BIOCHEMICAL ACTIONS OF HORMONES

Edited by Gerald Litwack

Volume I

Biochemical Actions of Hormones

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Preface

This collection of papers by researchers in the field of hormone action surveys the significant developments in our progress toward understanding the primary effects of hormones in cellular receptors at the molecular level. During the last six years, there have been enormous developments in this field. The extent of progress is reflected in the size of this two-volume work. An advantage in having two volumes is the prompt publication in Volume I of those manuscripts completed at an early date, an important consideration in a rapidly expanding area of research.

Some informational overlap between contributions was unavoidable, but, hopefully, has been held to a minimum. It seemed more sensible to tolerate a small degree of redundancy than to tamper with cohesiveness. There are certain areas in which relatively little progress has been made. Accordingly, a few gaps in coverage will be evident, such as the absence of a contribution on intestinal hormones.

The coverage is broad enough to make this work useful as a modern reference text for the endocrinologist. In many cases, new data from the contributors' laboratories are presented. Thus, the purpose of these two volumes is to provide in one source an up-to-date survey of molecular and biochemical approaches bearing on the problem of hormone mechanism.

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Carlos O. Miller

CHAPTER 1

Hormonal Responses in Amphibian Metamorphosis

Earl Frieden and John J. Just

I. INTRODUCTION[1]

Amphibian metamorphosis is a postembryonic developmental process in which nonreproductive structures and organ systems of the amphibian larva change drastically to an adult form during a relatively brief and discrete period. The dramatic nature of the morphological transformations during this transition has excited biologists since the beginning of this century. The basic hormonal controls of this process were established by Gudernatsch, Allen, and others in the period 1912 to 1930. Our knowledge of the chemical and molecular changes associated with the biological transitions of amphibian metamorphosis is of a much more recent vintage. The process of metamorphosis is one of the classic examples of differentiation and of comparative and developmental biochemistry. In many of the amphibians there is a remarkable physiological adaptation during the transformation of an aquatic larva to a terrestrial adult. It also illustrates one of the most dramatic effects of the thyroid hormone, or any hormone for that matter. The rapid changes in function and in some cell types frequently have been compared to changes occurring in tumor cells. Much of our current knowledge in this field has been summarized in two recent volumes, one devoted principally to amphibians, edited by Moore (1964) and a second devoted to metamorphosis, edited by Etkin and Gilbert (1968). Numerous additional

[1] In addition to the standard abbreviations used for amino acids, nucleotides, and polynucleotides, the following more specialized abbreviations are used in this review: A. A., amino acid; Act D, actinomycin D; CM, carboxymethyl; GC ratio, guanylic acid to cytidylic acid ratio; Hb(s), hemoglobin(s); i-T_2, L-3′-isopropyl-3,5-diiodothyronine; LMC, lateral motor column; M-cells, Mauthner's cells; mRNA, messenger RNA; MSH, melanocyte-stimulating hormone; M-V, mesencephalic V nucleus; ppb, parts per billion; RBC(s), red blood cell(s); rRNA, ribosomal RNA; T_3, triiodothyronine; T_4, thyronine; TRH, thyrotropin-releasing hormone; tRNA, transfer RNA; TSH, thyroid-stimulating hormone.

reviews have appeared during the last decade (Bennett and Frieden, 1962; P. P. Cohen, 1966; Frieden, 1967; Weber, 1967a,b). Thus a significant literature characterizing the biological and biochemical aspects of this developmental phenomenon has accumulated in recent years.

Our objective in this chapter is to survey the metamorphic process in Amphibia using several recent reviews (Frieden, 1967, 1968; Weber, 1967a,b), as a point of reference. We attempted to include tissues that have been shown to respond directly to thyroid hormones or, conversely, which may not be under the sole control of the thyroid hormone, and finally, to bring up to date several of the very active areas of research, e.g., developmental changes in the liver, tail, etc.

In order to use various phases of the metamorphic process as points of reference in our discussion, the key morphological events are summarized in Fig. 1. Leg growth and tail regression are correlated with the stages of development and other significant structural changes. This description appears to fit *Rana pipiens* (leopard frog) and *Rana catesbeiana* (bullfrog) specifically and is probably representative of many anuran species. A comprehensive survey of the variation in amphibian life histories was published by Dent (1968). There are four distinct periods in the life history of amphibians: embryonic, larval, juvenile, and adult forms. In our treatment of hormonal responses during metamorphosis, we arbitrarily accept for discussion all changes that occur in the larval period, although we recognize that this inclusion may eventually prove to be too broad. Because of the availability of useful data principally on anuran metamorphosis, most of the discussion in this chapter necessarily emphasizes research on this group of amphibians.

A major source of interest in amphibian metamorphosis stems from its use as a model system for studying the mechanism of thyroid hormone action. Although there is no doubt that thyroid hormones play a dominant role in amphibian metamorphosis, it is likely that as for most vertebrates, amphibian larvae have an intricate system of endocrine interplay. Caution should be exercised in interpreting current data because few typical glandular ablative experiments combined with hormone replacement have been reported using amphibian larvae. Until such experiments, or adequate perfusion or tissue culture experiments are performed, it is not certain that the observed responses are indeed direct effects or are indirect effects triggered by the thyroid hormone.

