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Chromatophores and Color Change in the Lizard, *Anolis carolinensis**

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Summary. The skin of the lizard, *Anolis carolinensis*, changes rapidly from bright green to a dark brown color in response to melanophore stimulating hormone (MSH). Chromatophores responsible for color changes of the skin are xanthophores which lie just beneath the basal lamina containing pterinosomes and carotenoid vesicles. Iridophores lying immediately below the xanthophores contain regularly arranged rows of reflecting platelets. Melanophores containing melanosomes are present immediately below the iridophores. The ultrastructural features of these chromatophores and their pigmentary organelles are described. The color of *Anolis* skin is determined by the position of the melanosomes within the melanophores which is regulated by MSH and other hormones such as norepinephrine. Skins are green when melanosomes are located in a perinuclear position within melanophores. In response to MSH, they migrate into the terminal processes of the melanophores which overlie the xanthophores above, thus effectively preventing light penetration to the iridophores below, resulting in skins becoming brown. The structural and functional characteristics of *Anolis* chromatophores are compared to the *dermal chromatophore unit* of the frog.

Key-Words: Pigment cells — Melanophore stimulating hormone.

Introduction

The lizard, *Anolis carolinensis*, adapts readily to a light- or to a dark-colored background by becoming either a bright-green on the former or a dark-brown on the latter. The change from a green to a brown color requires only a few minutes for completion and apparently involves the stimulation of melanophores by melanophore stimulating hormone (MSH, Intermedin) from the pituitary gland (Kleinholz, 1938).

Color changes of poikilothermic vertebrates involve the response of dermal chromatophores to either (or both) nervous or endocrine stimulation (Parker, 1948). Recently, we described (Bagnara *et al.*, 1968) the structural and functional characteristics of color change in amphibians. The integrated nature of responses of xanthophores, iridophores, and melanophores, which together constitute the *dermal chromatophore unit*, provides the means for rapid color change of amphibians in response to hormonal stimulation.

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In the present report we describe light and electron microscopic details of chromatophore inter-relationships in the skin of the lizard, *Anolis carolinensis*, that are responsible for color change in response to hormonal stimulation. The anatomical relationships of chromatophores in reptilian skin have been described at the light microscopic level (Keller, 1891; von Geldern, 1921) but it is not clear from these earlier studies whether only the melanophores respond to hormonal stimulation, or, as in amphibians, all chromatophores respond and contribute to the phenomenon of color change. Although von Geldern (1921) correctly characterized the stratigraphic positions of the dermal chromatophores in the skin of *Anolis*, his interpretations of chromatophore structure as drawn from light microscopic studies are in need of clearer definition as determined by electron microscopy. Electron microscopic studies of Breathnach and Poyntz (1966) of the skin of the lizard, *Lacerta vivipara*, were concerned with structure and development of melanosomes and only provided superficial observations on lipophore (xanthophore) and guanophore (iridophore) structure.

Materials and Methods

Physiological Studies. The lizards, *Anolis carolinensis*, used in this study were obtained from the Snake Farm, Laplace, La. Both males and females were used. Lizards were sacrificed by decapitation and skins from each animal were prepared for photometric reflectance studies following the general methods of the *in vitro* frog skin bioassay for MSH (Shizume *et al.*, 1954) as modified for *Anolis* (Goldman and Hadley, 1969; Hadley and Goldman, 1969). Reflectance changes result from color changes in the skin and relate to the mobilization and distribution of pigments within dermal chromatophores (Hadley and Bagnara, 1969) in response to hormonal stimulation. Porcine β-MSH (obtained from Dr. Aaron B. Lerner) was used to darken skins and *l*-norepinephrine bitartrate was used to lighten skins. All agents and solutions used in these experiments were prepared as previously described (Goldman and Hadley, 1969; Hadley and Bagnara, 1969).

Tissue Preparation for Microscopy. The skins employed in this study were taken from the dorsal surface of non-injected (control) and MSH-injected lizards. A dosage of 0.2 ml of porcine β-MSH (1×10^{-7} g/ml) in Ringer's solution was used for injection to darken the skins. Lizards became maximally dark within five minutes after injection and were sacrificed shortly after that time.

All tissues were fixed for 2 hours with 4.37% glutaraldehyde in 0.1 *M* s-collidine buffer at pH of 7.4, followed by post fixation in 2% osmium tetroxide in 0.1 *M* s-collidine buffer at the same pH. Following fixation, tissues were dehydrated in an ethanol series and infiltrated in Epon 812 (Luft, 1961) under vacuum and then polymerized. Tissue blocks were sectioned on a Porter-Blum MT-2 ultramicrotome using a diamond knife. These sections were mounted on formvar-coated grids and were stained with a saturated aqueous solution of uranyl acetate (Watson, 1958) and alkaline lead citrate (Reynolds, 1963). Electron micrographs were taken on a Philips EM 200 operating at 60 kv. Thick sections (1 to 2μ) were stained with toluidine blue and were examined by light microscopy (Bennet and Radimska, 1961).

