THE DERMAL CHROMATOPHORE UNIT

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ABSTRACT

Rapid color changes of amphibians are mediated by three types of dermal chromatophores, xanthophores, iridophores, and melanophores, which comprise a morphologically and physiologically distinct structure, the dermal chromatophore unit. Xanthophores, the outermost element, are located immediately below the basal lamella. Iridophores, containing light-reflecting organelles, are found just beneath the xanthophores. Under each iridophore is found a melanophore from which processes extend upward around the iridophore. Finger-like structures project from these processes and occupy fixed spaces between the xanthophores and iridophores. When a frog darkens, melanosomes move upward from the body of the melanophore to fill the fingers which then obscure the overlying iridophore. Rapid blanching is accomplished by the evacuation of melanosomes from these fingers. Pale coloration ranging from tan to green is provided by the overlying xanthophores and iridophores. Details of chromatophore structure are presented, and the nature of the intimate contact between the chromatophore types is discussed.

INTRODUCTION

Dermal melanophores are generally considered to be responsible for the rapid color changes that occur in some amphibians, notably the hylids (see Parker, 1948). At first glance this concept seems reasonable since dermal melanophores are profoundly sensitive to intermedin, the major chromatophore-regulating hormone. However, when the precise location of dermal chromatophores in species exhibiting rapid color change is taken into account, serious doubt arises that dermal melanophores alone are the principal pigmentary elements involved in the color-change mechanism. It has been clearly demonstrated by Schmidt's elegant observations (1920) on adult *Hyla arborea* that these melanophores are found deep in the dermis and are covered by a layer of xanthophores and iridophores. Accordingly, in order that dermal melanophores might become manifested fully it would seem that some arrangement must exist so that the masking effect of the overlying chromatophores can be diminished. It is implicit in such a suggestion that chromatophores function together in a unified fashion. The association of certain pigment cells with one another is consistent with this unity and is exemplified by the close apposition of xanthophores with iridophores to form the so-called xantholeucosome (for discussion of chromatosomes and other pigment-cell terminology, see Bagnara, 1966). The involvement of melanophores in such an association is known from previously published literature (Gaupp, 1904) and has been confirmed by Hadley (1966) who reported that the dendrites from underlying dermal melanophores terminate on the surface of iridophores in skin of *Rana pipiens* and who described the termination. A physiological demonstration of this unity is the fact that responses of both melanophores and iridophores are mediated by intermedin (Bagnara, 1958, 1964; Hadley, 1966). Presumably, the action of this hormone as well as the specific morphology and location of the three dermal chromatophore types are the major factors responsible for the specific shade of color manifested by the skin. In

view of the unity of dermal chromatophores, with respect to both structure and function, the present study was undertaken to provide a clearer cytological picture of the structural characteristics of chromatophore interaction in color change responses. These studies have led to the development of the concept of the "dermal chromatophore unit," which provides the vehicle by which adult amphibians can color-adapt rapidly.

MATERIALS AND METHODS

The majority of observations were made on skins of three hylids: *Hyla cinerea* which varies in color from lemon yellow to dark green; *Agalychnis dachnicolor*, a large, light to dark green species collected near Alamos, Sonora, Mexico; and *H. arenicolor*, a light tan to brown-colored resident of southwestern canyons which was obtained in the vicinity of Tucson. Observations were also made on skins of adult *Rana pipiens*. Some of the animals were injected with intermedin (porcine β-MSH) at a concentration of 1×10^{-7} g/ml of Ringer solution. Because of their smaller size, *H. cinerea* and *H. arenicolor* received dosages of 1 ml whereas *A. dachnicolor* and *R. pipiens* were injected with 2 ml of the intermedin solution. These frogs were sacrificed 2 hr after hormone administration. Observations were also made on control frogs which were not injected. Tissues used for electron microscopy were fixed for 1–2 hr in 6% glutaraldehyde in 0.1 M *s*-collidine buffer (pH 7.3) and postfixed in 2% OsO_4 in the same buffer. After fixation, tissues were dehydrated in an ethanol series and embedded in Epon 812 (Luft, 1961). Sections were made on a Porter-Blum MT-2 ultramicrotome with a diamond knife. They were mounted on Formvar-coated grids, stained with either alkaline lead citrate (Reynolds, 1963) or the double stain of saturated uranyl acetate and alkaline lead citrate, and examined in a Philips EM200 electron microscope. Thick sections (1–2 μ) were also cut and then stained with toluidine blue for light microscopy (Bennett and Radimska, 1966). Further observations were made from paraffin sections or from whole mounts of skins either fixed in FAA (two parts formaldehyde, one part acetic acid, 17 parts 70% ethanol) or unfixed and mounted in clear Karo syrup (Bagnara, 1958). The latter method is effective in preserving yellow pigments which are dissolved by fat solvents.

OBSERVATIONS

Consistent with light microscope observations, by previous workers, on amphibians, electron micrographs reveal clearly that, in the dermis, xanthophores (*X*) are outermost in location, just beneath and in intimate contact with the basal lamella (Figs. 1–4). In cross-section the xanthophore presents a flattened profile; its nucleus is usually centrally located, polymorphic in outline, and also flattened. The most prominent pigmentary organelle is the pterinosome (*PT*) (Matsumoto, 1965). These pteridine-containing structures are distributed uniformly throughout the cytoplasm. Interspersed between them are droplets (*CV*) which probably represent the carotenoid complement of xanthophore pigments (Bagnara, 1966). Just beneath the xanthophore is found the iridophore (*I*). The latter is often shaped like an inverted cone and contains a rounded nucleus which is generally found near the base of the cell. The characteristic feature of iridophores is a series of empty spaces (*RP*), which are usually oriented in stacks. These spaces contain reflecting platelets which Taylor (1966, 1967) and Setoguti (1967) have shown to be the pigmentary organelles of iridophores. In species with many xanthophores, such as *H. cinerea* or *A. dachnicolor*, a xanthophore is found generally above every iridophore. The deepest dermal chromatophore is the melanophore, the body of which is found below the iridophore. In *H. cinerea* and *A. dachnicolor*, the melanophore body is in intimate contact with the lower surface of each iridophore; however, in *H. arenicolor*, melanophores are somewhat more deeply situated and often lose this basal contact. In *R. pipiens*, a relatively large space may separate the base of the iridophore from the melanophore body. In all four species, melanophore processes or arms extend around the sides of each iridophore and terminate in fingers which lie between the iridophore and its overlying xanthophore, as is shown in Figs. 2 and 4 *b*.

