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An Electron-microscopic Study on Six Kinds of Color Variants Induced by Radiation in *Rana nigromaculata*

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(With 1 Text-figure and 5 Plates)

INTRODUCTION

While immature *Rana nigromaculata* are green or brown in ground color of the dorsal integument, nine kinds of color variants have been produced by NISHIOKA (1977) by means of irradiation of eggs or sperm with X-rays or neutrons. She has determined the inheritance of these color variants as well as that of the green and brown colors in normal frogs. The nine kinds of color variants are albino, gray-eyed, black-eyed, blue, grayish-brown, greenish-olive, yellowish-olive, bluish-olive and brownish-olive ones. In the former three, the gene for each variant is recessive and epistatic in the homozygous condition to the genes for the other colorations. Differing from these three variants, the colors of the remaining six kinds of color variants as well as the green and brown of normal frogs occur from the cooperation of three pairs of alleles, *X*, *x* for normal or abnormal xanthophores, *I*, *i* for normal or abnormal iridophores and *E*, *e* for expansion or contraction of all the three kinds of dermal chromatophores.

In the present research, the integuments of the six kinds of variants, the blue, grayish-brown, greenish-olive, yellowish-olive, bluish-olive and brownish-olive as well as normal green and brown frogs were examined under light and electron microscopes to clarify the functions of the above three pairs of alleles on dermal chromatophores.

MATERIALS AND METHODS

Eggs of *Rana nigromaculata* HALLOWELL were obtained after injecting frog pituitaries. Sperm suspension was made by crushing a testis in about 10 ml of distilled water. Unfertilized eggs and sperm suspension were exposed to 90～240 rads of X-rays or 50～130 rads of neutrons. Artificial insemination was performed between the irradiated eggs or spermatozoa and untreated gametes. While the fertilized eggs became partly or mostly abnormal and died, the remaining ones developed normally and grew into mature frogs. From females of the latter, gynogenetic diploids were produced in order to obtain color variants, which

should appear in the half number of them, if any color variants were induced by irradiation of gametes. Besides these color variants, pure strains of wild-colored frogs should be obtained by such a method. The authors have so far obtained two kinds of wild-colored frogs, green-type and brown-type, and nine kinds of color variants, that is, albino, gray-eyed, black-eyed, blue, grayish-brown, greenish-olive, bluish-olive, yellowish-olive and brownish-olive (NISHIOKA, 1977).

Of these eleven color strains, eight other than albino, gray-eyed and black-eyed variants were used as materials in the present study. The three kinds of chromatophores, xanthophores, iridophores and melanophores, in their dorsal skins were observed under an electron microscope as well as under a light microscope with transmitted or reflected light. The frogs used were immature ones at the age of 3 months after metamorphosis; they were 3~4 cm in body length. Before making microscopic preparations from their skins, they were kept for about 48 hours in a vessel whose bottom and side walls were whitened and illuminated with a 15 watt fluorescent lamp, in order to adapt them to a light environment.

For light microscopic observation, a piece of skin was cut off from the dorsal surface of a frog, put into a drop of RINGER's salt solution on a slide glass and covered with a deck glass. The margin of the deck glass was sealed with vaseline. This procedure and the subsequent observation were made as quickly as possible. For electron microscopic observation, a piece of skin taken out of the dorsal or ventral surface of a frog was put in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for fixation, cut into smaller pieces at once and then kept for two hours by renewing the fixing fluid once more. After washing in 0.1 M phosphate buffer, the pieces were postfixed in 2% osmic acid in the same buffer at 2~6°C. The tissue was dehydrated in an ethanol series and embedded in Epon 812 (LUFT, 1961). Sections were cut on a Porter-Blum M-1 ultramicrotome with a glass knife at the thickness of silver or silver-gold. The thin sections were double stained with saturated uranyl acetate and alkaline lead citrate and photographed with a Hitachi Hs-8 electron microscope.

OBSERVATION

I. Two color types in normal frogs

1. Green-type frog

Green-type frogs used as materials were third-generation offspring of normal frogs collected from the field in 1967 together with the ancestors of the above brown-type frogs. When they were adapted to a light environment for 48 hours, their backs were yellow-green in ground color. The dorso-median stripe was dull yellow-green. The dorso-lateral folds, irises and dermal protuberances on the back were pale brown, although the marginal portion of each of the protuberances was yellow-green.

a. Light microscopy

When a piece of the dorsal skin is observed under a light microscope, the three kinds of dermal chromatophores are horizontally expanded; adjacent chromatophores are partly overlapped and there is scarcely any interspace between them. Xanthophores have no yellow spots and are yellow as a whole by lighting with both reflected and transmitted light. Iridophores look pale blue with metallic luster by lighting with reflected light, while dull purple by lighting with transmitted light. Melanophores have many long arms filled with melanosomes and are black when observed with reflected or transmitted light.