Observations

General Description of Anolis Chromatophores and Color Change

Chromatophores responsible for color change in *Anolis* are located in the dermis and their stratigraphic positions have been described by both light (von Geldern, 1921) and electron microscopy (Forsdahl, 1959; Alexander and Fahrenbach, 1969). Xanthophores are outermost in position (Fig. 1) and overlie iridophores (reflecting cells) which in turn are above melanophores. The spatial relationships of these cells are similar to those previously described for frogs (Hadley,

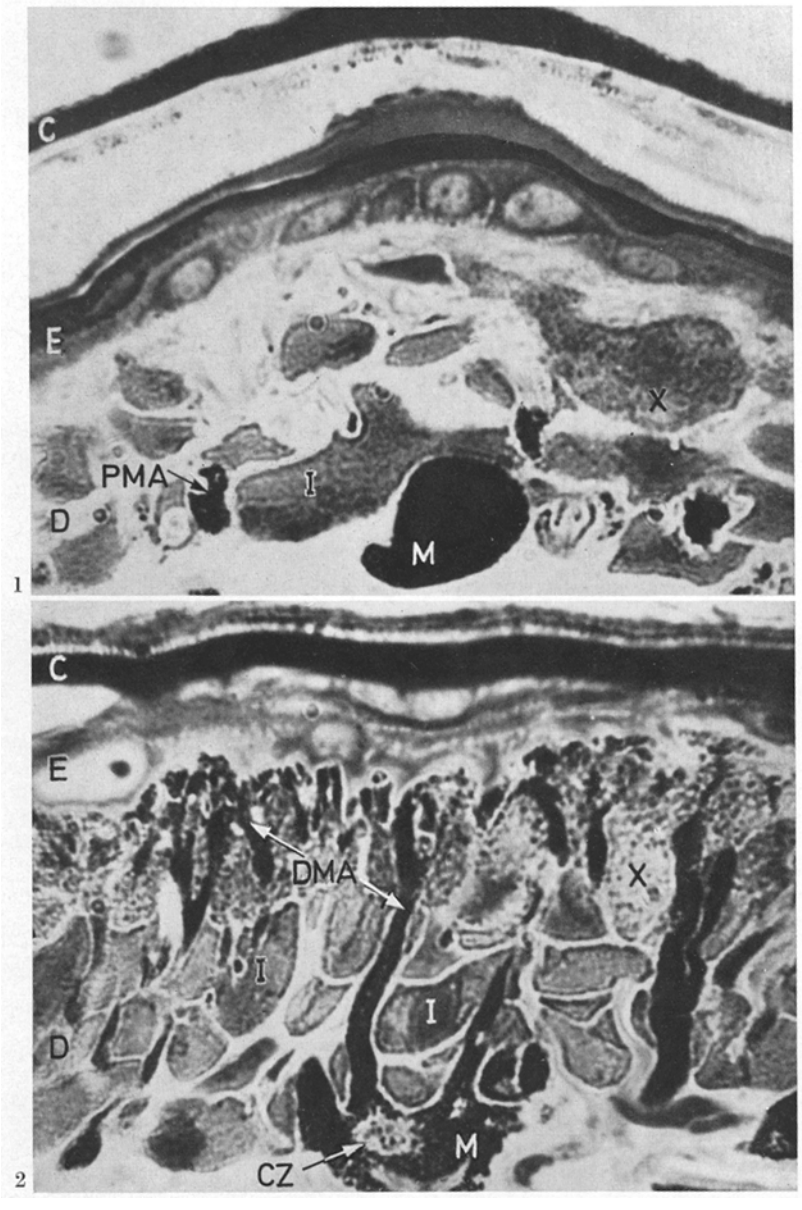

Fig. 1. Transverse section of *Anolis* skin from the dorsal surface. In the bright-green state, melanophore pigments are concentrated in the perinuclear region (*M*) and in proximal processes or arms (*PMA*). *C* cornified cells, *E* epidermis; *D* dermis; *X* xanthophore; *I* iridophore. ×1,200

Fig. 2. Same as Fig. 1 except skin is in a dark-brown state due to MSH treatment. Melanophore pigments have dispersed into the distal arms (*DMA*) leaving a clear zone (*CZ*) in the perinuclear region. ×1,200

1966; Bagnara *et al.*, 1968; Hadley and Bagnara, 1969). Unlike amphibians, however, which have only one layer of iridophores above the melanophores, two to four layers are present in this lizard. Epidermal melanophores are present within