Localized morphological changes associated with thyroid hormone implants were first reported 30 years ago, strongly suggesting a direct effect of thyroid hormone on certain anatomical responses (Hartwig, 1940; Kollros, 1942). These early observations have been extended to other morphological systems (see review of Kaltenbach, 1968). It should

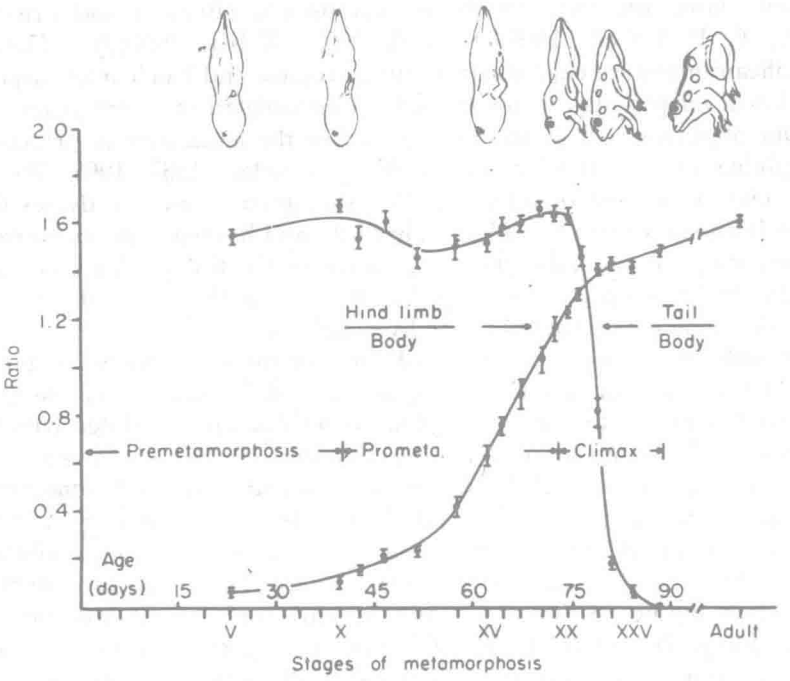

FIG. 1. Morphological events during spontaneous metamorphosis of *R. pipiens* larvae. The ages and stages are taken directly from A. C. Taylor and Kollros (1946). The illustrations depict several striking anatomical changes correlated with appropriate stages. The ratios represent the means of ten or more tadpoles at 19 ± 1° with the standard error indicated (Just, 1968). Pre-, prometamorphosis, and climax are as defined by Etkin (1964).

be emphasized that only a few of the biochemical changes that have been associated with metamorphosis have been shown to be directly affected by thyroid hormone.

II. INTERRELATIONSHIPS OF HORMONES DURING ANURAN METAMORPHOSIS

Although the major purpose of this chapter is to review the peripheral responses associated with amphibian metamorphosis, we will comment briefly on the endocrine relationships involved. Most of the previous work was concerned with demonstrating that the hypothalamus–pituitary–

thyroid axis was necessary for metamorphosis (see review by Etkin, 1968). Confirmation of this hypothesis by Etkin (1964) and Voitkevich (1962) led to further examination of the role of other hormones in the control of metamorphosis. The contributions of Bern, Etkin, and their co-workers implicated other pituitary hormones in amphibian development, an idea that had been inferred earlier from the effects of pituitary transplants. Berman *et al.* (1964) first demonstrated that prolactin promoted tadpole growth and retarded metamorphosis, and Bern *et al.* (1967) have summarized their view on endocrine relationships in the peripheral tissues. Numerous recent papers have established the following facts.

1. Mammalian prolactin and growth hormones accelerated growth rates in normal (Berman *et al.*, 1964; Remy and Bounhiol, 1965, 1966; Etkin and Gona, 1967) and in hypophysectomized tadpoles (Etkin and Gona, 1967; Just and Kollros, 1969).

2. Hypophysectomized tadpoles attain normal or larger than normal size, indicating that pituitary hormones are not essential for larval growth (Hanaoka, 1967; Just and Kollros, 1968).

3. Prolactin and growth hormone counteracted tail reduction (Bern *et al.*, 1967; Etkin and Gona, 1967) and inhibited urea excretion stimulated by thyroid hormones (Medda and Frieden, 1970). Prolactin did not inhibit the thyroxine-stimulated appearance of carbamyl phosphate synthetase, a liver urea cycle enzyme (Blatt *et al.*, 1969).

4. Prolactin also inhibits thyroid gland function in tadpoles (Gona, 1968).

Although these data suggest a possible role for prolactin and growth hormone in delaying the initiation of metamorphosis, it must be emphasized that neither hormone has been demonstrated in the tadpole pituitary or blood. The amounts of mammalian prolactin and growth hormone frequently used to produce the foregoing effects appear to be massive and nonphysiological. Furthermore, these hormones usually are administered to intact animals, and the observed effects could be caused by numerous hormonal interactions. Clearly, many decisive experiments remain to be done.

