CIRCULATING IODOTYROSINES AND IODOTHYRONINES IN AMPHIBIANS

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ABSTRACTS—Circulating thyroid hormone patterns as determined by thin layer chromatographic analysis of butanol extracts of whole blood of amphibians showed that Necturus maculosus differed from the other urodeles in not having monoiodotyrosine as a major fraction. Thyroxine was detected in late tadpole and adult anurans. Free iodide was visualized in both anurans and urodeles except in Amphiuma means.

A recent study, undertaken to determine the differences between pre and postmetamorphic Mexican axolotls (*Ambystoma mexicanum*) showed that all or most of the circulating thyroid hormone was in the form of monoiodotyrosine (T_1) (Prahlad, 1968). Thyroidectomy or hypophysectomy abolished the presence of monoiodotyrosine in the blood indicating that it was formed in the thyroid gland under the influence of pituitary hormones. Recently (unpublished observations) it has been noticed that even a single injection of a large amount (4000 μg) of monoiodotyrosine to the premetamorphic Mexican axolotl does not induce metamorphosis. Thus, the role played by T_1 in body metabolism remains enigmatic not only in the Mexican axolotl but also in mammals (Rhodes, 1968). As there was very little information on the circulating thyroid hormones (Etkin, 1968, Rhodes, 1968, Thornburn, 1967) in various amphibians which differ in their metamorphic Status, a preliminary investigation was undertaken to determine the pattern of these circulating hormones.

METHODS AND MATERIALS

Several examples of urodeles and anurans were investigated (Table 1). These were obtained from commercial sources. However *Ambystoma mexicanum* used in this study was raised in our laboratory. It should be emphasized that some of these animals metamorphose spontaneously. All of the animals were anaesthetized in MS-222 prior to bleeding. In most cases blood was obtained by exposing the heart after opening the pericardium and cutting the sinus venosus. The blood was then drained into a vial. In some animals a 27 guage needle was thrust into the truncus and a quantity of blood was withdrawn into a syringe. In all cases a minimum of one cc of blood was obtained. These samples were analyzed by thin layer chromatography (TLC) according to previously described methods (Milstein and Thomas, 1966, Prahlad, 1968).

RESULTS

The technique used in the present investigation is capable of detecting the following fractions of iodinated

tyrosines and thyronines, from the point of origin to the solvent front: (1) Diiodotyrosine (at the point of origin (0)). (2) Monoiodotyrosine (T_1). (3) Free iodide (I). (4) Thyroxine (T_4). (5) Diiodothyronine (T_2) or triiodothyronine (T_3). (6) An unidentified fraction moving close to the solvent front. According to the investigations of Milstein and Thomas (1966), the TLC method is sensitive to approximately 0.1 μg of DIT, T_1, T_3 and T_4. The results of the current studies are shown in Figure 1. It is seen that the predominant fraction in Amphiuma and Ambystoma is T_1. This compound is detectable quite easily in late tadpole stages. All of the animals except Amphiuma show varying concentrations of I. T_4 is clearly visible in late tadpole, adult Rana pipiens and Xenopus laevis. T_2 is seen only in late tadpole. The absence of T_2 in the early tadpole

Thus, it is seen from the above observations that three urodeles, *A. mexicanum, A. tigrinum* and *Amphiuma means,* show similar patterns is enigmatic. No attempt was made to identify the fractions moving with the solvent front.

even though the three differ with respect to their life history and response to thyroid hormones. *Amphiuma means* is incapable of metamorphosis either natural or induced. *A. mexicanum* does not metamorphose spontaneously but is capable of responding to exogenous thyroid hormones (T_4 or T_3 or T_2). Even though *A. tigrinum* is capable of spontaneous metamorphosis no T_2 or T_3 or T_4 was detected within the limits of sensitivity of the technique used. *Necturus* maculosus, which is supposed to be permanently larval like *Amphiuma,* resembles the early bullfrog tadpole (an anuran) in its pattern of circulating thyroid hormones. It may be pertinent to point out that the postmetamorphic forms of both *A. tigrinum* and *mexicanum* show sloughing of skin frequently.

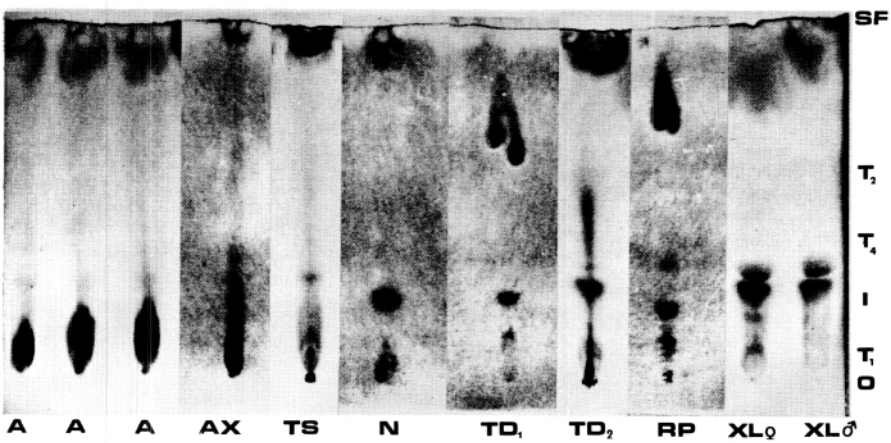

FIGURE 1. Thin layer chromatogram of butanol extracts of blood of Amphibians. From left to right on bottom: A-Amphiuma means. AX-Ambystoma mexicanum. TS-Ambystoma tigrinum. N-Necturus TD_1-young (no hind limbs but with limb buds) bullfrog tadpole. TD_2-older (small hind limb) bullfrog tadpole. RP-Rana pipiens. XL-Xenopus laevis. See the text for the rest of the abbreviations.

TABLE 1.—Amphibians used in the Present Investigation

Group	Genus and Species	Blood Obtained from Larvae or Adults	Metamorphic Status	Response to T_3 or T_4
Urodele	Amphiuma means (2)[1]	Larva	Permanently larval[2]	No metamorphosis
	Necturus maculosus (4)	Larva	" "	No metamorphosis
	Ambystoma mexicanum (6)	Larva	Permanently larval[2]	Metamorphoses
	Ambystoma tigrinum (3)	Adult	Temporarily larval[2] Metamorphoses Spontaneously	Metamorphoses
Anuran	Rana catesbeiana (6)	Tadpole	Temporarily larval Metamorphoses Spontaneously	Metamorphoses
	Rana pipiens (4)	Adult	....	....
	Xenopus laevis (4) ♀ and ♂	Adult	....	....

[1] Numbers in Parenthesis indicate the number of animals.
[2] Dent (1968).

Although this response has been clearly shown to be under the influence of thyroxine in *A. mexicanum* (Prahlad 1968) no T_4 was detected in the blood. Any explanation as to the significance of the present observations would only be speculative. Further investigations may clarify these enigmatic similarities and differences.

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