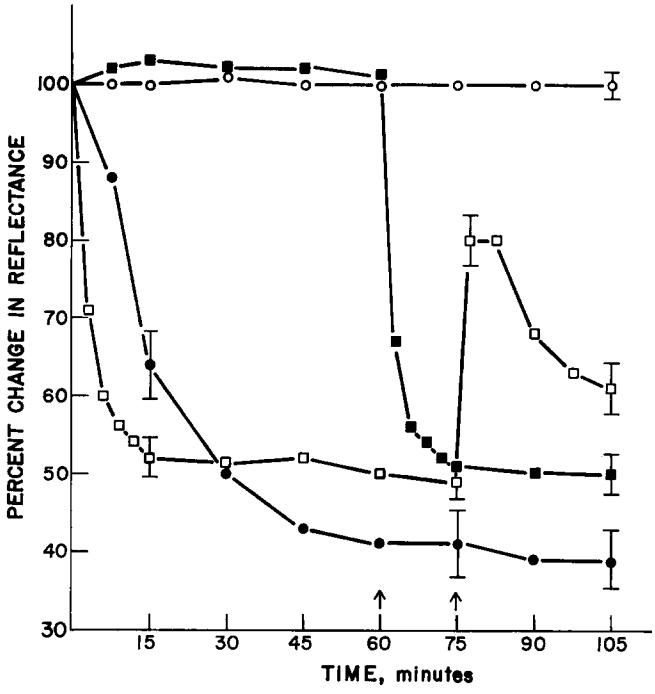

Fig. 3. Comparative *in vitro* response of *Anolis* and *Rana pipiens* skins to hormonal stimulation. *Anolis* skins (□) darken much more rapidly in response to MSH than do *Rana* skins (●). The response of *Anolis* skins is almost maximal by seven minutes as is demonstrated by the fact that a higher concentration (1.8×10^{-8} gm/ml) of MSH added at 60 minutes (arrow) to a control group of *Anolis* skins (■) darkens them to the same degree. Norepinephrine added to a group of darkened *Anolis* skins (□) caused an extremely rapid lightening. One group of *Rana* skins (○) was maintained as a control throughout the experiment. Each point on the graph is the mean of at least eight reflectance measurements

the epidermis of *Anolis* and, as in other reptiles (Bartley, 1966; Breathnach and Poyntz, 1966), synthesize and release melanosomes into surrounding epidermal cells similar to that as described for amphibians (Hadley and Quevedo, 1966, 1967) and mammals (Fitzpatrick and Breathnach, 1963). These cells are few in number and do not contribute to color change in this reptilian species.

Integumental color changes in *Anolis* result mainly from movements of melanosomes within the dendritic processes of melanophores in response to hormonal stimulation. In the absence of MSH, melanosomes are located in a perinuclear position and in the adjacent proximal portions of the melanophore processes (Fig. 1). In response to MSH, they migrate into the terminal extensions of the processes which lie above iridophores and xanthophores (Fig. 2). Melanosomes which now overlie iridophores below restrict the amount of light reaching them and thereby reduce reflection back through the above yellow layer of xanthophores. This results in the skin becoming very dark brown in color. Color change in *Anolis* is much more rapid than that of the frog, and the response is maximal either *in vivo* (Kleinholz, 1938) or *in vitro* (Horowitz, 1958; Goldman and Hadley, 1969; Hadley and Goldman, 1969), as demonstrated here (Fig. 3), within a very short time.

