AM. ZOOLOGIST, 10:201-216 (1970).

Endocrinology of the Amphibian Pineal

JOSEPH T. BAGNARA AND MAC E. HADLEY

Department of Biological Sciences, University of Arizona, Tucson, Arizona 85721

SYNOPSIS. McCord and Allen (1917) found that extracts of mammalian pineal glands contain a potent contracting agent of larval amphibian melanophores. Lerner and his co-workers determined the chemical structure of this principle and named it melatonin. This agent contracts dermal melanophores at a concentration as low as 10^{-10} g/ml. Both intact and eyeless larval amphibians blanch when placed in the dark, and the melanophore contraction which causes this lightening response is abolished by pinealectomy. The amphibian pineal contains photoreceptive elements similar to those found in the vertebrate lateral eyes, and these elements are inhibited by light but are stimulated in its absence. There is evidence for the presence of both HIOMT and melatonin in the amphibian pineal. It has been proposed that the body-blanching response results from a direct stimulation of the pineal under conditions of darkness leading to a release of melatonin into the general circulation which is then responsible for a direct contracting effect on dermal melanophores. The cytophysiological effects of melatonin mimic those that take place in the body-blanching response. Since no other hormone or pharmacological agent duplicates this response, this is strong evidence that melatonin is a hormone that normally regulates body blanching. Other evidence for the support of this hypothesis is presented.

Cytological features of both normal and melatonin-induced lightening indicate that the effects of melatonin are at the effector cell level rather than at either the hypothalamus or the pituitary. An inhibition of MSH-release by melatonin is not involved. Melatonin plays a normal role in young larvae to regulate the lightening response that takes place in darkness (the primary chromatic response). Neither melatonin nor the pineal play a role in the later (secondary stage) adaptive background responses of amphibians. As McCord and Allen first noted, the pineal may contain other substances which may have other physiological roles in amphibians as well as other vertebrates. These have been little studied.

In 1917, McCord and Allen observed that the feeding of desiccated beef pineal glands to anuran tadpoles resulted in a rapid and profound lightening in color of these larvae. This change in color resulted from the perinuclear aggregation of melanosomes within dermal melanophores, leading to the punctate state (contracted state) of the melanophore. The alteration in color was so dramatic that the integument of the tadpoles became transparent, allowing the intestines and other internal organs to become visible. The lightening response was rapid in onset and transient in duration but could be repeated with each feeding of pineal material. Feeding of other desiccated glandular materials was without such a pigmentary effect. While the substance responsible for the color change was extractable in acetone, the residue remaining after extraction with acetone was without effect on color change but did have an influence on growth and development. McCord and Allen (1917) concluded that the pineal gland contains more than one active substance.

Although the chromatic effects of pineal extracts on anuran larvae are quite dramatic, McCord and Allen (1917) concluded that "while the pineal does not act in the role of its ancient ocular function, it contains within itself an active principle capable of inducing pigment changes independent of and wholly apart from environmental conditions." These authors were so impressed by the convincing arguments of their contemporary, Henry Laurens, who considered the pineal not to be involved in control of melanophores (see below), that they apparently considered their observa-

Supported by grants GB-8347, GB-4923 and GB-3330 from the National Science Foundation.

tion to be of only pharmacological interest.

The possibility that the pineal might play a role in chromatic as well as other physiological regulations in vertebrates was revived by a number of observations implicating a functional photic role for the pineal and/or associated epithalamic areas (Scharrer, 1928; Young, 1935). The possibility was further strengthened by the electron microscopic observations of the reptilian pineal (parietal) eye which revealed the presence of photoreceptive elements similar to those present in the lateral eyes (Eakin and Westfall, 1959, 1960; Steyn, 1959, 1960). Lerner and his colleagues (Lerner, *et al.,* 1958) then isolated a specific pineal substance, which they identified and named melatonin, that was extremely potent in causing melanophore contraction. This strongly suggested that the pineal might be considered an endocrine organ, in addition to any sensory functions it might serve.

In this paper we will try to bring together the recorded literature on the amphibian pineal and try to show that this gland through both its photoreceptive and endocrine capabilities is able to regulate chromatic behavior in amphibians. In addition we will suggest that the pineal is important in regulating color only in early developmental stages. Certain phylogenetic implications arise from such a consideration and provide a basis for an understanding of the pineal's role in regulating amphibian chromatophores. Other studies on a possible role for the pineal in nonchromatic physiological regulations in amphibians are also to be discussed.

HISTORICAL BACKGROUND TO THE PROPOSED ROLE OF THE PINEAL AND MELATONIN REGULATION OF PIGMENT CELLS IN AMPHIBIANS

To appreciate a role for the pineal in the regulation of melanophore responses it is necessary to review the early experiments on melanophore regulation in larval amphibians. The melanophores of very young urodelan amphibians (salamanders) do not respond to light or darkness but remain expanded whether larvae are on a light- or a dark-colored background (*Ambystoma punctatum* and *A. opacum;* Laurens, 1915). Thus, young larvae are dark and are apparently unaffected by any environmental photic conditions to which they are subjected. At a later developmental stage, although larvae remain dark on any background over which they are kept, they do blanch (become transparent) when they are placed in darkness and become dark again when they are returned to illuminated conditions (Laurens, 1915, 1916). At a still later developmental stage, these larvae acquire the ability to background-adapt (become light or dark) under conditions of illumination, and they retain the ability to lighten when placed in darkness. They lose the ability to background-adapt when blinded and thus remain permanently dark under conditions of illumination, but these larvae blanch when placed in the dark.

Babak (1910) considered that in all stages of development melanophores are directly responsive to photic stimulation, but that at a later developmental stage the eye, through a central nervous system regulation from retina to melanophore, takes over control of melanophore responses by overriding the direct effects of light on the pigment cell. Extirpating the eyes was considered to remove such an overpowering control, and the melanophores were then, again, as at an earlier developmental stage, only regulated by direct photic influences. Laurens (1915), however, demonstrated that melanophores are insensitive to direct photic stimulation *in vitro,* and that melanophores remain expanded irrespective of whether light (daylight or artificial) is present or not. He still believed, however, that in the intact animal, the general response of melanophores is to expand when illuminated and to contract when in darkness. Laurens (1917) felt that when the melanophores come "under the influence of the nervous system" the reception

of light by the eyes results in a stimulus which, when animals are on a white background, is antagonistic to and reverses the direct expanding effect of light on melanophores. He provided no information as to how larvae could adapt to a black background, which also takes place under lighted conditions, and he admitted that he had no explanation of the mechanism by which melanophores contract in the dark. Fuchs (1914), however, had provided the following explanation. Substances, possibly inner secretions, produced by the ordinary processes of life are elaborated and these can cause contraction of melanophores, but only in the dark. Light stimulates the pineal, setting up an impulse which, in some unknown manner, inhibits contraction of melanophores. Thus, the pineal was considered to be actively stimulated by light. Accordingly, he discarded the direct photic regulation of melanophores, as suggested by Babak (1910), in favor of a form of humoral regulation, which could be inhibited through the intervention of light stimulating the pineal. At a later developmental state, the eyes, as in the other theories (Babak, 1910; Laurens, 1915, 1916, 1917), took on an overpowering control of melanophore regulation, in this case (Fuchs, 1914), by inhibiting the pineal. When the eyes were removed, the pineal was then again in complete control, as it is at an earlier developmental stage, and the melanophores expanded.