The arrangement of these three pigment cells forms a discrete structural unit. The functional relationships in this unit become obvious in intermedin-treated animals. In skins from these animals, melanosomes migrate to the most distal extensions of the melanophore. Altogether, these structural and functional relationships have led us to formulate a concept, the dermal chromatophore unit, which is depicted in Fig. 3 as a schematic interpretation of the situation in those species of amphibians that have been studied. The salient feature of the unit is that each melanophore can modify the bright colors imparted by iridophores above it by obscuring the light-reflecting capacity of these cells. This color modification is accomplished by migration of

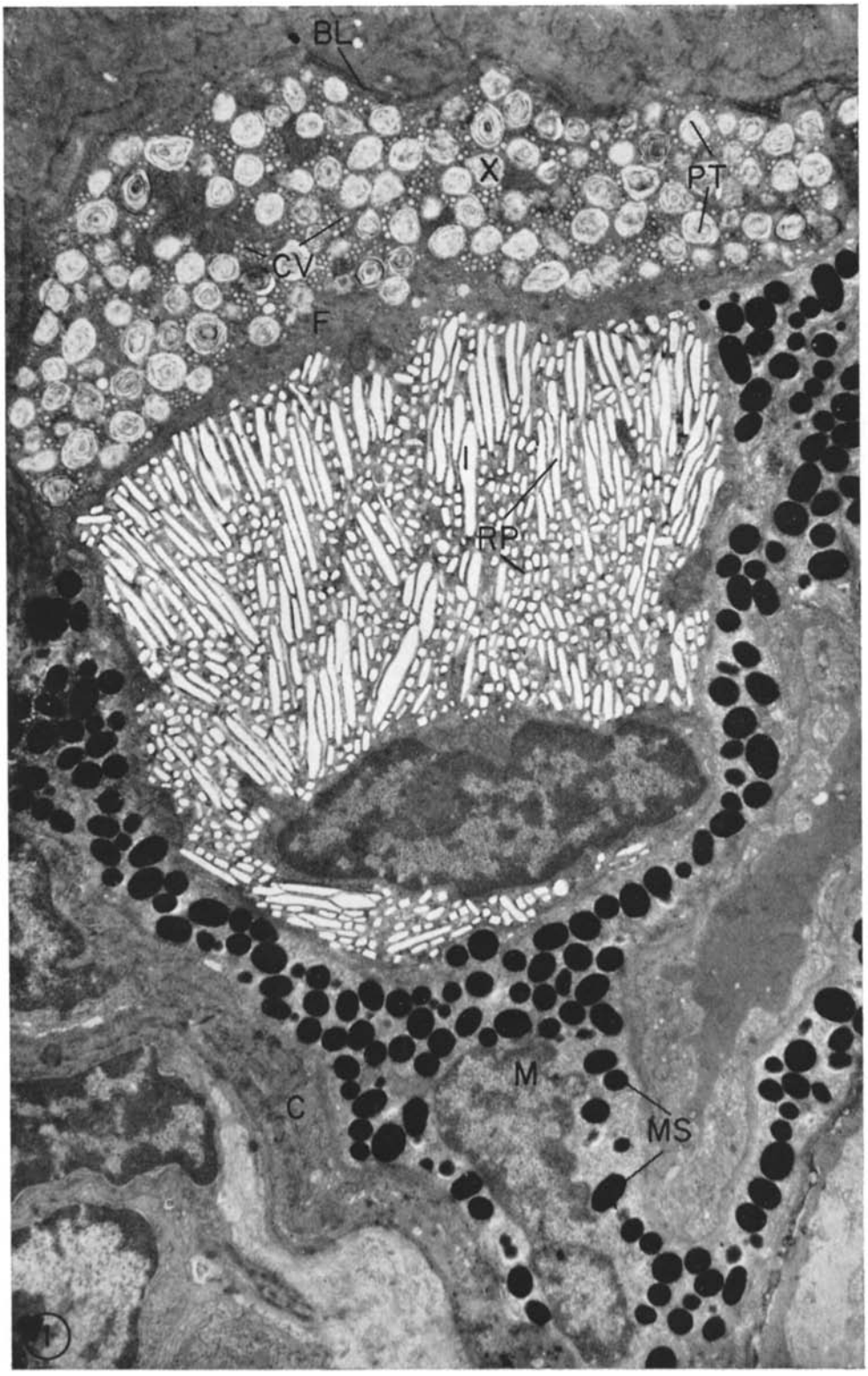


FIGURE 1 Transverse section of *H. cinerea* skin from dorsal surface showing dermal chromatophore unit in white background-adapted state. Melanophore *M;* iridophore, *I*; xanthophore, *X*. Melanosomes (*MS*) are uniformly distributed in arms around sides of iridophore, but fingers (*F*) over iridophores are empty. *BL*, basal lamella; *RP*, reflecting platelets; *CV*, carotenoid vesicles; *C*, collagenous masses; *PT*, pterinosome. × 9,700.