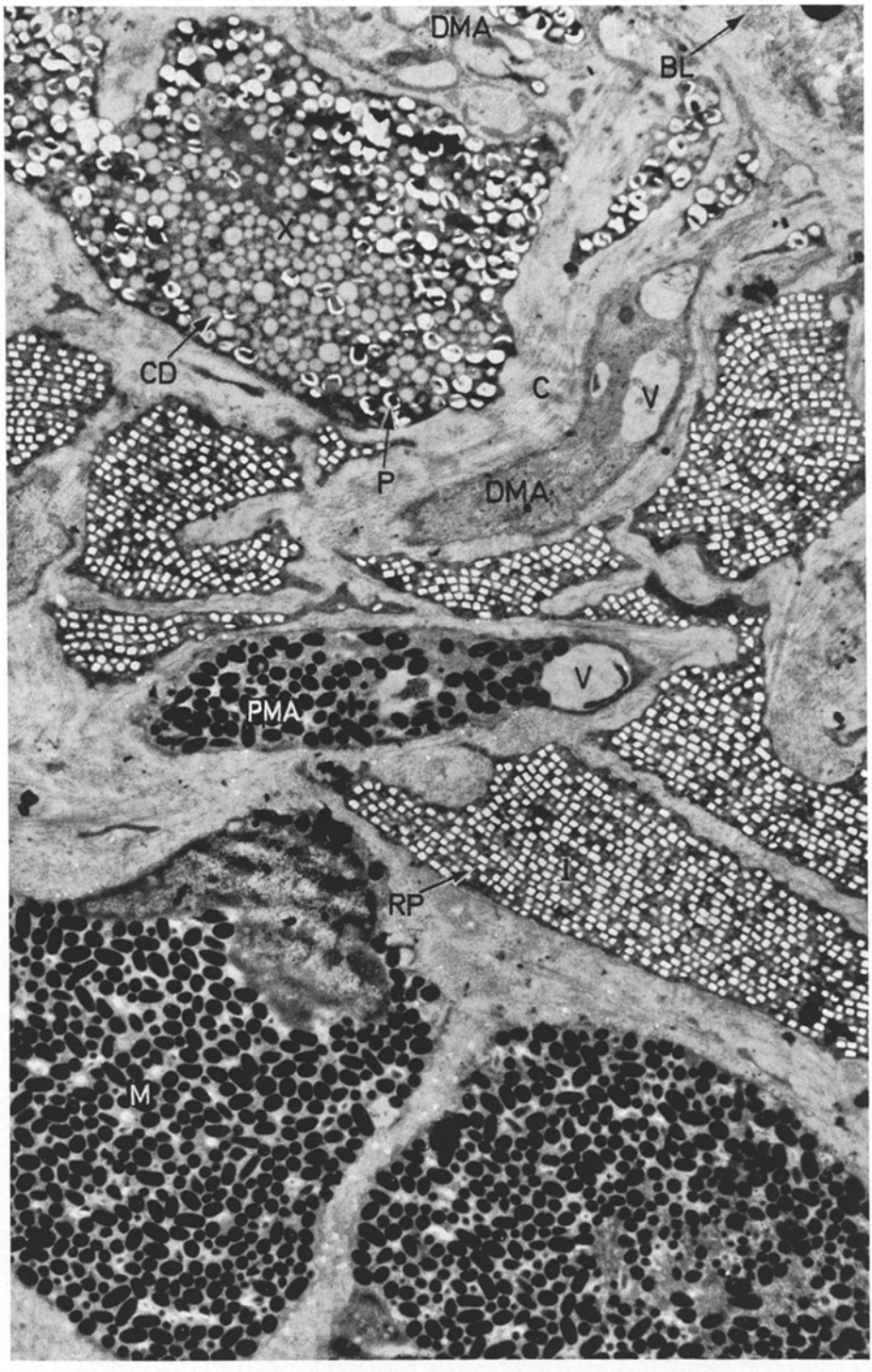

Fig. 4

Electron Microscopy of Chromatophores

Electron microscopic observations of *Anolis* chromatophores confirm earlier light microscopic descriptions. Melanophores are basal in position relative to the other chromatophores (Fig. 4). They are characterized by their specific intracellular organelle, the melanosome. Melanosomes appear to be fully melanized, no premelanosomes were observed. Above melanophores are several layers of iridophores containing their specific intracellular organelle, the reflecting platelet, which has been previously described for amphibians (Taylor, 1966, 1967, 1969; Setoguti, 1967) and reptiles (Breathnach and Poyntz, 1966; Alexander and Fahrenbach, 1969). The reflecting platelets assume a remarkable regularity of position within the iridophore; they are arranged in rows parallel to each other. They seem to be uniform in size and measure approximately 0.18×0.25 μ. Above iridophores are located the yellow pigment cells, the xanthophores, containing their characteristic pigmentary organelles, pterinosomes, which have been described in fish (Matsumoto, 1965; Matsumoto and Obika, 1968), amphibians (Bagnara *et al.*, 1968; Obika and Matsumoto, 1968) and reptiles (Breathnach and Poyntz, 1966; Alexander and Fahrenbach, 1969). Unlike amphibians, sectioning seems to be deleterious to *Anolis* pterinosomes for in many cases fragments are missing. Also present within the xanthophores are vesicles which are probably carotenoid in nature as described for amphibians (Bagnara, 1966; Obika and Matsumoto, 1968).

In the absence of MSH the skin of *Anolis* is bright green. Melanosomes within melanophores are concentrated in perinuclear areas and in the adjacent proximal portions of the melanophore processes (Fig. 4). Melanophore processes devoid of melanosomes extend up through the iridophores above and continue on to terminate above the xanthophores and just beneath the basal lamina. They can be distinguished on the basis of their cytoplasmic densities which are similar to those of other melanosome containing portions of the melanophore processes. In addition, all processes observed contain characterisitic large empty vacuoles which appear as tubules in sagittal sections (Fig. 4) and as vacuoles in frontal sections (Fig. 6). They are seen extending distally from the proximal portions of the processes. Microtubules are found in the processes but not the vacuoles of the processes. Large bundles of collagen separate melanophore processes and also separate one chromatophore from another.

In response to MSH, melanosomes migrate from a perinuclear position and come to fill all portions of the melanophore processes, including those above the xanthophores (Fig. 5). These terminal arborizations are separated from the basal lamina by a thin layer of collagen fibers. Melanosome migration into the processes

Fig. 4. Electron micrograph showing chromatophores from the bright-green state. Xanthophores (*X*) are outermost, several layers of iridophores (*I*) are situated below the xanthophores and melanophores (*M*) are below the iridophores. Xanthophore pigmentary organelles consist of pterinosomes (*P*) and carotenoid vesicles or droplets (*CD*); iridophore organelles are reflecting platelets (*RP*) and those of the melanophores are melanosomes. Melanosomes are concentrated in the perinuclear region and adjacent proximal processes or arms (*PMA*) leaving the distal arms (*DMA*) devoid of melanosomes. Vacuoles (*V*) are found in the arms which actually seem to be tubules extending distally from the melanophore body. *BL* basal lamina; *C* collagen. ×8,600

