according to the technique of Awapara (21), were used to determine the free amino acid concentration of that tissue. Unidimensional descending chromatograms, with the solvent of Partridge (22), on Whatman No. 1 filter paper were employed for analysis. Controls prepared according to Levy and Chung (23) were run concurrently with extracts at 25–29°.

The method of Fisher et al. (24, 25), employing the measurement of the area developed after treatment with ninhydrin, gave semiquantitative values of the concentration of each amino acid.

Results

In Fig. 1, the comparative morphological changes induced by equal doses of thyroxine and triiodothyronine are shown at 120 hours after the initiation of treatment. The same developmental stage reached by the triiodothyronine-treated animals required approximately 420 hours of thyroxine treatment.

Arginase Activity

B. terrestris—Fig. 2 summarizes the variation in arginase activity during normal and induced metamorphosis. The line is drawn on the basis of least squares. In each case, an approximate linearity exists between the morphological stage and the enzymatic activity. A final liver arginase activity of 25 ± 1 μmoles of urea produced in 30 minutes per gm. of liver tissue occurred in each case. The rate of increase of liver arginase activity is augmented during induced metamorphosis. In this species, metamorphosis normally results in a 3.5-fold increase in arginase activity. Feeding experiments, as summarized in Fig. 3, showed that liver, given prior to and during the course of metamorphosis, resulted in a greater increase in arginase activity than is normally found. Eggs and greens did not induce this 5-fold change. It is possible that liver contains an additional factor which is active in the synthesis or activity of the enzyme.

R. hecksheri, Wright—The greater premetamorphic and metamorphic
ARGINASE ACTIVITY DURING METAMORPHOSIS

periods in this species may account for the non-linearity in Fig. 4 in comparison with Fig. 2. Here again, the enzymatic change with thyroxine proceeds more rapidly than the morphological change. The final concentration of the arginase activity in the liver is 63 µmoles of urea produced in 30 minutes per gm. of dry tissue, with a maximal error of ±5 per cent.

To distinguish between the phenomena of morphological and enzymatic change, experiments were designed to isolate them from each other if possible. Fig. 5, plotted with the curves of Fig. 4, shows that the morphological changes of metamorphosis do not necessarily parallel activity of liver arginase. Of the agents tested, only dinitrophenol appeared to induce metamorphosis as measured by the allometric index. Treatment with the other agents was initiated at approximately the same morphological stage indicated in Fig. 5. Some of these data are summarized in Table II. Testosterone propionate, estrone, and progesterone, given with or without thyroxine, had no appreciable effect on the liver arginase activity.

**Amino Acid Pattern**

The larger size of the tadpoles of *R. hecksheri* facilitated the investigation of changes in the free amino acids of the liver that might clarify
changes in the arginase concentration. Of particular interest were the changes in concentration of arginine and tyrosine among the seventeen amino acids identified. None of the amino acids that were found was unique for this species.

![Graph of Arginase Activity](image)

**Fig. 2.** Arginase activity during normal and induced metamorphosis in *B. terrestris*. Arginase activity in micrograms of urea produced in 30 minutes per gm. of liver. The allometric index $^{-1}$ is the ratio of the hind limb length to the tail length. $\Delta$, control; $X$, triiodothyronine; $O$, thyroxine; $\blacksquare$, thyroxine plus liver; $\bullet$, young toad.

**Fig. 3.** Time sequence of the changes in arginase activity and allometric index $^{-1}$ during induced metamorphosis in *B. terrestris*. The effect of liver resulting in a greater final enzymatic activity is also presented. The arginase activity is expressed in micromoles of urea produced in 30 minutes per gm. of tissue. Solid bar, control; crossed bar, thyroxine; clear bar, thyroxine plus liver.

The concentrations of arginine and tyrosine decrease during metamorphosis (Fig. 6). Normally, the decrease was found to be more rapid for tyrosine than for arginine. A more rapid decrease in concentration of tyrosine and arginine is observed in thyroxine- and triiodothyronine-induced metamorphosis. In fact, no free arginine could be detected at very early morphological stages in the livers of tadpoles subjected to triiodothyronine-induced metamorphosis. Some hormonal effects on arginine and tyrosine are included in Table II.

No decisive alterations in the concentrations of most of the other amino
ARGINASE ACTIVITY DURING METAMORPHOSIS

Fig. 4. Arginase activity during normal and induced metamorphosis in R. hecksheri. Arginase activity in micromoles of urea produced in 30 minutes per gm. of tissue. ○, control; □, thyroxine; ●, triiodothyronine.

Fig. 5. Effects of metabolic agents on the arginase changes of normal and induced metamorphosis. ●, dinitrophenol; ○, growth hormone; ×, growth hormone plus thyroxine; Δ, insulin; ■, insulin plus thyroxine; □, epinephrine; ●, hydrocortisone. Arginase activity in micromoles of urea produced in 30 minutes per gm. of tissue.