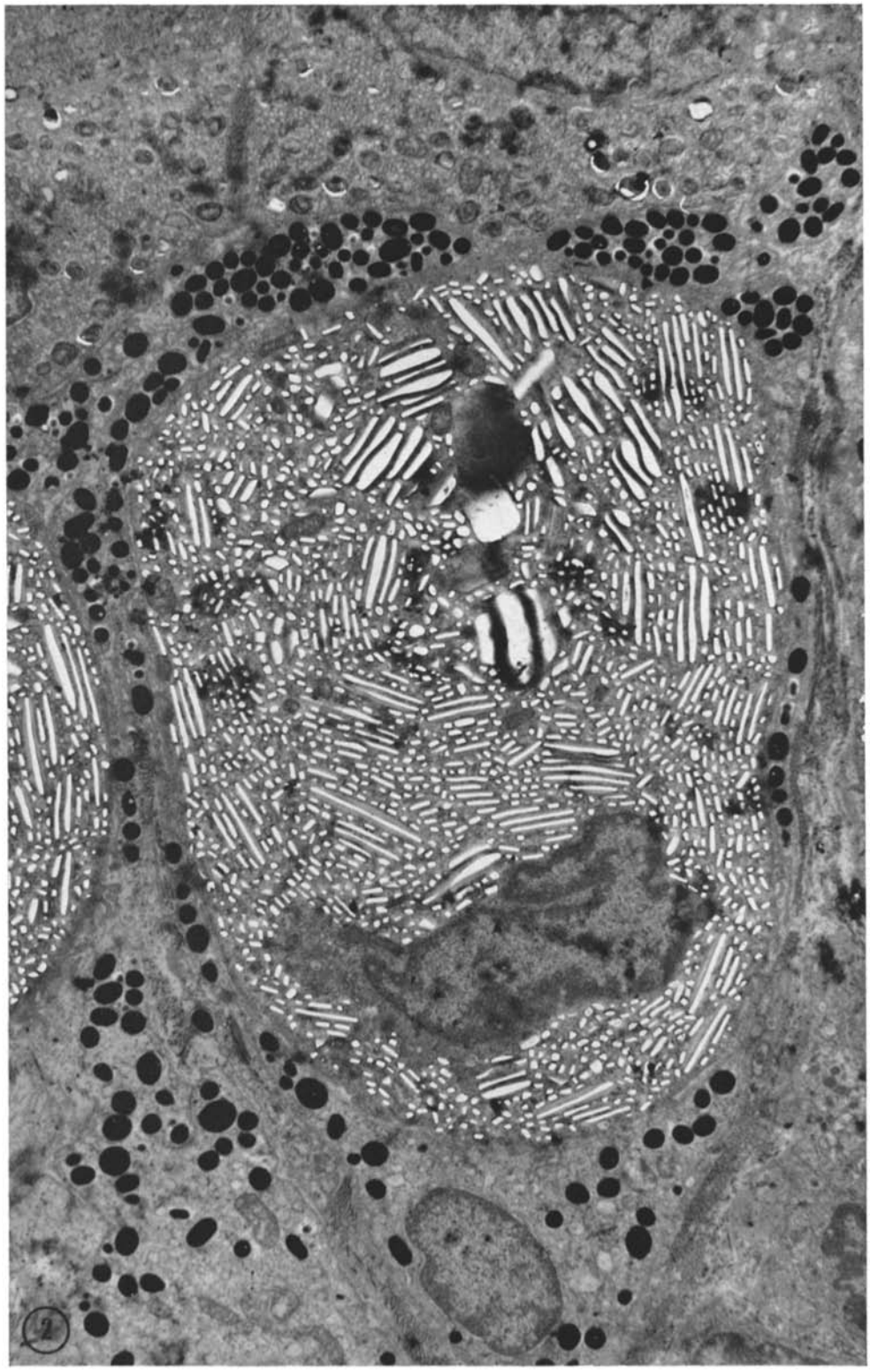


FIGURE 2 Same as Fig. 1, except treated with intermedin. Melanosomes now fill fingers over iridophore at the expense of arms and body of melanophore. × 7,300.

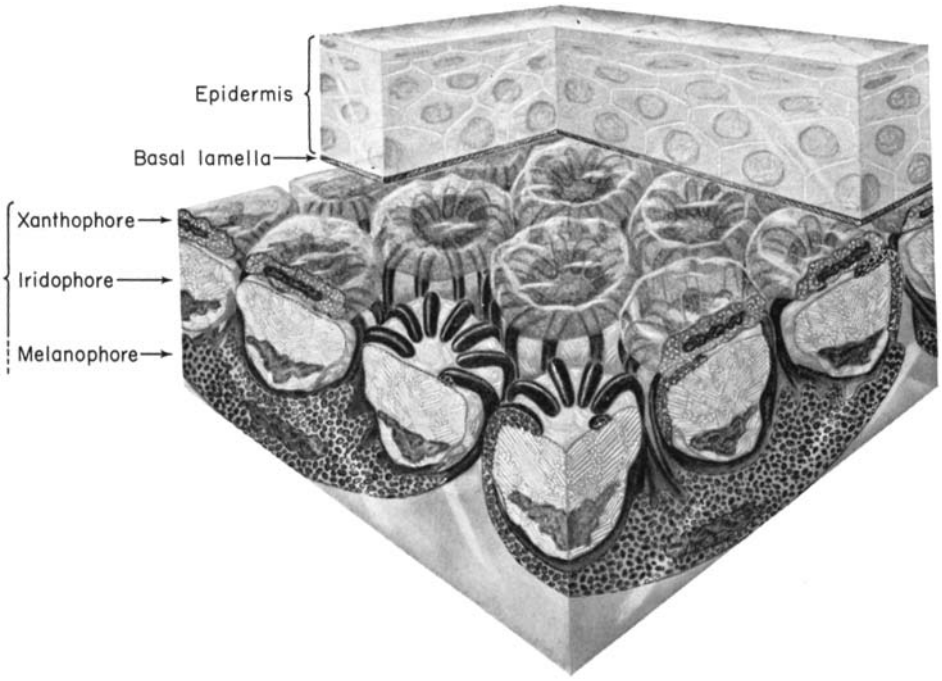


FIGURE 3 Schematic interpretation of the dermal chromatophore unit from several anurans. Adaptation to a dark background is represented.

melanosomes into melanophore fingers between the xanthophore and the iridophore. In this way, not only is the iridophore obscured but the xanthophore-iridophore relationship is interrupted or modified (Fig. 4 *e, f*).