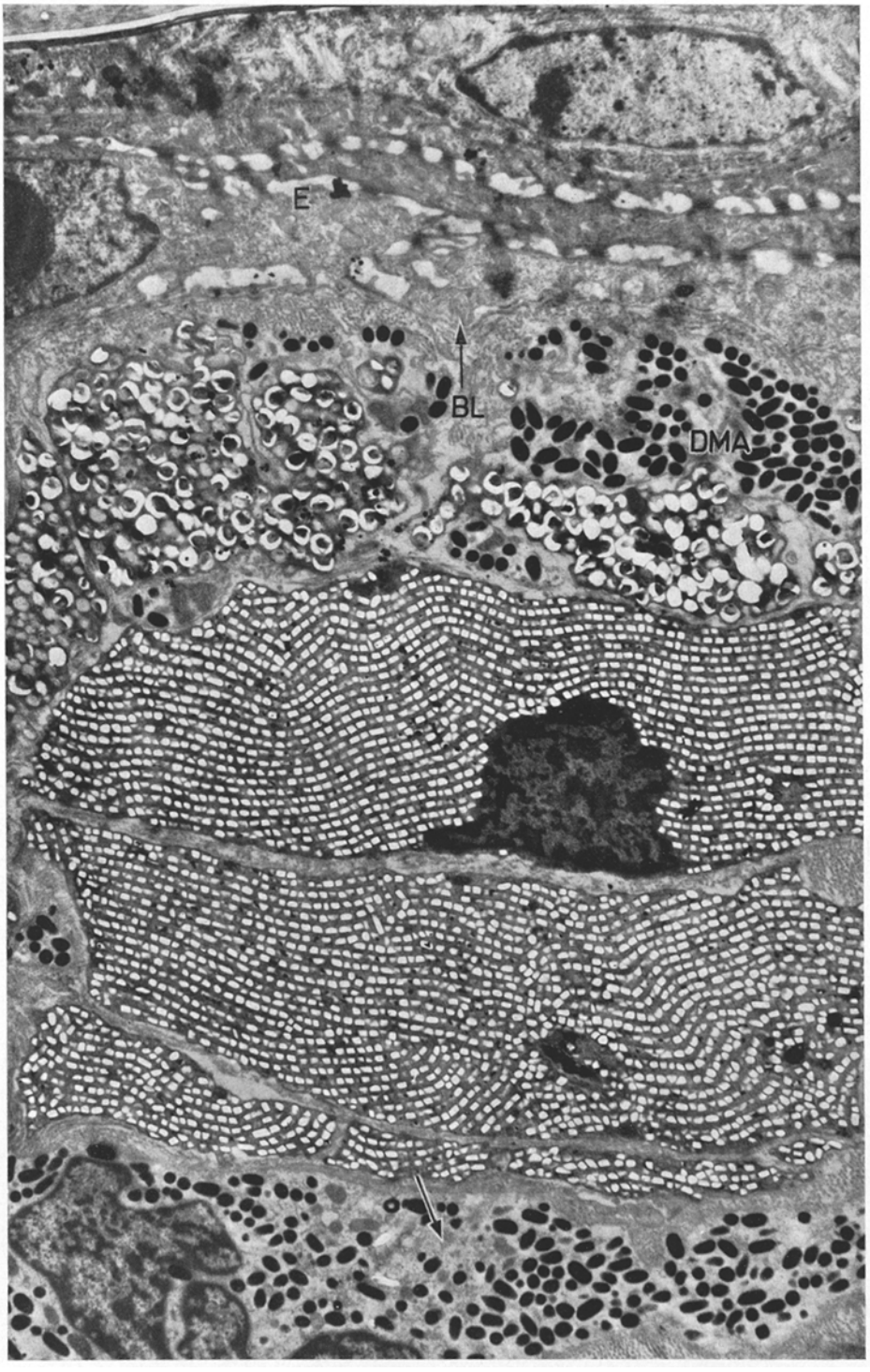

Fig. 5. In the MSH-treated state, melanosomes disperse to the distal arms (*DMA*) which terminate over the xanthophore layer. A reduction in the number of melanosomes is seen in the melanophore body (arrow). *E* epidermis; *BL* basal lamina. ×8,600