Fuchs (1914) was apparently influenced by the earlier work of von Frisch (1911) who had demonstrated that stimulation of the epithalamus of the minnow, *Phoxinus laevis* (= *Phoxinus phoxinus*), with light causes expansion of melanophores, whereas lack of such illumination results in melanophore contraction. Von Frisch (1911) demonstrated that stimulation of the epithalamus by light was not limited to the pineal organ, because this organ could be removed without interfering with the responses of the melanophores that followed from photic stimulation of the epithalamus. The fact that von Frisch suggested that the pineal organ might have an influence in other vertebrates probably led to such an acceptance by Fuchs (1914). Von Frisch (1911), himself, however, had obtained rather negative results in similar studies on urodeles (larval *Salamandra* and adult newts).

Laurens (1917), however, had become convinced that at later developmental stages only the eyes were involved in overriding the direct expanding effects of light on melanophores, and considered them to be entirely responsible for regulating both background responses and the blanching response that took place in darkness. He was able successfully to epiphysectomize later-stage larvae (demonstrated by histological observation), allowing him to perform experiments on both normal and eyeless larvae, with and without the epiphysis. In no case did these operations cause the chromatic responses of these larvae to differ from those of intact controls. Direct local illumination of the epiphysis while larvae were in the dark did not lead to expansion of melanophores. Of additional interest, was the observation that even larvae deprived of both the diencephalic roof and epiphysis were still able to background adapt and to blanch in the dark. Such responses could be prevented, however, by cutting the lateral walls of the diencephalon. These experiments led Laurens (1916, 1917) to refute the earlier suggestion of Fuchs (1914) that the epiphysis plays a role in the regulation of melanophore responses in larval urodeles.

Although these early studies left much unanswered about the mechanism of chromatic control in larval amphibians, they did reveal two important general observations: (1) the eyes are not needed for the body-lightening response which takes place in darkness, but (2) the eyes are necessary to regulate the lightening response that takes place in the light in adaptation to a light-colored background. Thus, two seemingly similar responses appear to be controlled by two quite different mechanisms. In addition, these early observations indicated that these two mechanisms of chromatic control are acquired sequentially in

development, with the intervention of a melanophore regulation through the retina as the later acquisition. Since a humoral control of melanophores was only beginning to be alluded to at this time, there was no suggestion that either the pineal, or the eyes, either released a hormonal factor or were instrumental in regulating the release of such a factor from some other source.

At this point in history, the role of the pineal in controlling pigmentation was not followed up, even though the observations of McCord and Allen (1917) on the potent lightening effects of pineal extracts on anuran larvae were well known. This may have been because the role of the pituitary (Smith, 1920) particularly the pars intermedia (Swingle, 1921), was just beginning to be implicated as the source of a humoral agent important, and indeed necessary, for control of chromatophores (Smith, 1920). Swingle did note, however, that there was a "possible relationship of the pars intermedia to the pineal gland in the production of pigmentation changes in anuran larvae." Implanting a reptilian (Chelonian) pineal into darkly pigmented tadpoles caused them to lighten rapidly as a result of melanophore contraction (Swingle, 1921). Similar results were obtained following the introduction of desiccated mammalian pineal material into the abdominal cavity. These lightening effects were dramatic, but transient.

Scharrer (1928) confirmed the earlier observations of von Frisch (1911) on the photic effects of epithalamic stimulation on color change in *P. phoxinus*. Young (1935) observed that the pallor occurring in larval lampreys when transferred from light to darkness was abolished after removing the pineal. Both Scharrer (1928) and Young (1935) suggested that the chromatic effects of photostimulation of the pineal were mediated through nerves, leading either through other neural connectives to innervate the melanophores directly (Scharrer, 1928) or else to the hypothalamus to regulate (inhibit) the release of a chromatophorotropic principle from the pituitary. Beall, *et al.* (1937) extended the earlier observations of McCord and Allen (1917) and showed that the melanophore-contracting substance present in beef and sheep pineals was an unsaturated nitrogenous base. Bors and Ralston (1951) next observed that pineal extracts of both pig and man induce melanophore contraction in larval and adult African clawed toads, *Xenopus laevis*. Lerner, *et al.* (1958) then isolated the potent melanophore-contracting agent from beef pineal glands, identifying it as melatonin (N-acetyl-5-methoxytryptamine). Thus, at last, there was identified a specific agent which might be considered important in regulating pigmentary events in the amphibian, as well as in other vertebrates, including man. It was yet to be established experimentally, however, whether this agent indeed played such a role.

PINEAL ROLE IN THE BODY-LIGHTENING RESPONSE OF LARVAL AMPHIBIANS

Although numerous workers had observed that larval amphibians and many other larval as well as adult poikilotherms blanched in darkness, no detailed observations had been made to fully investigate the temporal aspects of this response. As a result of the discovery that the tail-darkening reaction of *Xenopus laevis* tadpoles (Bagnara, 1957, 1960*a*), which occurs when larvae are placed in darkness, is mediated by the direct action of light on tail-fin melanophores, it was considered possible that a similar photochemical mechanism might mediate the body-lightening reaction. The temporal events in body lightening and subsequent recovery do not, however, seem to be consistent with a direct control of melanophores by a photochemical system (Figs. 1 and 2). Instead, they imply that a hormonal mechanism is involved with relatively rapid onset of melanophore contraction corresponding to release of a stored hormone and slow recovery concordant with gradual loss and/or breakdown of this principle from the circulation. With the implication of a

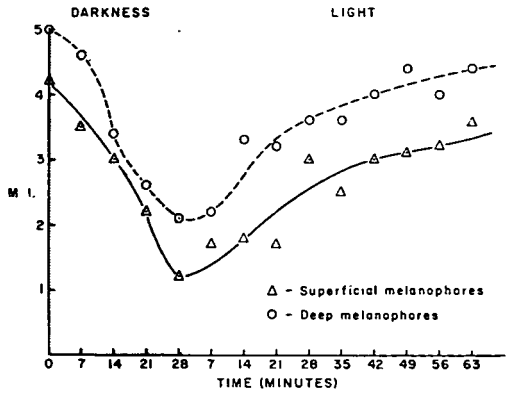

FIG. 1. Melanophore responses, expressed as a melanophore index (M.I.) (Hogben and Slome, 1931), of *Xenopus laevis* larvae (stages 48-49) during development of, and recovery from, the body-lightening response induced by a 28-minute exposure to darkness (shaded area). (From Bagnara, 1963).

hormonal mechanism in the body-blanching reaction, suspicion arose that possibly the melanophore-contracting principle of the pineal might be involved in the response. This suspicion grew even stronger because the paling reaction occurred when eyeless larvae were placed in darkness (Laurens, 1915, 1916, 1917; Bagnara, 1960*b*). It seemed possible, therefore, that the pineal might be the photoreceptor necessary for the paling reaction. Accordingly, a hypothesis was established which explained the mechanism of the body-blanching reaction completely in terms of two aspects of pineal physiology: photoreception and endocrine function. Briefly, the hypothesis states that when amphibian larvae are placed in darkness the pineal is stimulated by the absence of light, causing it to produce and release a melanophore-contracting agent, which is then directly responsible for mediating the blanching reaction. The first publication of this hypothesis (Bagnara, 1960*b*) included data showing that pinealectomized *Xenopus* larvae lack the ability to pale when placed in the dark.