**Summary of Effects of Metabolic Agents on R. hecksheri**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Change in arginase activity from normal metamorphosis per cent</th>
<th>Change in arginase activity from induced metamorphosis per cent</th>
<th>Liver-free arginine concentration</th>
<th>Liver-free tyrosine concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroxine</td>
<td>+0–33</td>
<td></td>
<td>Decrease</td>
<td>Decrease</td>
</tr>
<tr>
<td>Triiodothyronine</td>
<td>+0–33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth hormone</td>
<td>+0–15</td>
<td>+30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>+100</td>
<td>+67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>+50–100</td>
<td>+100</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>−60</td>
<td>−70</td>
<td>&quot;</td>
<td>Increase</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>+100</td>
<td>+80</td>
<td>&quot;</td>
<td>No effect or slight increase</td>
</tr>
</tbody>
</table>

acids were noted. The amino acids were identified as alanine, asparagine, aspartic acid, cystine, glutamic acid, glycine, histidine, leucine, methionine, phenylalanine, proline, serine, tryptophan, and valine.
DISCUSSION

Various laboratories have reported on the comparative efficacy of thyroxine and triiodothyronine on amphibian metamorphosis (14, 26-28). In both species used in this study, it was found that the potency of triiodothyronine is between 3- and 4-fold that of thyroxine. Data obtained with triiodothyronine and thyroxine fit the same curve, suggesting that these agents have the same mode of action on liver arginase.

The percentage increase in urea production through metamorphosis as reported by Munro (8, 9) and the arginase activities reported for R. heck-

![Graph showing changes in liver free amino acid pattern of arginine and tyrosine during normal and induced metamorphosis. The control tadpoles represent normal animals whose livers were analyzed at the indicated allometric index⁻¹.](http://www.jbc.org/)

Fig. 6. Changes in the liver free amino acid pattern of arginine and tyrosine during normal and induced metamorphosis. The control tadpoles represent normal animals whose livers were analyzed at the indicated allometric index⁻¹.

Arginase activity was not demonstrable in the livers of newly hatched R. hecksheri tadpoles, owing perhaps to the extremely large amount of tissue required, the Archibald-Van Slyke method being subject to interference due to protein in the regions of low enzyme activity.

The effect of the diet on arginase activity has been previously reported (10-13). Kochakian found that the protein content of the diet influenced arginase activity only when cobaltous ion was employed for activation. Since manganous ion has no effect in this last instance, the suggestion has been made of the existence of two arginase enzymes. Manganous ion was found to be an activator for the arginase enzyme observed in these species, but was not employed for study of the dietary factors.
The agents studied for their effect on liver arginase to distinguish it from morphological changes may have their actions explained by the concept presented by Rosenthal et al. (29, 30). This concept states that the greater the protein catabolism, the more rapid is the increase in liver arginase. Hydrocortisone and epinephrine best exemplify agents fostering protein catabolism, and their effects on liver arginase support Rosenthal's thesis (Fig. 5 and Table II). Growth hormone, an anabolic agent, would not seem to fit this pattern, but treatment with it does result in an increase in liver size. Rosenthal's group found that changes in liver arginase are always initiated by simultaneous gain or loss of hepatic protein.

The lack of change in the arginase activity following treatment with dinitrophenol may be due to its well known effect on oxidative phosphorylation (31). In agreement with Bruice, Winzler, and Kharasch (26), we have found it necessary to administer dinitrophenol continuously to instigate apparent morphological changes. These changes are primarily catabolic and may reflect the interference of the agent on protein synthesis. The contrasting arginase response of the tadpole to thyroxine and dinitrophenol represents a significant qualitative difference in the metabolic action of these two agents.

Other than the accord between Munro's work (8, 9) and that reported here, there is little agreement among workers on the effect of thyroid preparations on liver arginase (32-34). Thyroidectomy decreases the liver arginase in most species (35). Using premetamorphic Anurae as a test system, one is dealing with an animal devoid of all or part of the adult thyroid activity.

The more rapid increase in arginase activity in induced metamorphosis correlates with the more rapid decrease in free arginine in the liver. The change in the tyrosine level, possibly indicating more rapid incorporation into liver protein, perhaps arginase, is also augmented. The interpretation of these results, as well as those of the agents employed not of thyroidal origin, has led to the conclusion that thyroid preparations do not directly initiate changes in liver arginase. This is in agreement with the enhancement by hydrocortisone of thyroxine-induced metamorphosis (14). To substantiate this, the effects of thyroid preparations on adrenalectomized premetamorphic Amphibia are being examined.

**SUMMARY**

An increase in liver arginase activity during normal and induced metamorphosis has been observed in two species of tadpoles. A relationship has been found to exist in these species between the morphological stage and arginase activity. In both species, arginase activity responds more
rapidly to thyroxine and triiodothyronine than do the morphological changes.

Immersion of animals in a solution of dinitrophenol does not cause an increase in liver arginase activity. Treatment with hydrocortisone results in a 2-fold increase in liver arginase without morphological changes. Other hormones tested had little or no effect on the arginase activity.

The concentration of free arginine and tyrosine in the liver decreases very appreciably during normal metamorphosis, but even more rapidly during induced metamorphosis. The concentrations of the other free amino acids tested underwent no pronounced changes during metamorphosis.

BIBLIOGRAPHY

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