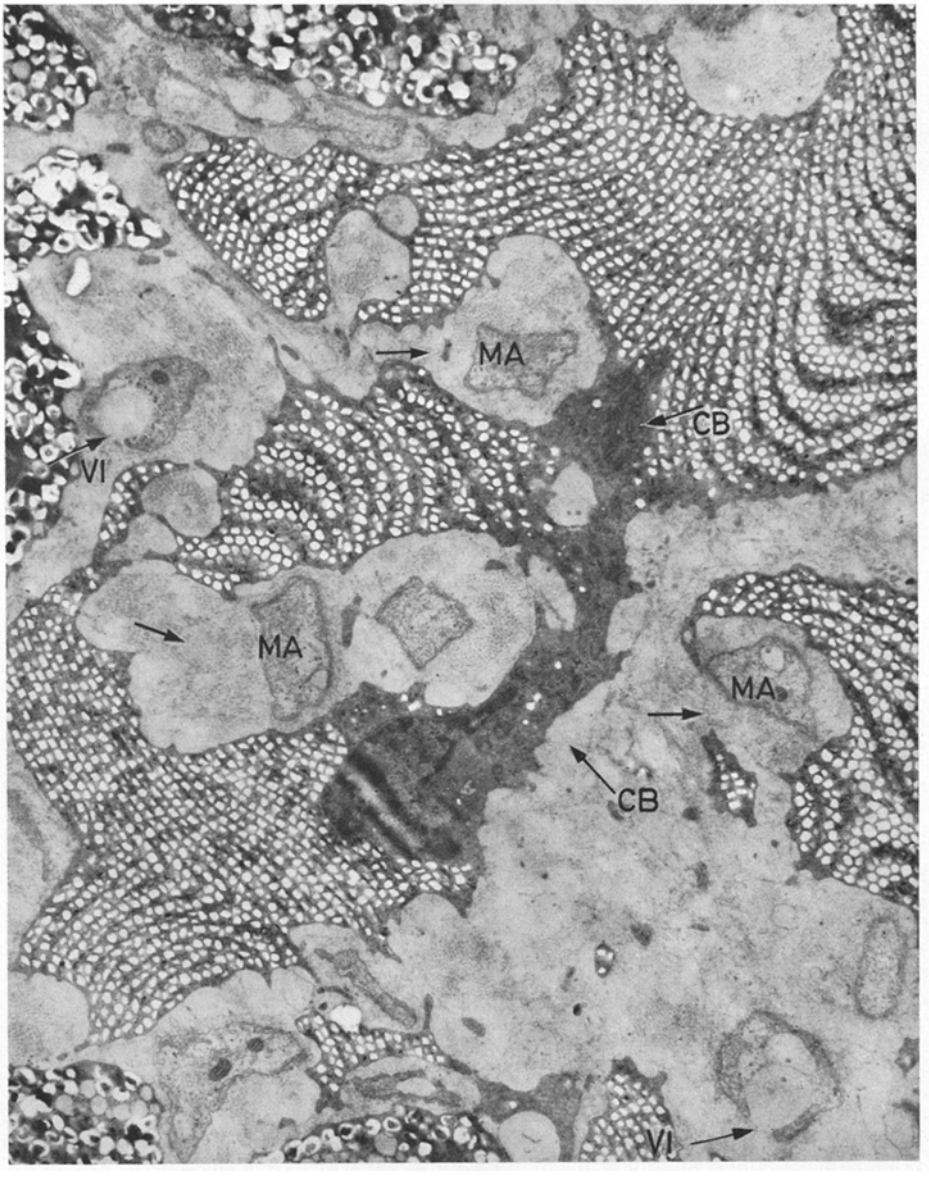

Fig. 6. Frontal section of *Anolis* skin showing the dendritic state of the iridophores with indentations or holes (arrows) which allow melanophore arms (*MA*) to penetrate the iridophore layers. Cytoplasmic bridges (*CB*) link various segments of the iridophores together. With this plane of sectioning, vacuoles in the melanophore arms appear as invaginations of the cell membranes. (*VI*). ×8,600

results in a reduction in number present in the body of the melanophore. Melanosomes were not observed inside the tubules of the processes.

Thus, as in the frog *dermal chromatophore unit*, color changes are mainly regulated by the movements of melanosomes within melanophores (Fig. 5). Mela-

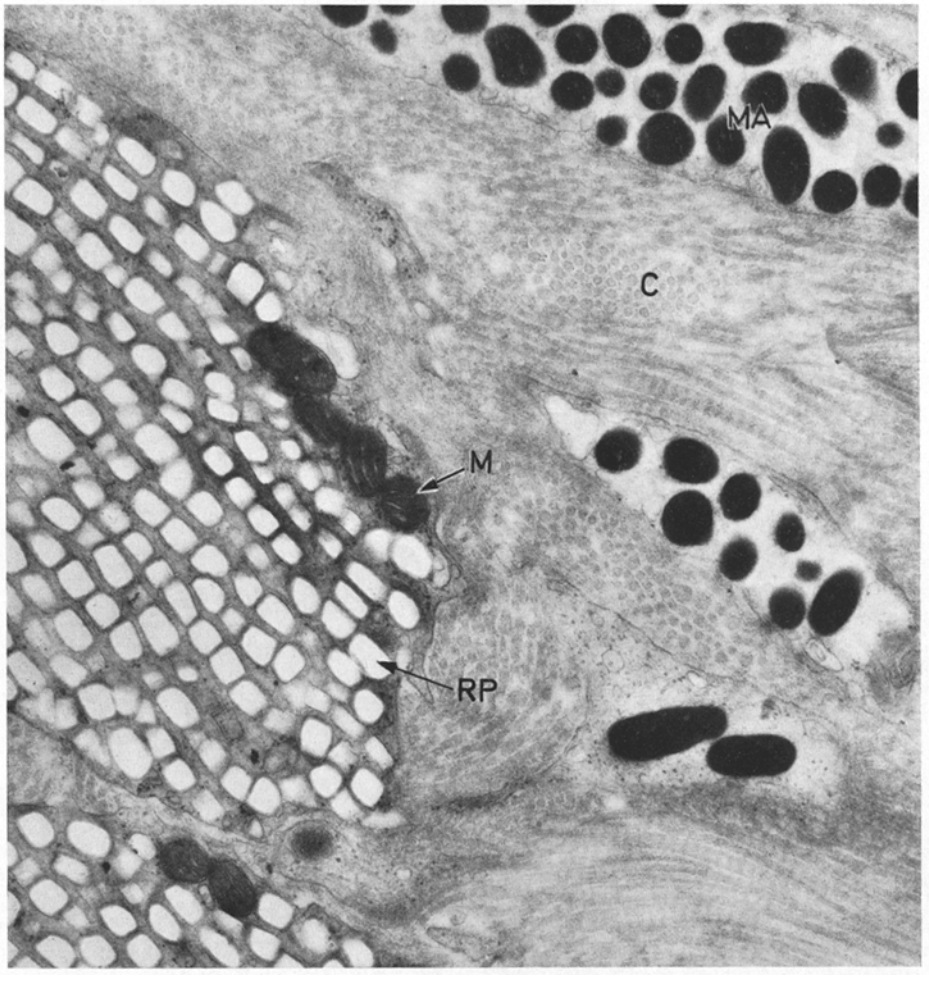

Fig. 7. Mitochondria (*M*) are usually seen on the periphery of the iridophore. Reflecting platelets (*RP*) are arranged in rows parallel to each other. *C* collagen; *MA* melanophore arm. ×22,500

nosome migration to a position above the xanthophores effectively prevents light from reaching the iridophores below. As in frogs, this apparently reduces the reflection of short wavelengths of blue light back through the yellow pigments of the xanthophores above. The partial movement of melanosomes to intermediate positions within the melanophore processes allows for a graded change in color from a green to an olive then to a light-brown and finally to a dark-brown color under maximal melanosome migration.