Evidence that the pineal might release a hormonal agent which is responsible for the blanching reaction comes from several sources. The first direct experiments (Bagnara 1960*b*, 1961*a,b*, 1963) involved cautery of the diencephalic roof. Older *Xenopus* larvae "pinealectomized" in this manner consistently failed to blanch when placed in the dark. Similar results have been obtained by others working with larval urodeles (Brick, 1962; Kelly, 1962, 1963) and both larval and adult *Xenopus* (Charlton, 1966*a*). Such experiments as these, however, have been looked upon with some degree of skepticism (Kelly, 1962), particularly since the ability to blanch appears to return eventually despite the loss of the pineal. Laurens (1916) had similarly noted that epiphysectomy did not impair the chromatic responses of larval *A. punctatum*. As a check against the possibility of operative trauma, young larvae of *Xenopus* were deprived of their epiphysis at early embryonic stages. In order to prevent regeneration of the pineal, the whole top of the prosencephalon was removed. Such tadpoles were subsequently unable to blanch (Bagnara, 1963).

THE PINEAL AS PHOTORECEPTOR

A salient feature of the photoreceptive aspect of the blanching reaction is that the response is triggered by darkness. Undoubtedly, this is one reason that Laurens (1916) discounted any influence of light on the pineal organ. He attempted to obtain an active melanophore response by

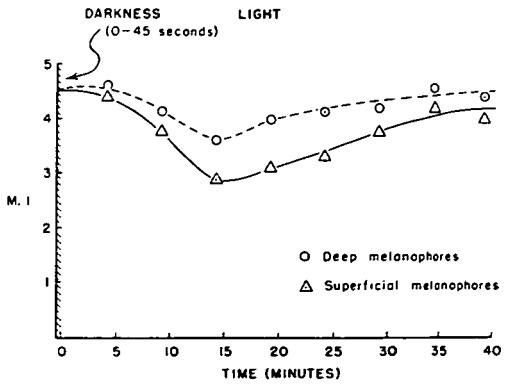

FIG. 2. Melanophore responses, expressed as in Figure 1. Body-lightening response induced by a 45-second exposure to darkness. (From Bagnara, 1963).

illuminating the pineal directly, as he considered that this structure should respond to a positive photostimulus. Recently, the concept that the pineal is activated by darkness has gained support from other experiments (Dodt and Heerd, 1962; Dodt and Jacobson, 1963). After removing the lateral eyes and the Stirnorgan, photic responses were recorded from a very localized region in the diencephalic roof of the frog's brain. Systematic exploration with a recording electrode indicated that the photic responses originated from the epiphyseal stalk. A sustained discharge of action potentials was recorded from the epiphyseal stalk in darkness. This activity was inhibited by light of all wavelengths.

Essential for a hypothesis that the pineal might be responsible for body blanching would be the presence of photoreceptors in some part of the pineal complex. Such structures have been found in amphibian pineal elements, resembling those found in retinas of the vertebrate lateral eyes (Kelly, 1962, 1963; Kelly and Smith, 1964; Eakin, *et al.*, 1963). In addition, the frontal organ (Stirnorgan, "brow spot"; Eakin, 1961; Eakin and Westfall, 1961) and its reptilian homologue, the parietal eye (Steyn, 1959, 1960; Eakin and Westfall, 1959, 1960) both possess cells which are structurally similar to those of photoreceptors of the retina of the lateral eye. Both organs are connected to the brain by a nerve or pineal tract that in the amphibian passes dorsally through the epiphysis. The photoreceptive elements within these organs appear to be functionally photoreceptive in that they are capable of discriminating wavelengths as determined by measurements of the electrical activity of the surface of the parietal eye itself (Hamasaki, 1968) or from the efferent nerves from the Stirnorgan (Dodt and Heerd, 1962). Whether these organs regulate either pineal or other brain function by way of direct efferent nervous pathways or by secretions is not known. Removing the Stirnorgan has no effect on the ability of either larval or adult frogs to background-adapt (Kleine, 1930; Stebbins, *et al.*, 1960; Charlton, 1966*a*) or to blanch in the dark (Eakin, 1961; Charlton, 1966*a*). Removing both the Stirnorgan and the eyes in tadpoles (*Hyla regilla*; Eakin, 1961), however, either eliminates or reduces the body-lightening response. It is of further interest that in *Xenopus* the skin above the frontal organ, unlike the pigmented adjacent integument, is unobscured by overlying dermal melanophores, and is, therefore, always accessible to light (Bagnara, 1961*a*; van de Kamer, *et al.*, 1962). Also, cytological observations have revealed some histochemical staining differences in frontal organs of frogs adapted to darkness compared to those of frogs adapted to light (Eakin, *et al.*, 1963).

ENDOCRINE ROLE OF THE PINEAL

All of the experiments described so far allude to mediation of the blanching reaction by a "pineal hormone." The identity of the specific hormone active in this response is unknown; however, it has been suggested (Bagnara, 1960, 1963) that it is melatonin. No hormone appears so likely a candidate as melatonin, whose physiological effects on melanophores fit all the prerequisites for the blanching reaction. In addition, the specific localization of melatonin within the pineal (Lerner, *et al.*, 1958), or other photoreceptors (see below), can be taken as very strong support of this suggestion.

Of direct importance is the fact that melatonin is a powerful melanophore-contracting agent. The minimal effective dose of melatonin required for contraction of melanophores in larval *Xenopus laevis* or *Rana pipiens* (Fig. 3) is 0.0001 μg per ml of water in which tadpoles swim (Bagnara, 1963; Hadley and Bagnara, 1969). Other agents so far tested (catecholamines, acetylcholine, hydrocortisone) are either ineffective, or are only effective at much higher concentrations. Both Quay and Bagnara (1964) and Quay (1968) have demonstrated a rather specific relationship between chemical structure and biological activity for indole derivatives in melano-

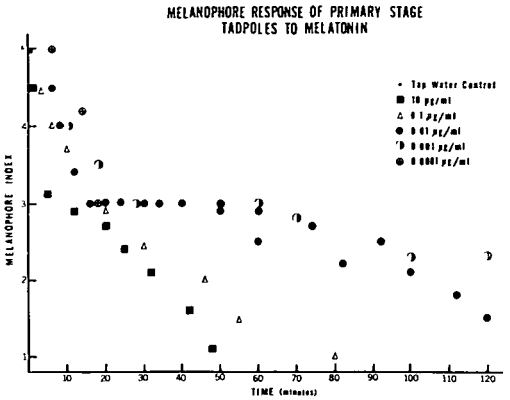

FIG. 3. Dermal melanophore responses of larval *Rana pipiens* to various concentrations of melatonin. Each point on the graph is the mean melanophore index of 15 or more tadpoles. (From Hadley and Bagnara, 1969).

phore contraction. On the basis of several observations, including the onset of body blanching during the presence of circulating MSH from the pituitary, the rapid onset of the blanching reaction, the relatively long period required for inactivating the "pineal hormone" during recovery from the blanching reaction, and the occurrence of the tail-darkening reaction concomitant with blanching, it seems that the natural melanophore-contracting agent is one that is active at a very low concentration. Melatonin certainly fulfills this requirement. It should be noted also that the character of pallor induced by melatonin is identical to that which occurs during normal blanching. Deep melanophores on blood vessels, nerves, and various organs, as well as those in the integument, contract markedly. Moreover, the temporal response of *Xenopus* melanophores to melatonin bears striking resemblance to the naturally occurring blanching reaction (Burgers and van Oordt, 1962).