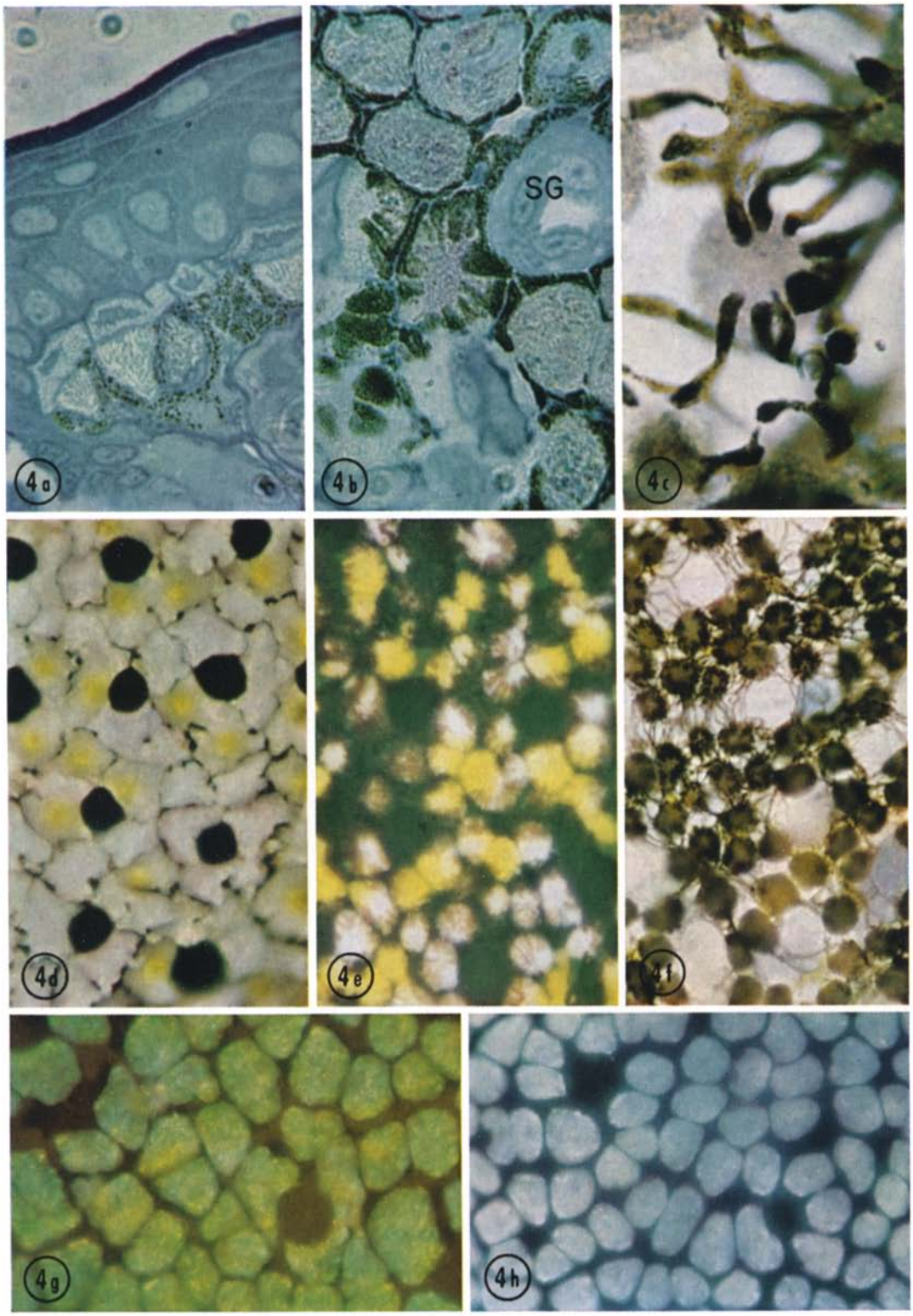
Melanophore fingers, which become obvious in intermedin-treated animals, apparently are fixed structures. There is no indication of an actual movement of fingers to and from the area between the iridophores and xanthophores; instead, it seems that fingers remain static and that melanosomes actively migrate into and out of these fingers. In white background-adapted animals in which melanosomes are concentrated in the body of the melanophore, the area between the iridophore and the xanthophore contains numerous membranes (Fig. 5). In all probability these membranes are collapsed melanophore fingers which are now devoid of melanosomes. By contrast, in intermedin-treated animals, these fingers are distended by an invasion of melanosomes (Fig. 6). The relationship between cell membranes of melanophore fingers and iridophores is complex. Where the membranes of each of these two cells are in intimate contact, as on the upper surface or along the sides of the iridophore, they are folded and give a rugose appearance (Figs. 7 and 8). No such complex contact was found to exist between xanthophores and melanophore fingers; although the membranes of these chromatophores are in close apposition, the contact is smooth and without indication of foldings (Fig. 7). That melanophore fingers remain permanently in place is further suggested by indentations which can be seen around the edges of iridophores in *H. arenicolor* (Fig. 4 *d*). In this species, iridophores are somewhat expanded in the absence of intermedin so that the reflecting material is spread throughout the iridophore except for the indentations which presumably represent vacant fingers through which melanosomes migrate from beneath to the upper surface of the iridophore. In *R. pipiens*, iridophores are capable of considerable expansion, and indentations of the iridophore surface are quite prominent. It appears that these indentations exist because the position of the fingers is permanent; thus, whenever the iridophore expands, it must do so around these fingers which provide a localized barrier (Hadley, 1966). The external form of the iridophore is not visibly

affected in *H. cinerea* or in *A. dachnicolor*; that is, iridophores exhibit no perceptible expansion or contraction (Taylor, 1967). Accordingly, deep indentations were not found. Frontal sections of iridophores of *H. cinerea* (Fig. 9) reveal that fingers are uniformly distributed around the iridophore and that they do not actually indent its surface. These sections also demonstrate that some individual chromatophore units are often in contact with one another, whereas others are often separated by collagenous masses (*C* in Fig. 10) which extend from the basal lamella to the hypodermis (Figs. 1 and 2).