There is no evidence from these studies that the distribution of reflecting platelets within iridophores changes in response to hormonal stimulation. Iridophores are highly dendritic in shape (Fig. 6) whether in the presence or absence of MSH. Although the intracellular disposition of iridophore pigment in the frog is apparently radically changed in response to hormonal stimulation (Hadley,

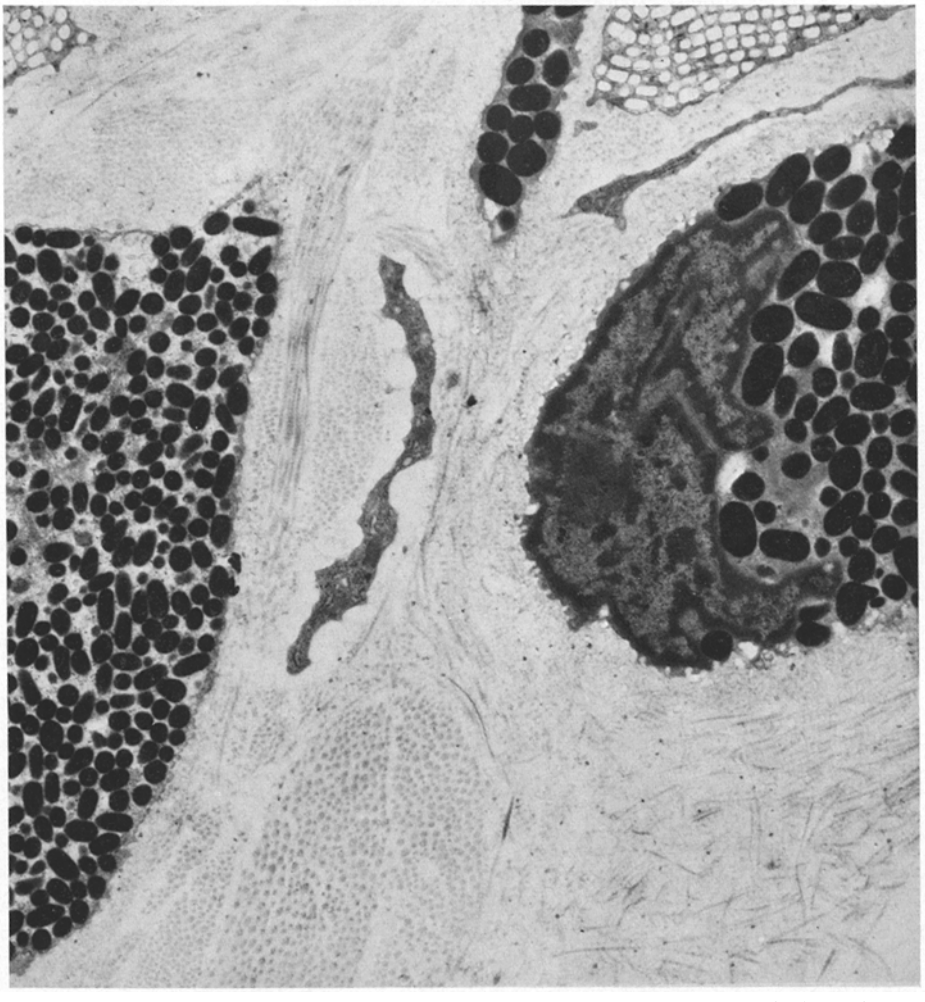

Fig. 8. Two distinct morphological types of melanophores can be distinguished by the size of their melanosomes. These types are found in approximately the same level of the dermis and their gross appearances seem to be similar. They both react to MSH by dispersing their melanosomes. ×8,300

1966; Hadley and Bagnara, 1969; Taylor, 1969), there is no such evidence for a similar situation in *Anolis* iridophores as studied by either light or electron microscopy.

The dendritic morphology of the iridophore is such that indentations (possibly "holes") in them allow the melanophore processes to penetrate through the iridophore layers to xanthophores above. Cytoplasmic bridges (Fig. 6) link the various segments of the iridophore together. Mitochondria, when observed, are generally seen at the periphery of the iridophore (Fig. 7). Two morphological types of melanophores are found (Fig. 8). The difference between these is in the size of their melanosomes. Melanosomes of one melanophore have an approximate diameter of 0.5 μ whereas they measure approximately 0.9 μ in the other type.