There are other data which also indirectly support the pineal hypothesis. Charlton (1964, 1966*b*) demonstrated a somewhat preferential uptake of a radioactive product from radioactive ^{14}C 5-hydroxytryptamine creatinine sulfate by the epiphysis of young adult *Xenopus*. Since 5-hydroxytryptamine is apparently the precursor of melatonin, this might be taken as evidence for such a synthesis in the frog's pineal (Axelrod, *et al.*, 1965). Also, the enzyme responsible for this conversion, hydroxyindole-O-methyl transferase (HIO MT) has been found in the pineal gland (and the eyes) of both urodeles and anurans, as well as in the third eye of the lizard (Quay, 1965; Baker, *et al.*, 1965). By chromatographic methods followed by bioassay on larval *Xenopus* melanophores, van de Veerdonk (1967) demonstrated the presence of melatonin in the diencephalon of *Xenopus* larvae. It is interesting that photoreceptive elements in the pineal (Kelly, 1965; Bagnara, 1965) are present at an early developmental stage as is the presence of melatonin (as measured in whole embryos: Baker, 1969) and that the body-lightening response is also prominent at this early time (Bagnara, 1965).

Although the cytological studies of the amphibian pineal have clearly established a morphological basis for the photoreceptive function of the pineal, there has been less conclusive evidence for a secretory role (Kelly, 1962, 1963; Kelly and Smith 1964). However, histochemical and electron-microscopical observations of the pineal of *Xenopus* (Charlton, 1968) provided data that might support the suggestion of a secretory function, as have the earlier studies of others (Oksche and von Harnack, 1963; Eakin, *et al.*, 1963) on other anurans. There is yet, however, no direct evidence for the release of secretory materials from the amphibian pineal (Kelly, 1965).

SITE OF ACTION OF MELATONIN IN THE BODY-LIGHTENING RESPONSE

Although there is probably a general consensus of opinion that the body-blanching response results from the release of melatonin from the pineal under conditions of darkness, there have been a number of hypotheses as to the locus or site of action of melatonin (or other "pineal hormone") in this response. The body-lightening response has been considered to result either from: (1) the direct effect of

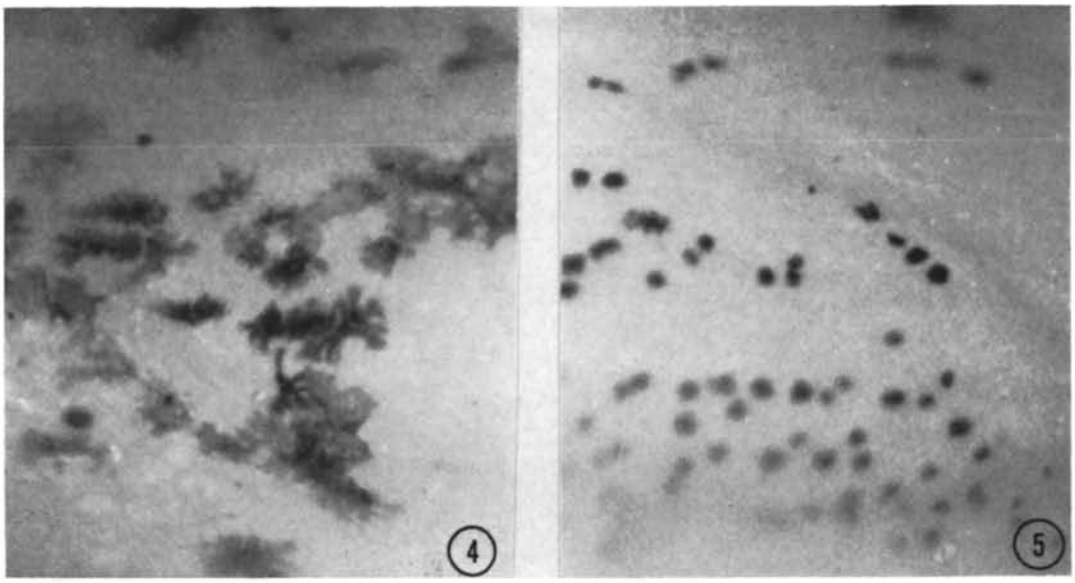

FIG. 4. Melanophores of hypophysectomized *Hyla arenicolor* larvae expand in response to MSH.
FIG. 5. These melanophores then contract when larvae are placed in darkness for one hour. (From Bagnara, 1964).

melatonin at the effector cell (chromatophore) level, or (2) from the inhibition by melatonin of MSH-release.

Experiments utilizing hypophysectomized tadpoles of both *Rana pipiens* and *Hyla arenicolor* show clearly that the control of the blanching reaction does not operate through the hypophysis (Bagnara, 1964). As a result of the lack of a pituitary chromatophorotropin in such larvae, melanophores are contracted and iridophores expanded. When hypophysectomized tadpoles are placed in darkness, melanophores, which are already contracted become even more contracted. A clearer picture of this reaction is obtained by immersing hypophysectomized tadpoles in water containing MSH; the melanophores expanded prominently. These MSH-darkened, hypophysectomized tadpoles blanch when they are placed in darkness, but the melanophores of other tadpoles left under normal illumination as controls remain expanded (Figs. 4 and 5). Clearly then, the mechanism of the blanching reaction in these experiments does not involve an inhibition of the release of a chromatophorotropic hormone from the hypophysis, for the reaction occurs perfectly well in its absence. These experiments demonstrate that in the blanching reaction of normal larvae, the "pineal hormone" overrides the melanophore-expanding stimulation of endogenous chromatophorotropic hormone, and does so *at the effector cell level.*

Further evidence to support such an interpretation comes from the observation that epidermal melanophores remain expanded during the body-lightening response of *Rana pipiens* tadpoles (Hooker, 1914; Hadley, 1966), whereas the dermal melanophores contract. Similarly, when tadpoles are immersed in melatonin all dermal melanophores contract, but epidermal melanophores remain expanded. These epidermal melanophores are expanded in the presence of MSH but do have the capacity to contract, and do so in its absence, as effected by hypophysectomy. Thus, the expanded condition of the epidermal melanophores during the body-lightening response is good evidence for maintained circulating levels of intermedin, for they would have contracted if

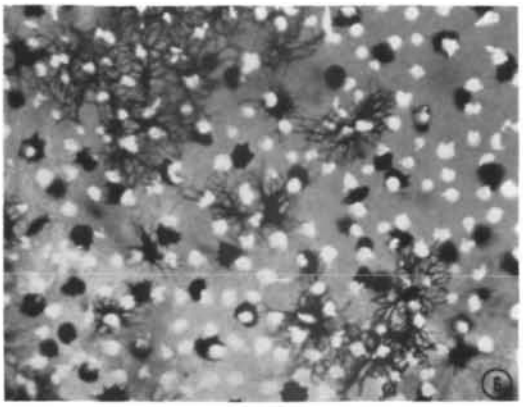

FIG. 6. Differential response of dermal chromatophores of frog skin to melatonin. Melanophores expand and iridophores (white-colored cells) contract in response to MSH. Melatonin contracts some dermal melanophores but is without effect on iridophores, all of which remain contracted. × 170. (From Hadley and Bagnara, 1969).

MSH-release had been inhibited during the response.

Additional support for such a suggestion is also available from studies on adult amphibians. Melatonin has no effect on iridophores or epidermal melanophores when injected into frogs adapted to a black background or on iridophores or epidermal melanophores of frog skins darkened by MSH, *in vitro* (Hadley, 1966; Hadley and Bagnara, 1969). The very minimal lightening effect of melatonin on frog skins, either *in vivo* or *in vitro,* is due solely to melanophore contraction occurring within dermal melanophores. Here, the maintenance of the expanded state of the epidermal melanophores and the contracted state of the iridophores indicates that the effects of intermedin still persist and are not antagonized by melatonin (Fig. 6). Since neither iridophores nor epidermal melanophores of either adult (Hadley and Bagnara, 1969) or larval amphibians (Bagnara, 1964; Hadley, 1966) are directly responsive to melatonin, *in vivo* or *in vitro,* the lightening effects of this agent, or other "pineal hormone" during the body-lightening response must be ascribed to their direct effects solely on dermal melanophores, rather than to any lightening resulting from an inhibition of MSH-release from the pituitary.