A hypothesis to account for the endocrine control of amphibian metamorphosis has been proposed by Etkin (1963). The postulated interaction of endocrine factors in determining the time and pattern of anuran metamorphosis is illustrated in Fig. 2. We quote from Etkin's most recent review in 1968:

> "In the early premetamorphic period the thyroxine level is very low and remains so until just before prometamorphosis begins. At

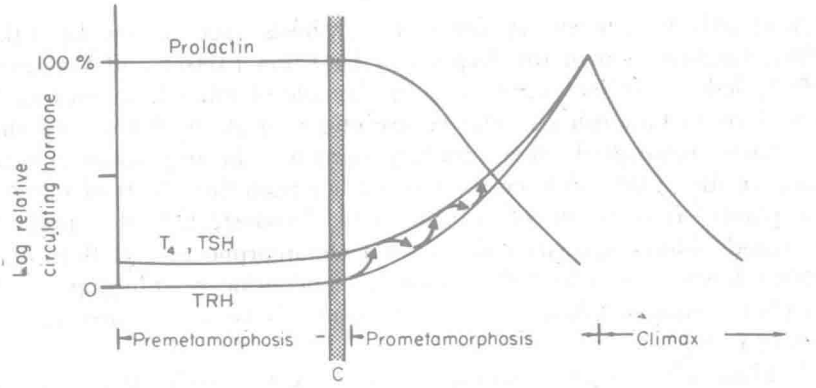

FIG. 2. Diagram of the relationships between the thyroid, pituitary, and hypothalamic hormones as proposed by Etkin (1968). C refers to the stage when the hypothalamus acquires capability for a response to thyroxine. TRH—thyrotrophin-releasing hormone; TSH—thyroid-stimulating hormone; T_4—thyroxine.

this time, the hypothalamic TRH mechanism becomes sensitive to positive thyroxine feedback, thereby initiating prometamorphosis. The increase in the TRH provoked by the action of the initial thyroxine level upon the hypothalamus stimulates increased TSH release, which acts back to raise the thyroxine level. This leads to a spiraling action which raises the thyroxine level and thereby induces prometamorphosis with its characteristic sequence of changes. The positive feedback cycle leads to maximal activation of the pituitary–thyroid axis, thereby bringing on metamorphic climax. During early premetamorphosis, prolactin is produced at a high rate. With the activation of the hypothalamus, the production of prolactin drops under the inhibitory influence of hypothalamic activity. As the level of TSH rises during prometamorphosis, that of prolactin decreases. The growth rate of the animal therefore falls, and the metamorphosis-restraining activity of prolactin diminishes. Thus the premetamorphic period in which growth is active and metamorphosis is inhibited is characterized by the predominance of prolactin over TSH. The reverse holds during metamorphosis. The time of shift in hormone balance is determined by the initiation of positive thyroid feedback to the hypothalamus. This varies greatly between species. The pattern of change during metamorphosis is regulated by the pattern of the feedback buildup and is much the same in most anurans."

This highly speculative proposal lacks confirmation at a number of key

points as follows: (1) circulating levels of thyroid hormones in the tadpole do not increase as described (Just, 1968); (2) the presence of prolactin and TRH in their respective glands or in the blood have not been demonstrated; and (3) TSH has not been identified in larval blood.

Evidence that appears inconsistent with Etkin's hypothesis includes (1) the lack of a requirement of the hypothalamus for metamorphosis in one species, *X. laevis* (Guardabassi, 1961); (2) the failure of mammalian TRH to induce metamorphosis (Etkin and Gona, 1968); and (3) the large difference in the half-lives in mammals of blood TSH (< 2 hours) and blood T_4 (20 hours) would probably not account for the correspondence of blood TSH and T_4 levels (Pittman and Shimizu, 1966; Odell *et al.*, 1967). Although this hypothesis or some modified form (Just, 1968) is valuable in that it serves as a basis for experimental design, we emphasize that it should not be accepted without further confirmation.

III. CHANGES IN BLOOD

A. Immune Response

The demonstration of immune responses by skin rejection has been made in numerous anurans (see Bovbjerg, 1966, for earlier references) and urodeles (N. Cohen, 1966; Charlemagne and Houillon, 1968), and many early investigators demonstrated allograft destruction of various tissues in larval amphibians. The immunological basis for graft rejection was shown by Hildemann and Haas (1959), who reported a significantly earlier rejection of second-set grafts, compared with first grafts.

There are indications that very young larvae do not have a fully developed immune response (Harris, 1941; Eakin and Harris, 1945; Hildemann, 1966; Bovbjerg, 1966). During the critical period of immune response maturation, the numbers of small lymphocytes have been found to be increased as much as tenfold (Hildemann and Haas, 1962). Antibody production in response to a variety of antigens has been demonstrated in larvae (Cooper *et al.*, 1964; Cooper and Hildemann, 1965a), and recently it was shown that lymphocytes and histocytes produce antibodies in amphibians (Du Pasquier, 1967).

Although little work has been done on the role of the thymus in the immune response in larval amphibians, the development of the thymus