The nature of melanophore involvement in the dermal chromatophore unit is somewhat variable among species. For example, there are fewer fingers over each iridophore in both *H. cinerea* and *A. dachnicolor* than in *H. arenicolor* in which as many as 25 fingers are often found. In *H. arenicolor*, however, the fingers are considerably thinner than those in the other two hylids. But the net effect of almost completely obscuring the upper iridophore surface is the same. The number of iridophores served by one melanophore is also variable. In dorsal skin of *A. dachnicolor* there appears to be a melanophore for every iridophore. This melanophore seems primarily to serve its overlying iridophore; however, it may also serve neighboring iridophores, as is especially obvious in the flank where single melanophores serve several iridophores. Often an individual iridophore receives dendritic processes from several melanophores. In the dorsal skin of both *H. cinerea* and *H. arenicolor* there are about 10–15 iridophores for every melanophore; this ratio is even higher in *R. pipiens*. Just as with *A. dachnicolor*, in the flank of these three species there are fewer melanophores than iridophores. Thus, individual melanophore processes are more widespread, allowing for contact with distant iridophores (Fig. 4 *f*).

Thus far, it has been implied that hormonal action on the dermal chromatophore unit mainly involves the movement of melanosomes into and out of melanophore fingers. Actually, intermedin affects iridophores as well. Hadley (1966) has shown that in the absence of intermedin, the iridophores of adult *R. pipiens* are dendritic. Thus, a maximum of reflecting surface is presented, unmasked by melanosomes which, under these conditions, are concentrated toward the center of the melanophore beneath the iridophore. A similar situation prevails in *H. arenicolor* except that the degree of iridophore expansion is far less than that in *R. pipiens*. Nevertheless, iridophores are so numerous that they provide practically a continuous sheet of reflecting material (Fig. 4 *d*). In intermedin-treated animals (Fig. 4 *e*) the skin becomes dark not only because melanophore fingers partially mask the iridophore, but also because the hormone causes iridophores to become somewhat punctate; this results in the exposure of less reflecting surface. In *H. arenicolor*, even xanthophore pigments are concentrated toward the center of the cell (Fig. 4 *d*); however, in the intermedin-treated animal this yellow pigment disperses and covers a larger surface area (Fig. 4 *e*). It was not possible to observe xanthophore response in the other species that were studied.

FIGURE 4 *a*, Thick section (1 μ) of skin from *H. cinerea* showing dermal chromatophore unit during adaptation to a white background. × 800. *b*, Thick section (1 μ) of skin cut parallel to dorsal surface, from *H. cinerea* treated with intermedin. Arms containing melanosomes surround iridophores. Melanophore fingers are visible over some iridophores. *SG*, skin gland. × 1,240. *c*, Termination of melanophore fingers on iridophore in skin of intermedin-treated male *R. pipiens*. × 1900. *d*, Whole mount of skin from *H. arenicolor* in the absence of intermedin, photographed with reflected light. Iridophores are expanded and are indented at their edges. Yellow spots over some iridophores are xanthophores in the contracted state. Black circles represent openings of skin glands. × 520. *e*, Same as *d*, except treated with intermedin. Iridophores are contracted, xanthophores are expanded and melanophore fingers are seen above iridophores. × 520. *f*, Whole mount of skin from flank of *H. arenicolor*. Melanophore processes are widespread and show contact with several iridophores. × 230. *g*, Whole mount of dorsal skin from *H. cinerea* adapted to light background. Mounted in Karo syrup to preserve xanthophores. Green represents combined effect of xanthophores and iridophores. × 1040. *h*, Same as *g*, except treated with alcohol. Xanthophore pigments are removed and underlying iridophores blue (Tyndall effect). × 1040.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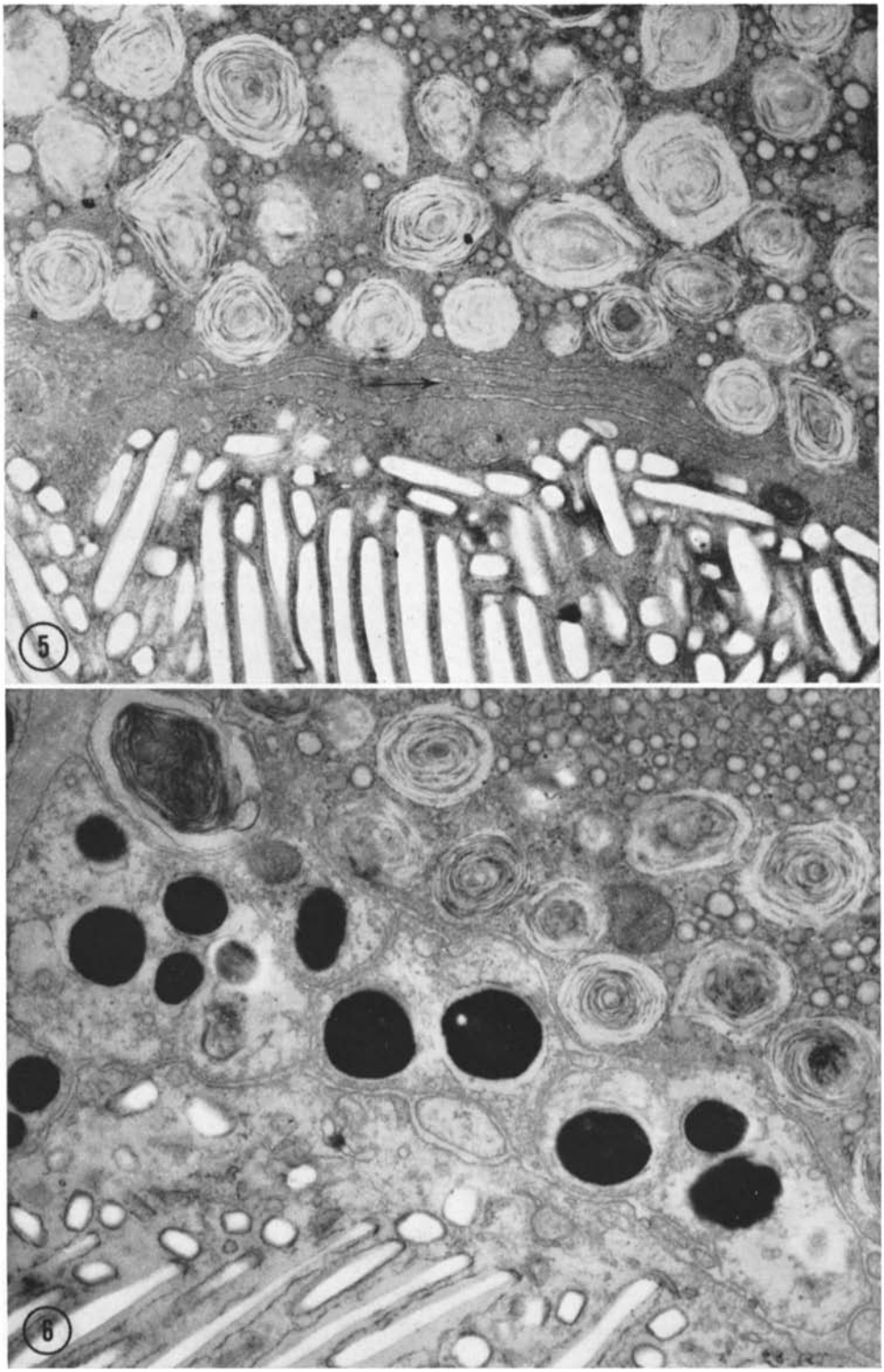