What is of great interest here is that the cytological events that normally take place in the dark mimic those that can be induced by melatonin. Since no other hormonal or pharmacological agent has such a differential reaction on amphibian pigment cells, this would seem very strong evidence, indeed, that melatonin is the normal hormonal factor in the body-lightening response. It is of historical interest to also note, and again record here, that McCord and Allen (1917) clearly demonstrated that the lightening effects of pineal extracts on tadpole melanophores were on dermal melanophores and not on epidermal melanophores (Figs. 7 and 8). Both Smith (1920) and Atwell (1921) confirmed the observations of McCord and Allen (1917), and in addition, they noted that although immersing tadpoles in pineal extracts caused contraction of dermal melanophores, the iridophores were unaffected and remained contracted. These early observations are consistent with our present observations today using pure melatonin (Bagnara and Hadley, 1969; Bagnara, *et al.*, 1969).

Notwithstanding these observations that adequately explain the site of action of melatonin in the body-blanching reaction of larval amphibians, a number of other pertinent hypotheses have been formulated. Both Brick (1962) and Charlton (1966*a*) have suggested that the blanching response might come from a pineal inhibition of pituitary release of MSH. The argument by Brick is apparently based on the observations that melanophores of pinealectomized larvae (*A. opacum*) are no more expanded than those of unoperated controls. In addition, melanophores of larvae which are both pinealectomized and hypophysectomized are no more expanded than those of hypophysectomized controls. Such arguments as these are valid only for an hypothesis wherein the pineal, through its direct effect on melanophores, exerts its effects when illuminated. Such a suggestion is inconsistent with past experi-

FIG. 7. Drawing of a whole mount of skin from a normal tadpole (*Rana pipiens*). The epidermal melanophores (Ep. M.) of the epidermis (Ep.) are expanded as are the sub-epidermal (dermal) melanophores (Sub. M.) of the integument (Int.). (From McCord and Allen, 1917).

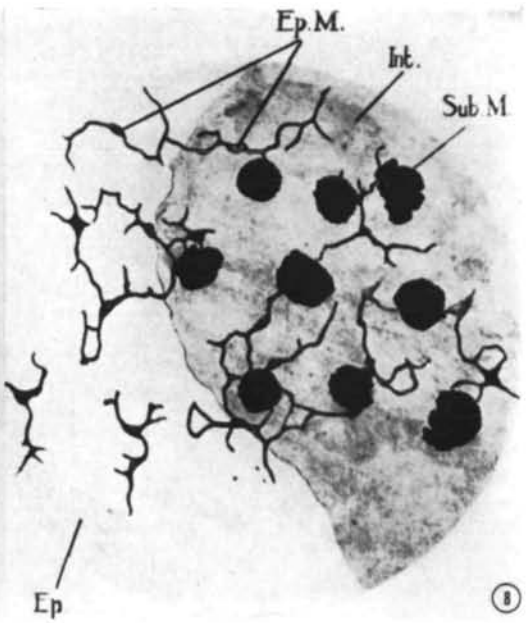

FIG. 8. Feeding pineal extracts to tadpoles causes a rapid blanching of the skin because the dermal melanophores contract. The epidermal melanophores are unresponsive and remain expanded. Compare with Figure 7. (From McCord and Allen, 1917).

mental evidence as well as with Brick's own experimental results. Thus, this interpretation of the experimental data led to an alternate hypothesis, that the effects of the pineal are mediated at the pituitary level rather than at the effector-cell level. Historically, such reasoning had its precedent in a similar hypothesis proposed by Young (1935) to explain the role of the pineal in chromatic regulation in cyclostomes.

According to Charlton (1966*a*), the release of melatonin from the pineal inhibits release of MSH from the pituitary, and in addition directly causes contraction of melanophores. Melatonin was considered to inhibit the release of MSH by either causing the release of an MSH-inhibiting factor from the hypothalamus or by activating inhibitory neurons innervating the pars intermedia. Such a hypothesis, however, is without any supporting experimental data and is incompatible with the cytological features of the lightening response as described earlier. An essential feature of the interpretation of experimental results that led to the formulation of this hypothesis by Charlton (1966*a*) was that melatonin could not cross the blood-brain barrier in vertebrates. No evidence was presented, however, that there is such a brain barrier to melatonin. Moreover, it has been indicated recently that melatonin can easily gain access to the brain (Wurtman and Anton-Tay, 1969).

ROLE OF THE PINEAL AND THE LATERAL EYES IN THE PRIMARY AND SECONDARY STAGES OF CHROMATIC REGULATION

During the ontogeny of most amphibians, the young larvae pass through a primary stage of chromatic control which at later developmental stages is succeeded by a secondary stage (Parker, 1948). In the primary stage, larvae are dark in the light and become pale or transparent in the dark. During this stage, the eyes are said to be nonfunctional (Prosser and Brown, 1961) or, as better stated (Waring, 1942),

the eyes are not the receptor for the primary response. The control of integumental coloration during the secondary stage involves information received through the eyes (Parker, 1948). Although the primary response may still be potentially operative, it is apparently masked by control mechanisms of the secondary stage. When blinded, larvae of the secondary stage have been thought to lapse into a condition approximating the primary stage (Babak, 1910). According to Parker (1948), no clear explanation has been given for either the biological significance of the primary color phase or the physiological basis for the establishment of these two stages of chromatic control. Our experimental observations explain in physiological terms the control mechanisms involved in the regulation of the primary stage and the ensuing developmental transition to the secondary stage (Hadley, 1966).

The body-lightening response of tadpoles in the primary stage apparently involves release of melatonin from the pineal gland (Bagnara, 1960*b*). Melatonin exerts its lightening effect directly on the dermal melanophores by overriding the melanophore-expanding effect of circulating MSH. This body-lightening response does not inhibit the release of MSH from the pituitary. Tadpoles in the primary stage fail to adapt to a white background, not because they cannot see that background, but, rather, because there is apparently no mechanism for inhibiting release of MSH from the pituitary gland during this early stage of chromatic control. When larvae are placed in darkness, paling does take place, and this is effected by the release of melatonin into the circulation. Pinealectomized tadpoles, therefore, no longer have any means of regulating integumentary color changes.

Apparently, the pituitary comes under a control mechanism wherein the release of MSH is inhibited when tadpoles in the secondary stage or metamorphosed frogs are placed on a white background. The transition from the primary stage involves, therefore, the gradual to complete acquisition of a means of inhibiting the release of MSH from the hypophysis. The transition from the primary stage to the secondary stage may involve either the development of background perception through the eyes, or, more likely, the development of a means of inhibiting the release of MSH from the pituitary when larvae are placed over a white background. In either case, the eye, rather than the pineal, is now the initial photoreceptor for the regulation of changes in color.

It is only within the Amphibia that thorough studies on the physiological significance of the primary and secondary stage have been undertaken. Here, it is clear that the pineal is the important photoreceptor in chromatic regulation during the primary stage in which there is an inability to inhibit MSH-release; during this stage the pineal hormone, melatonin, appears to play a role in chromatic responses. The developmental stage at which individuals pass from the primary to the secondary stage of chromatic control appears to differ greatly between species. *Xenopus laevis* is able to background-adapt almost from the time that pigment cells first appear. *Rana pipiens* does not develop the ability to background-adapt until about half way through its larval development. In some species of toads (*Bufo*), there is complete inability to background-adapt during the tadpole's life.