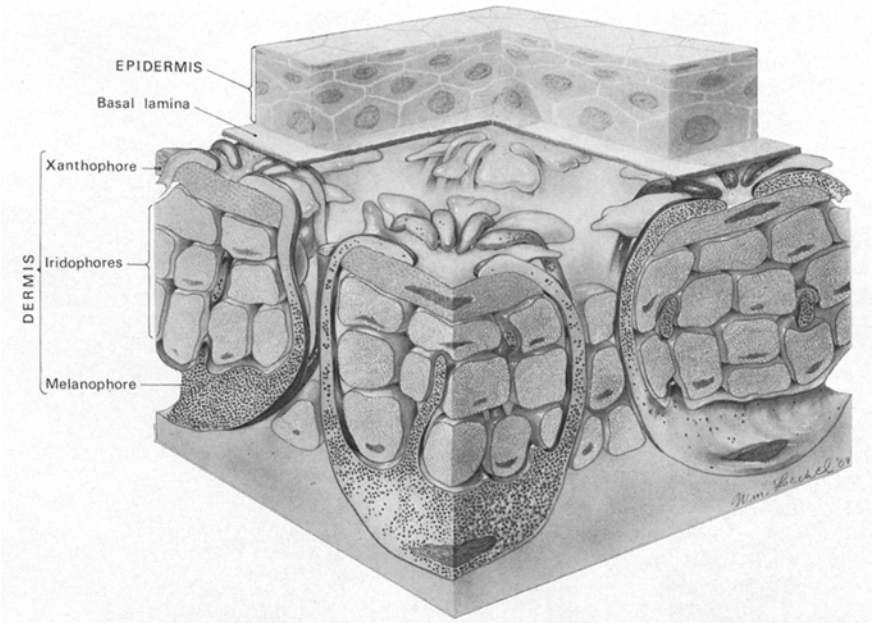

Fig. 9. A composite schematic interpretation of *Anolis* dermal chromatophores found under three physiological color states. The melanophore on the left, with its melanosomes concentrated in the perinuclear region, represents melanophores found in the bright-green state. The one in the middle, with its melanosomes partially dispersed, represent melanophores found in the olive to light-brown state. Finally the melanophore on the right, with its melanosomes completely dispersed, represents melanophores found in the dark-brown state. In all three color states, arms or processes of melanophores remain static as they penetrate the iridophore layers via indentations or holes and terminate over the xanthophore layer. The gross appearance and intracellular pigmentary organelles of the xanthophores and iridophores remain unchanged in all three states

Fig. 9 demonstrates a composite schematic interpretation of chromatophores found in the dermis of *Anolis*. Included are three melanophores with melanosomes either aggregated, partially dispersed or completely dispersed.

Discussion

The electron microscopy of the chromatophores of *Anolis carolinensis* confirms the structural and functional characteristics of color change as so beautifully described in great detail at the light microscopic level by von Geldern (1921). These electron microscopic studies add an ultrastructural description of chromatophores and their morphological response to hormonal stimulation. Color change is effected in a manner quite similar to that described for the *dermal chromatophore unit* of frogs (Bagnara *et al.*, 1968). There are some structural and functional differences of interest, however. Instead of a single layer of iridophores above the melanophores, a number of layers of these reflecting cells are present. In addition, instead of the melanophore processes terminating between iridophores and xanthophores, as in frogs, they mainly terminate above the xanthophores in *Anolis*. Nevertheless, melanosomes within the terminal arborizations of the melanophores in both the frog and the lizard effectively prevent light from

reaching the iridophores below. Also, although iridophores of some frogs undergo structural alterations in response to hormonal stimulation (Hadley and Bagnara, 1969; Taylor, 1969), a similar response has not been noted in *Anolis*. Whether the ultrastructural and functional features of chromatophore regulation in other reptilian species are similar to those of *Anolis* has not been reported.

In the teleost, *Fundulus heteroclitus*, melanosome movements are very rapid. Microtubules have been noted within *Fundulus* melanophores and have been suggested to play a role in the regulation of melanosome movement (Bikle *et al.*, 1966). Melanosome aggregation within *Anolis* melanophores in response to norepinephrine stimulation after maximal dispersion by MSH is often complete within 90 seconds after catecholamine stimulation. One might expect some kind of microtubular apparatus to accomplish this feat as suggested for *Fundulus* melanophores.

Of interest in the present studies are vacuoles or tubules (possibly tubular invaginations of the cell membrane) extending distally within the dendritic processes of the melanophores. In our earlier ultrastructural studies on frog melanophores (Bagnara *et al.*, 1968) we did not observe any similar structures and they were not reported for the teleost melanophore (Bikle *et al.*, 1966). Further studies are needed to determine whether they play a role in the regulation of melanosome movements.

One additional difference between the structure of the dendritic processes of frog and reptilian melanophores is that the membranes of some processes in the frog collapse in the absence of melanosomes (Wise, 1966; Bagnara *et al.*, 1968), but apparently all remain filled in *Anolis*. These observations, as for fish (Bikle *et al.*, 1966) and amphibians (Bagnara *et al.*, 1968; Taylor, 1969), again point out that melanophore processes are static structures and are not amoeboid as many early workers suggested (Parker, 1948).

In one of the two last major papers on reptilian chromatophore structure and function, Sand (1935) stated that it was not known whether the bright pigment cells "play a dynamic or merely static role in the pigmentary responses of reptiles". In *Anolis*, the xanthophores and iridophores do not appear to respond to hormonal stimulation as they do in some frogs (Bagnara *et al.*, 1969).

In the lizard, *Anolis carolinensis*, and the frogs we have studied (Bagnara *et al.*, 1968), chromatophores function to control color changes in a rather similar manner although differences are apparent. These animals are noted for their ability to rapidly change color. Many other reptiles (snakes) and amphibians (urodeles) generally do not so rapidly regulate their color changes. In these animals, a *dermal chromatophore unit*-like structure may not control chromatic changes.

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