FIGURE 5 Transverse section of area between xanthophore and iridophore in skin of *H. cinerea*. Numerous membranes (arrow) are believed to represent collapsed melanophore fingers which are devoid of melanosomes. × 23,500.

FIGURE 6 Same as Fig. 5, except that frogs were darkened with intermedin. Melanophore fingers between xanthophore and iridophore are distended with melanosomes. × 23,500.

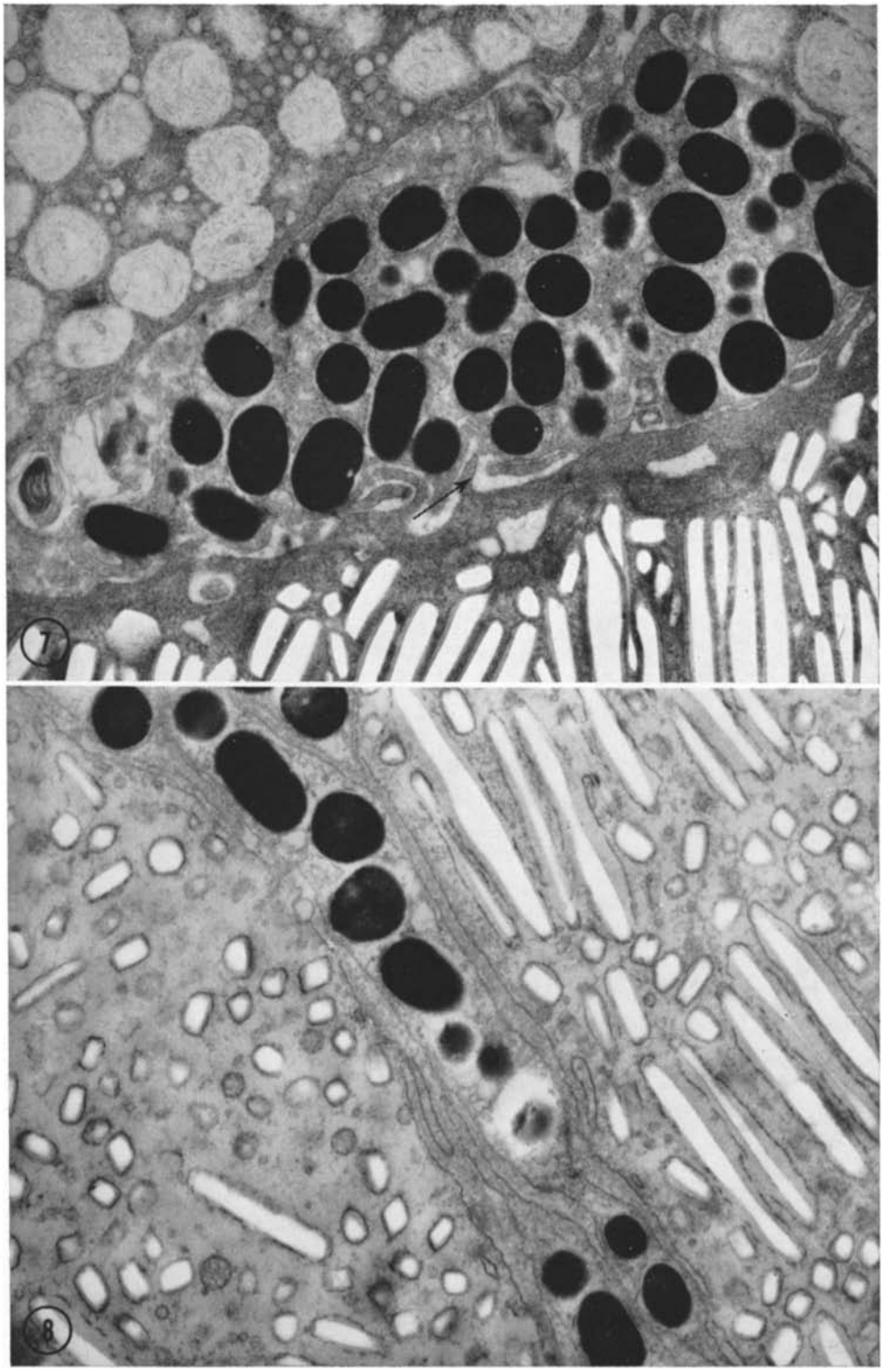

FIGURE 7 Same as Fig. 6, showing interdigitations between melanophore fingers and iridophore (arrow). Contact between melanophore and xanthophore is smooth. × 18,000.

FIGURE 8 Tangential section through dorsal skin of *H. cinerea* demonstrating melanophore process between two adjacent iridophores. The rugose nature of contact between melanophore and iridophore is obvious. × 23,500.