Of further interest, the cyclostomes, the phylogenetically oldest vertebrate group, are unable to background-adapt. They are permanently in the primary stage, and the pineal, or related complex, is apparently the only regulatory mechanism responsible for diurnal color change (Young, 1935). It is possible that in cyclostomes there had not yet evolved a mechanism for inhibiting MSH from the pars intermedia. The change from the primary stage to the secondary stage of chromatic regulation involved a switch from the pineal as initial photoreceptor to the lateral eyes as photoreceptors. In addition, this involved a switch from a direct hormonal control of chromatophores at the effector-cell level to

an indirect control involving regulation of the release of MSH by the pars intermedia.

Melatonin is considered the most potent melanophore-contracting agent known; it is said to be 10^5 times as effective as norepinephrine in lightening adult frog skin *in vitro* (Lande and Lerner, 1967; Lerner and Case, 1959). Such statements, unfortunately, may have led others not only to believe that this agent is indeed a very powerful lightening agent of adult frog skin but to the further extension of thought that this agent might also be involved in the normal, *in vivo,* mechanism of skin-lightening which takes place in the adaptive response of frogs to a light-colored background. Several observations indicate that this is clearly not the case. Injections of melatonin into black-adapted frogs (*R. pipiens)* are quite ineffective in causing skin-lightening in this species (Hadley, 1966). Other investigators also noted only a minimal lightening when melatonin is injected into *R. pipiens* (Kastin and Schally, 1966). These *in vivo* observations are consistent with *in vitro* results showing that melatonin has only a slight effect in lightening frog skins (Hadley and Bagnara, 1969). Similar *in vitro* studies on over a dozen other species of adult frogs have shown that melatonin is quite ineffective in lightening skins by antagonizing or reversing the melanosome-dispersing action of MSH. These negative results are quite remarkable in light of the melanophore-contracting action of melatonin on dermal melanophores of larval amphibians (Bagnara, 1960*b,* 1964; Burgers and van Oordt, 1962). These results, however, are reminiscent of the original observations on *R. pipiens* by McCord and Allen (1917) who showed that pineal extracts have a powerful melanophore-contracting effect on dermal melanophores of tadpoles. McCord and Allen also noted that the melanophores of recently metamorphosed *R. pipiens* tadpoles were no longer sensitive to the pineal extracts.

Numerous investigators (McCord and Allen, 1917; Bors and Ralston, 1951; Hadley, 1966; Charlton, 1966*a)* have noted that the melanophore-contracting effects of melatonin can be quite transient. This is true only for its lightening effects on melanophores of older larval or adult anurans. It is interesting, therefore, that this loss of sensitivity of melanophores to melatonin is a developmental phenomenon and coincides with the change from a pineal to a retinal control of melanophore responses, which is a switch from a direct hormonal regulation of melanophores by melatonin to an indirect control by a retinal inhibition of MSH-release.

Although there is strong evidence that melatonin may play a natural role in the control of chromatic changes (responses of dermal melanophores during the body-blanching response) in young larval amphibians (Bagnara, 1960*b*), no such role has been demonstrated for adult amphibians. Bogenschutz (1967) found that epiphysectomized adult *Rana esculenta* still become pale when transferred from a black to a white background. Histological examination of the midbrain confirmed that the pineal was entirely destroyed and no regeneration had taken place. These results appear to rule out further a role for the pineal in chromatic adaptations to background in older anurans.

QUESTIONABLE ASPECTS OF A PINEAL ROLE IN CONTROL OF CHROMATOPHORES

A number of problems remain to be answered about the proposed endocrine role of the amphibian pineal. Most important, one would like to be able to demonstrate not only circulating melatonin but to show that its level varies in accordance with the body-lightening response. This would clearly establish whether or not melatonin can be considered a hormone in chromatophoric control. The demonstration by Quay (1965) of HIOMT, an enzyme responsible for forming melatonin, in the lateral eyes of amphibians (and other vertebrates) raises the question whether melatonin is released from these photoreceptors and might play a regulatory role in am-

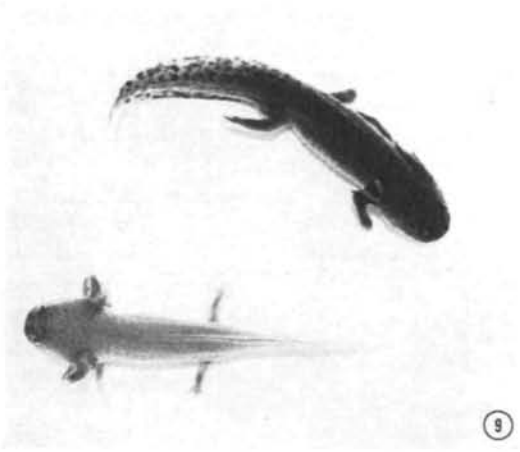

FIG. 9. A single injection of melatonin into the axolotl, *A. mexicanum,* causes a profound blanching which contrasts with the color of a control animal injected only with saline. This blanching response appears to be semi-permanent, at least in some individuals.

phibian pigmentation. The early observations of Laurens (1916) that urodelan larvae can still blanch in the dark after epiphysectomy might indicate some additional photic mechanism of chromatophoric control other than one mediated exclusively through the pineal.

Although we have emphasized that neither the pineal nor melatonin participates in the control of the secondary chromatic responses, it is premature to exclude the possibility that the pineal of adult frogs may play other roles (even pigmentary) during conditions of darkness. Recent electrophysiological studies (Oshima and Gorbman, 1969*a,b*) suggest such a possibility.

Although the pineal does not regulate color change in either larval or adult anurans by inhibiting the release of MSH, there is some evidence that melatonin may do so in certain species of mammals, and thus affect pelage coloration (Rust and Meyer, 1969). In other mammals (Snell, 1965), and man (Lerner, 1961), melatonin was ineffective in affecting pigmentary events. It would appear from the literature that there are great differences in species sensitivity to melatonin. It is interesting, therefore, that melatonin injected into the black axolotl (*A. mexicanum*) can have a dramatic lightening effect on adult individuals. Melatonin causes a maximal contraction of all melanophores, causing the animal to change from black to pink (Fig. 9). Of greatest interest, the effect appears to be semi-permanent; we have noted individuals to be maximally light in color two months after a single injection of melatonin. We can presently only account for these lasting effects by assuming that melatonin has permanently inhibited release of MSH. Similar concentrations of melatonin injected into the closely related salamander, *A. tigrinum,* are totally without such a lightening effect. Further, *A. mexicanum* is incapable of background-adaptation (the animals are always black), whereas *A. tigrinum* adapts readily.

OTHER POSSIBLE ENDOCRINE FUNCTIONS OF THE AMPHIBIAN PINEAL

In addition to discovering a potent melanophore-contracting substance in pineal extracts, McCord and Allen (1917) found that the residue contained a growth-stimulating principle. Tadpoles fed for two weeks on pineal tissue became twice the size of controls but showed no signs of differentiation. McCord (1914) claimed that small amounts of pineal material administered to young animals (chicks, dogs, guinea pigs) stimulated rapid growth of the body, but not beyond normal size. Addair and Chidester (1928) repeated the experiments on tadpoles and found that instead of a gain in weight, as reported by McCord and Allen (1917), the feeding of pineal glands hastened the rate of metamorphosis, accompanied by a loss in weight. Removing the pineal of tadpoles (*Alytes obstetricans*) stimulated the rate of development, which correlated histologically with a very active thyroid. It is difficult to draw any general conclusions from these experiments, as the results of different workers seem to be inconsistent with each other. Obviously, further studies are needed.

Kelly (1958) found that after discrete epiphysectomy of the salamander (*Taricha*

torosa) that there was normal growth, activity, and metamorphosis. As in earlier studies, there developed a post-metamorphic scoliosis, but it was concluded that this condition was not due specifically to absence of or damage to the epiphysis, but rather, possibly to a damage of some higher motor area of the central nervous system.

Injections of pineal homogenates from adult cows or female calves into the frog (*Rana esculenta*) inhibited the spermatogenic response to chorionic gonadotropin (Juszkiewicz and Rakalska, 1963, 1965). Homogenates from male calves were without effect. Because this spermatogenic inhibition could not be duplicated by injecting melatonin (Juszkiewicz and Rakalska, 1965), some other unidentified pineal component was considered to be the active factor.

It is possible that the pineal plays a role in muscular contraction. McCord and Allen (1917) found that pineal extracts "produced a typical though feeble" contraction of guinea-pig uterine muscle. Moreover, Morgalit and Rahamimoff (1965) found that melatonin inhibited spontaneous and serotonin-induced contractions of the estrogenized rat uterus. In this context, we have observed (unpublished) that amphibian larvae defecate when immersed in melatonin solutions; possibly muscular contraction is involved. It has also been observed that melatonin decreased the *in vitro* excitability of the frog's sciatic nerve (Rahamimoff; cited in Morgalit and Rahamimoff, 1965). Of further significance, it is known that melatonin causes a rapid contraction of retinal cones in larval *Xenopus laevis* (Quay and McLeod, 1968). Injections of melatonin into the adult frog (*Rana temporaria*) lead to a retraction of melanosomes within the retinal pigment cells (melanophores), and melatonin has a similar retinal melanophore-contracting effect on isolated eyes *in vitro* (Kraus-Ruppert and Lembeck, 1965). Since hydroxyindole-O-methyl transferase has been localized within a number of photic structures (Quay, 1965) and is apparently responsible for the synthesis of melatonin therein, it might be concluded that the effects of melatonin on retinal structures represent a phylogenetically early and localized role for this indole. The ability of melatonin to have systemic effects on other contractile events, in muscle or melanophores, may represent a later evolutionary regulatory role of this hormone.

REFERENCES

Addair, J., and F. E. Chidester. 1928. Pineal and metamorphosis: the influence of pineal feeding upon the rate of metamorphosis in frogs. Endocrinology 12:791-796.

Atwell, W. J. 1921. Further observations on the pigment changes following removal of the epithelial hypophysis and the pineal gland in the frog tadpole. Endocrinology 5:221-232.

Axelrod, J., W. B. Quay, and P. C. Baker. 1965. Enzymatic synthesis of the skin-lightening agent, melatonin, in amphibians. Nature 208:386.

Babak, E. 1910. Zur chromatischen Hautfunktion der Amphibien, Pflüger's Arch. Gesamte Physiol. 131:87-118.

Bagnara, J. T. 1957. Hypophysectomy and the tail-darkening reaction in *Xenopus*. Proc. Soc. Exp. Biol. Med. 94:572-575.

Bagnara, J. T. 1960*a*. Tail melanophores of *Xenopus* in normal development and regeneration. Biol. Bull. 118:1-8.

Bagnara, J. T. 1960*b*. Pineal regulation of the body lightening reaction in amphibian larvae. Science 132:1481-1483.

Bagnara, J. T. 1961*a*. The pineal gland and body lightening in *Xenopus* larvae. Anat. Rec. 139:204.

Bagnara, J. T. 1961*b*. Onset of pineal and hypophyseal regulation of melanophores in *Xenopus*. Amer. Zoologist 1:339-340.

Bagnara, J. T. 1963. The pineal and the body lightening reaction of larval amphibians. Gen. Comp. Endocrinol. 3:86-100.

Bagnara, J. T. 1964. Independent actions of pineal and hypophysis in the regulation of chromatophores of anuran larvae. Gen. Comp. Endocrinol. 4:299-303.

Bagnara, J. T. 1965. Pineal regulation of body blanching in amphibian larvae. Progr. Brain Res. 10:489-506.

Bagnara, J. T., and M. E. Hadley. 1969. The control of bright colored pigment cells of fishes and amphibians. Amer. Zoologist 9:465-478.

Bagnara, J. T., M. E. Hadley, and J. D. Taylor. 1969. Regulation of bright colored pigmentation of amphibians. Gen. Comp. Endocrinol. Suppl. 2:425-438.

Baker, P. C. 1969. Melatonin levels in developing *Xenopus laevis*. Comp. Biochem. Physiol. 28: 1387-1393.

Baker, P. C., W. B. Quay, and J. Axelrod. 1965.

Development of hydroxyindole-O-methyl transferase activity in the eye and brain of the amphibian, *Xenopus laevis*. Life Sci. 4:1981-1987.

Beall, D., H. A. Shapiro, and H. Zwarenstein. 1937. The melanophore contracting principle of the pineal. Chem. Ind. (London) 56:190.

Bogenschütz, H. 1967. Über den Farbwechsel von *Rana esculenta* nach Epiphysektomie. Experientia 23:967-968.

Bors, O., and W. C. Ralston. 1951. A simple assay of mammalian pineal extracts. Proc. Soc. Exp. Biol. Med. 77:807-808.

Brick, I. 1962. Relationship of the pineal to the pituitary-melanophore effector system in *Amblystoma opacum*. Anat. Rec. 142:229.

Burgers, A. C. J., and G. J. van Oordt, 1962. Regulation of pigment migration in the amphibian melanophore. Gen. Comp. Endocrinol. Suppl. 1:99-109.

Charlton, H. M. 1964. Uptake of labelled precursors of melatonin by the epiphysis of *Xenopus laevis*. Nature 204:1093-1094.

Charlton, H. M. 1966*a*. The pineal gland and colour change in *Xenopus laevis* Daudin. Gen. Comp. Endocrinol. 7:384-397.

Charlton, H. M. 1966*b*. The uptake of ^{14}C 5-hydroxytryptamine creatine sulfate and ^{14}C methylmethionine by the epiphysis of *Xenopus laevis* Daudin. Comp. Biochem. Physiol. 17:777-784.

Charlton, H. M. 1968. The pineal gland of *Xenopus laevis*, Daudin: a histological, histochemical, and electron microscopic study. Gen. Comp. Endocrinol. 11:465-480.

Dodt, E., and E. Heerd. 1962. Mode of action of pineal nerve fibers in frogs. J. Neurophysiol. 25:405-429.

Dodt, E., and M. Jacobson. 1963. Photosensitivity of a localized region of the frog diencephalon. J. Neurophysiol. 26:752-758.

Eakin, R. M. 1961. Photoreceptors in the amphibian frontal organ. Proc. Nat. Acad. Sci. 47:1084-1088.

Eakin, R. M., and J. A. Westfall. 1959. Fine structure of the retina in the reptilian third eye. J. Biophys. Biochem. Cytol. 6:133-134.

Eakin, R. M., and J. A. Westfall. 1960. Further observations on the fine structure of the parietal eye of lizards. Biophys. Biochem. Cytol. 8:483-501.

Eakin, R. M., and J. A. Westfall. 1961. The development of photoreceptors in the stirnorgan of the treefrog, *Hyla regilla*. Embryologia 6:84-98.

Eakin, R. M., W. B. Quay, and J. A. Westfall. 1963. Cytological and cytochemical studies on the frontal and pineal organs of the tree frog, *Hyla regilla*. Z. Zellforsch. 59:663-683.

Fuchs, R. F. 1914. Der Farbenwechsel und die chromatische Hautfunktion der Tiere. T. Winterstein, Handb. Vergl. Physiol. 3:1189-1657.