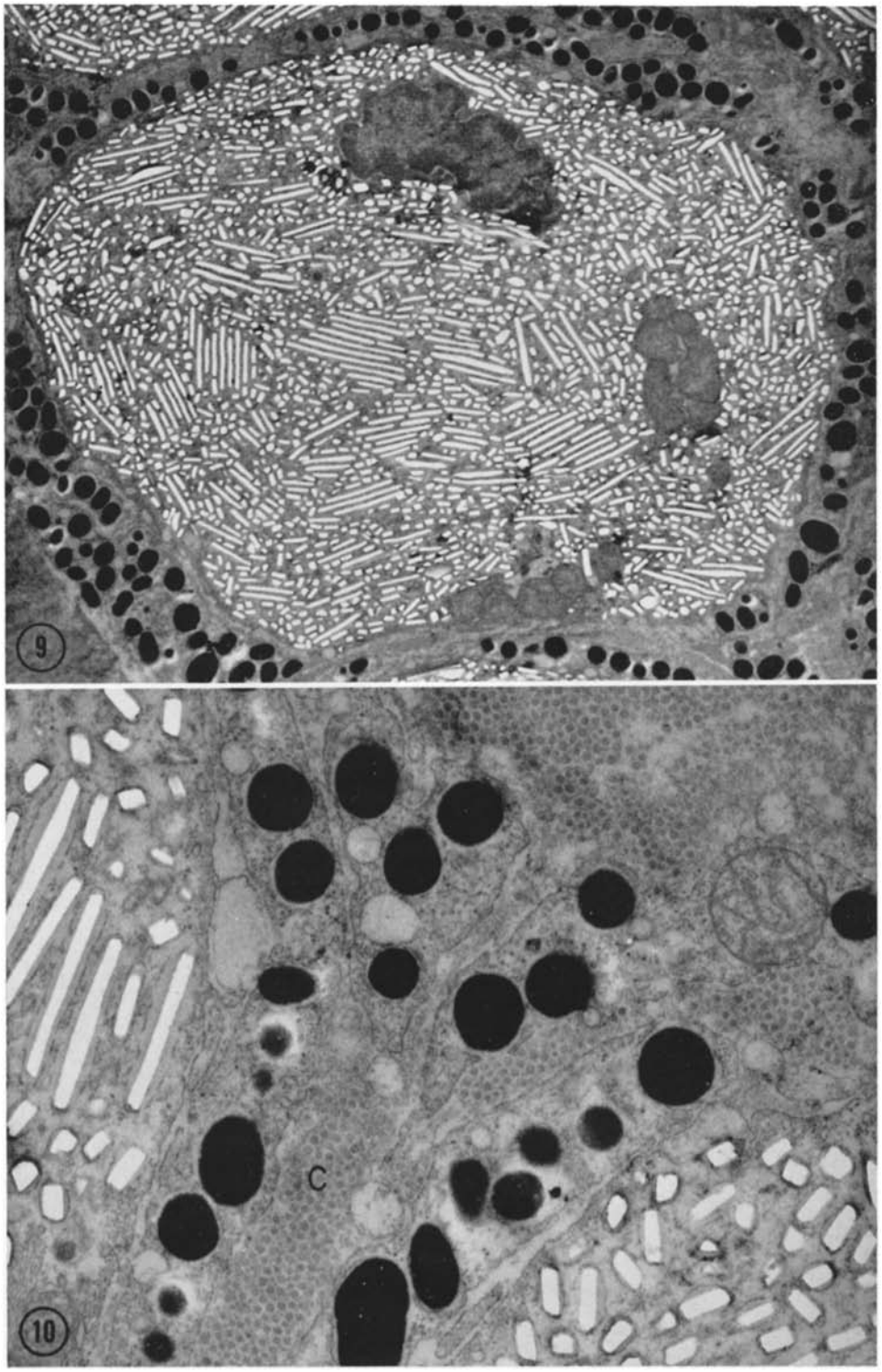

FIGURE 9 Same as Fig. 8, showing uniform distribution of melanophore processes around the iridophore. × 5,700.

FIGURE 10 Same as Fig. 9, demonstrating collagenous masses (*C*) extending between two dermal chromatophore units. × 23,500.

It should be emphasized, also, that in the other species of amphibians the number of xanthophores is far greater; iridophores are covered by xanthophores which form a more or less continuous yellow layer. In *H. cinerea*, the edges of adjacent xanthophores are always closely apposed to one another (Fig. 4 *a*).

DISCUSSION

The dermal chromatophore unit provides a remarkable example of the functional integration of several morphologically distinct cells, xanthophores, iridophores, and melanophores, to provide a uniform gross effect. Before the gross effect can be explained, the specific form and function of each chromatophore type must be understood. Xanthophores apparently are the least dynamic of the three chromatophore types. They contain yellow pigments; in the species studied these pigments presumably are carotenoid and pteridine in nature (Bagnara, 1966). Pterinosomes, the pteridine-containing organelles (Matsumoto, 1965), were obvious in every xanthophore. These organelles, together with small vesicles which probably contain carotenoid pigments, are distributed uniformly throughout the cytoplasm and give the xanthophore a uniform yellow appearance. Formerly, it was believed that xanthophores of amphibians played no active part in color change mechanisms because they were never observed to expand or contract as a result of hormonal or other stimulation. Our observation that xanthophores of adult *H. arenicolor* expand after administration of intermedin represents the first example of an amphibian xanthophore response to a hormone. It was not possible to extend this observation to xanthophores of the other three species studied, because they are usually obscured by the underlying melanophore fingers.