Hadley, M. E. 1966. Cytophysiological studies on the chromatophores of *Rana pipiens*. Ph.D. Thesis, Brown University, University Microfilms, Ann Arbor, Mich.

Hadley, M. E., and J. T. Bagnara. 1969. Integrated nature of chromatophore responses in the *in vitro* frog skin bioassay. Endocrinology 84.69-82.

Hamasaki, D. I. 1968. Properties of the parietal eye of the green iguana. Vision Res. 8:591-599.

Hogben, L., and D. Slome. 1931. The pigmentary effector system. VI. The dual character of endocrine co-ordination in amphibian colour change. Proc. Roy. Soc., B, 108:10-53.

Hooker, D. 1914. The reactions of light and darkness on the melanophores of frog tadpoles. Science 39:473.

Juszkiewicz, T., and Z. Rakalska. 1963. Anti-oestrogenic effects of bovine pineal glands. Nature 200:1329-1330.

Juszkiewicz, T., and Z. Rakalska. 1965. Lack of the effect of melatonin on the frog spermatogenic reaction. J. Pharm. Pharmacol. 17:189-190.

Kastin, A. J., and A. V. Schally. 1966. *In vivo* assay for melanocyte lightening substances. Experientia 22:389.

Kelly, D. E. 1958. Embryonic and larval epiphysectomy in the salamander, *Taricha torosa*, and observations on scoliosis. J. Morphol. 103:503-538.

Kelly, D. E. 1962. Pineal organs: Photoreception, secretion, and development. Amer. Scientist 50: 597-625.

Kelly, D. E. 1963. The pineal organ of the newt, a developmental study. Z. Zellforsch. 58:693-713.

Kelly, D. E. 1965. Ultrastructure and development of amphibian pineal organs. Progr. Brain Res. 10:270-287.

Kelly, D. E., and S. W. Smith. 1964. Fine structure of the pineal organs of the adult frog, *Rana pipiens*. J. Cell Biol. 22:653-674.

Kleine, A. 1930. Über die Parietalorgane bei einheimischen und ausländischen Anuran. Jena L. Med. Naturwiss. 64:339-376.

Kraus-Ruppert, R., and F. Lembeck. 1965. Die Wirkung von Melatonin auf die Pigmentzellen der Retina von Fröschen. Pflüger's Arch. Gesamte Physiol. 284:160-168.

Lande, S., and A. B. Lerner. 1967. The biochemistry of melanotropic agents. Pharmacol. Rev. 19:1-20.

Laurens, H. 1915. The reactions of the melanophores of *Amblystoma* larvae. J. Exp. Zool. 18:577-638.

Laurens, H. 1916. The reactions of the melanophores of *Amblystoma* larvae. The supposed influence of the pineal organ. J. Exp. Zool. 20:237-261.

Laurens, H. 1917. The reactions of the melanophores of *Amblystoma tigrinum* larvae to light and darkness. J. Exp. Zool. 23:195-205.

Lerner, A. B. 1961. Hormones and skin color. Sci. Amer. 205:98-108, (July).

Lerner, A. B., and J. D. Case. 1959. Pigment cell regulatory factors. J. Invest. Dermatol. 32:211-221.

Lerner, A. B., J. D. Case, Y. Takahashi, T. H. Lee, and W. Mori. 1958. Isolation of melatonin, the

pineal gland factor that lightens melanocytes. J. Amer. Chem. Soc. 80:2587.

McCord, C. P. 1914. The pineal gland in relation to somatic, sexual and mental development. Amer. Med. Assoc. 63:232-234.

McCord, C. P., and F. P. Allen. 1917. Evidences associating pineal gland function with alteration in pigmentation. J. Exp. Zool. 23:207-224.

Morgalit, H., and R. Rahamimoff. 1965. Effect of melatonin on uterine contractility. Life Sci. 4:1367-1372.

Oksche, A., and M. von Harnack. 1963. Elektronenmikroskopische Untersuchungen am der Epiphysis cerebri von *Rana esculenta* L. Z. Zellforsch. 59:582-614.

Oshima, K., and A. Gorbman. 1969*a*. Pars intermedia: unitary electrical activity regulated by light. Science 163:195-197.

Oshima, K., and A. Gorbman. 1969*b*. Evidence for a doubly innervated secretory unit in the anuran pars intermedia. I. Electrophysiologic studies. Gen. Comp. Endocrinol. 13:98-107.

Parker, G. H. 1948. Animal colour changes and their neurohumours. Cambridge Univ. Press. Cambridge, Eng.

Prosser, C. L., and F. A. Brown, Jr. 1961. Comparative animal physiology. W. B. Saunders Co., Philadelphia.

Quay, W. B. 1965. Retinal and pineal hydroxyindole-O-methyl transferase activity in vertebrates. Life Sci. 4:983-991.

Quay, W. B. 1968. Specificity and structure-activity relationships in the *Xenopus larval* melanophore assay for melatonin. Gen. Comp. Endocrinol. 11:253-254.

Quay, W. B., and J. T Bagnara. 1964. Relative potencies of indolic and related compounds in the body-lightening reaction of larval *Xenopus*. Arch. Intern. Pharmacodyn. 150:137-143.

Quay, W. B., and R. W. McLeod. 1968. Melatonin and photic stimulation of cone contraction in the retina of larval *Xenopus laevis*. Anat. Rec. 160:491.

Rust, C. C., and R. K. Meyer. 1969. Hair color, molt, and testis size in male, short-tailed weasels treated with melatonin. Science 165:921-922.

Scharrer, E. 1928. Die Lichtempfindlichkeit blinder Elritzen (Untersuchungen über das Zwischenhirn der Fische I). Z. Vergl. Physiol. 7:1-38.

Smith, P. E. 1920. The pigmentary, growth and endocrine disturbances induced in the anuran tadpole by the early ablation of the pars buccalis of the hypophysis. Amer. Anat. Mem. 11:1-151.

Snell, R. S. 1965. Effect of melatonin on mammalian epidermal melanocytes. J. Invest. Dermatol. 44:273-275.

Stebbins, R. C., W. Steyn, and C. Peers. 1960. Results of stirnorganectomy in tadpoles of the African ranid frog, *Pyxicephalus delalandi*. Herpetologica 16:261-275.

Steyn, W. 1959. Ultrastructure of pineal eye sensory cells. Nature 183:764-765.

Steyn, W. 1960. Observations on the ultrastructure of the pineal eye. J. Roy. Microsc. Soc. 79:47-58.

Swingle, W. W. 1921. The relation of the pars intermedia of the hypophysis and the pineal gland to pigment changes in anuran larvae. Anat. Rec. 21:87.

van de Kamer, J. C., C. Feekes, and A. C. J. Burgers. 1962. Histological investigation of the unpigmented meningeal spot on the brain of black background adapted *Xenopus laevis* larvae. Z. Zellforsch. 56:359-370.

van de Veerdonk, F. C. G. 1967. Demonstration of melatonin in Amphibia. Curr. Mod. Biol. 1:175-177.

von Frisch, K. 1911. Beiträge zur Physiologie der Pigmentzellen in der Fischhaut. Pflüger's Arch. Gesamte Physiol. 138:319-387.

Waring, H. 1942. The co-ordination of vertebrate melanophore responses. Biol. Rev. 17:120-150.

Wurtman, R. J., and F. Anton-Tay. 1969. The mammalian pineal as a neuroendocrine transducer. Recent Progr. Hormone Res. 25:493-514.

Young, J. Z. 1935. The photoreceptors of lampreys. II. The functions of the pineal complex. J. Exp. Biol. 12:254-270.