The precise contribution of iridophores to color-change mechanisms is difficult to ascertain because of the complex nature of these cells. Clearly, the principal function of this chromatophore is to reflect light. This is accomplished by the many stacks of purine-containing reflecting platelets which fill the iridophore. Taylor (1966, 1967) explains that these platelets are so hard that they cannot be properly infiltrated and, consequently, they shatter during sectioning, leaving behind their profiles in the form of empty spaces. The fact that stacks of platelets are often oriented at different angles introduces the possibility that light-scatter might play an active role in pigmentation. Whether or not this factor accounts for the red, green, or blue iridescence sometimes seen in individual iridophores is unknown. In *H. arenicolor*, light emanating from the skin laden with iridophores is white except at the location of a xanthophore where the yellow color of the xanthophore is expressed. Because there are relatively few xanthophores in the dorsal skin of this species, white light reflected from the iridophores predominates, and the xanthophore contribution is represented by a tinge of bronze or tan coloration. In *H. cinerea* and *A. dachnicolor*, light reflected from the iridophore layer of the dorsal surface appears to be blue; in fact, each individual iridophore appears to be blue. This blue is readily visible in whole mounts of skin from which the overlying yellow pigments have been leached (Fig. 4 *h*). Intact skin, either in the living frog or in whole mount preparations, appears green (Fig. 4 *g*). In all probability this green results from a filtering effect provided by the yellow of the overlying xanthophore layer. A hypothesis of this type to explain why some cold-blooded vertebrates are green was first made by Fox and Vevers (1960).

Whereas xanthophores and iridophores are responsible for imparting pale coloration, the melanophore plays a role in darkening the animal. The migration of melanosomes from their basal position within the melanophore to the fingers obscures the overlying iridophore and thus allows the animal to adapt readily to a dark-colored background. Rapid blanching is accomplished by the evacuation of melanosomes from these prominent fingers. In this way, the pigment is mobile while the chromatophore itself retains its permanent position. This observation is in agreement with the early observation of Spaeth (1913) that the arms of the teleost melanophore are static structures. In the absence of melanosomes, the fingers collapse; thus, in white background–adapted frog the fingers are observed as membranes between the xanthophore and the iridophore. An actual distension of the fingers occurs when they are occupied by melanosomes. A similar observation has been made for melanophores of larval *H. regilla* by Wise (1966). The mechanism for the migration of melanosomes into the fingers is unknown. Other than melanosomes, few organelles are found in the fingers; there is evidence that only a few microtubules are present.

Bikle et al. (1966) suggest that microtubules play a role in the concentration and dispersion of melanosomes in fish melanophores.

The close contact between the fingers of dermal melanophores and the iridophores presents several facets of interest. The actual terminations of these fingers were known previously in German literature (see Gaupp, 1904); however, their functional significance was not understood until the present investigation. On the basis of our first observation of the melanophore-iridophore relationship, it seemed attractive to suggest that in *R. pipiens*, at least, iridophore contraction after intermedin administration might be a secondary response resulting from the action of the hormone on the melanophore. In other words, the melanophore-iridophore junction (Fig. 4 *c*) might be analogous to a synapse which could act as a mechanism of communication between the two chromatophores. This suggestion was rejected for several reasons, the most important being that, from the standpoint of both absolute response and threshold of response to various hormonal and pharmacological agents, melanophore reactivity and iridophore reactivity can be separated (Hadley, 1966). Moreover, it is known that iridophores having no observable morphological association with melanophores respond to intermedin (Bagnara, 1958; Hadley, 1966). At any rate, the suggestion of communication between iridophores and melanophores need not be of great consequence to the generalized concept of the dermal chromatophore unit. In hylids, in which the unit is well developed, iridophores generally display no marked gross response to intermedin. They serve a pigmentary function in a passive way, through their location beneath the xanthophore layer and above the dermal melanophores. The contact between the membranes of the melanophore fingers and the iridophore membrane gives no indication of a communicating mechanism. The rugose membranes of these chromatophores appear to be interdigitated, but the interdigitations have no internal organization, such as that seen at synaptic junctions, which might be correlated with such sites of contact. It appears that this intimate junction serves to insure that melanophore fingers which are uniformly distributed over the iridophore remain consistently in place, allowing efficient masking of the iridophore to occur whenever the melanophore is stimulated to disperse its pigment. This interpretation is favored by the large area of contact between the two chromatophores that includes both the upper surface and the sides of the iridophore.

The concept of two or more cell types functioning as a discrete pigmentary unit is not a new one. In both cold-blooded vertebrates and mammals the epidermal melanocyte and its associated epidermal cells function as the "epidermal melanin unit" (Fitzpatrick and Breathnach, 1963; Hadley and Quevedo, 1966). Under appropriate stimulation, the epidermal melanocyte releases melanin granules into those surrounding epidermal cells which are serviced by dendrites from that particular epidermal melanocyte. With the dispersion into the epidermis of large numbers of melanin granules, the epidermis becomes dark. In contrast to the darkening effected by the response of the dermal chromatophore unit, the darkening produced by the epidermal melanin unit is not readily reversible, for the animal can become pale again only after the deposited melanin has been lost. Deposition of large amounts of melanin in the epidermis of animals with a well-developed epidermal melanin unit would obscure the action of the dermal chromatophore unit. It is noteworthy, therefore, that epidermal melanocytes are absent or minimally functional in species which exhibit rapid color changes. For example, the hylids used in the present study lack epidermal melanocytes.

The principal purpose of this paper has been to describe the dermal chromatophore unit found in some adult amphibians. The structural and functional arrangement of xanthophores, iridophores, and melanophores in the dermis provides a remarkably efficient mechanism for color adaptation among amphibians and probably other cold-blooded vertebrates. Simple as the mechanism appears, it has some obvious complexities which remain as a challenge for future investigation. Among the problems is the one of development of the unit during metamorphic climax. During this period of rapid changes in the skin, the adult pigmentary pattern is established. Other important problems to be solved include the mechanism of the movement of melanosomes in the melanophore fingers and the variation in form and function of each of the components of the dermal chromatophore unit among the various vetrebrates which utilize it.

This research was supported in part by grants GB-3681 and GB-4923 from the National Science Foundation.

We would like to thank Dr. Wayne Ferris for his advice in using the electron microscope facility which is supported by National Science Foundation grant GB-3330. We would also like to thank Mr. Gary Monheit for preparing the whole mount preparations of *H. arenicolor* skin and Mrs. Joanna McComb for preparing the stereogram entitled dermal chromatophore unit.

Received for publication 15 December 1967, and in revised form 20 February 1968.

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