The dorso-lateral folds and dermal protuberances of green-type frogs are the same as those of brown-type frogs in the microscopical structure of chromatophores as well as in their color as a whole.

b. Electron microscopy

i) Xanthophores Xanthophores are horizontally expanded, being closely contiguous and partially overlapped with one another. They are filled with pterinosomes and carotenoid vesicles, both of which are evenly distributed in each cell, differing from those of brown-type frogs. The pterinosomes are spherical or ellipsoidal, being $0.6 \sim 0.9\ \mu$ in the largest diameter. They are divided into type I and type II by their inner structure. While type I reveals concentric lamellae, type II has no lamellar structure. The carotenoid vesicles are about $0.2\ \mu$ in diameter. They are always mingled with pterinosomes, without being gathered into a mass (Plates I, 1 and V, 11).

ii) Iridophores Iridophores are flat and horizontally expanded as xanthophores are. They are closely contiguous and leave no interspaces between them. They are also closely contiguous to the xanthophores situated above. All the iridophores are filled with reflecting platelets each of which is a rectangle or parallelogram in shape and usually less than $2\ \mu$ in length and about $0.1\ \mu$ in thickness. They are mostly arranged in parallel with the surface of skin (Plate I, 1).

iii) Melanophores Each melanophore has several long arms filled with melanosomes. Such arms of adjacent melanophores form two to four layers under the iridophores. The melanosomes are ovoid or elliptical and reveal no inner structure; they are about $0.5\ \mu$ in the largest diameter. Premelanosomes are very scarce (Plate I, 1).

2. Brown-type frog

Brown-type frogs used for microscopic observation were third-generation offspring of normal frogs collected from the field in 1967. When they were adapted to a light environment for 48 hours, their backs were yellowish-brown in ground color. The dorso-median stripe, dorso-lateral folds, irises and dermal protuberances on the back were pale brown, although the marginal portion of each of the protuberances was black. The ventral surface of the body was white.

a. Light microscopy

When a piece of the dorsal skin is cut off and examined under a light microscope, all the three kinds of dermal chromatophores are contracted; an interspace is left between adjacent chromatophores of each kind. Xanthophores are not clear-cut in outline and contain a yellow spot. In contrast with xanthophores, iridophores are clear-cut and isolated from the neighbors by wide interspaces. They are pale brown with metallic luster by lighting with reflected light, while light blue or greenish-blue by lighting with transmitted light. Melanophores are of nearly the same size as or a little smaller than iridophores. Although they have several short arms filled with melanosomes, they are isolated from one another. Some melanophores look black, while the others are dark brown.

b. Electron microscopy

i) Xanthophores Xanthophores are stocky in shape and isolated from the adjacent ones by narrow interspaces. They are filled with pterinosomes and carotenoid vesicles. The pterinosomes are nearly the same as those of the xanthophores of green-type frogs in shape and size. The carotenoid vesicles are about 0.1 μ in diameter, being remarkably smaller and much more electron-dense than those of green-type frogs. They are mostly gathered to form a mass near the central area of the xanthophore and surrounded with pterinosomes. This central mass of carotenoid vesicles in each xanthophore corresponds to the yellow spot which was observed under a light microscope. The pterinosomes are divided into type I and type II, as those in the xanthophores of green-type frogs. They are invisible under a light microscope (Plates I, 2 and IV, 9).

ii) Iridophores Each iridophore appears like a massive, convex lens in shape and is isolated from the neighbors by wide interspaces. It is also isolated from the xanthophore situated above or from the melanophore situated below by a narrow interspace. The reflecting platelets contained in each iridophore are the same as those of green-type frogs in shape. They are mostly arranged irregularly, that is, nonparallel with the surface of skin (Plates I, 2 and IV, 9).

iii) Melanophores Although melanophores are irregular in shape, they have no long arms containing melanosomes. Nearly all the melanosomes are embedded in the body of each melanophore. The melanosomes are the same as those of green-type frogs in shape and size. Premelanosomes are very scarce (Plate I, 2).

II. Six kinds of color variants

1. Blue variant

Blue variants were produced in 1970 by the method of diploid gynogenesis from the following six females obtained in 1967: female No. 1 from a spermatozoon exposed to 90 rads of X-rays, female No. 2 from a spermatozoon exposed to 170 rads of X-rays, females Nos. 4 and 8 from spermatozoa exposed to 130 rads of neutrons and females Nos. 2 and 6 from eggs exposed to 50 rads of neutrons. The offspring produced from females of these blue variants by repeating the

method of diploid gynogenesis in 1973 were also all blue variants. Some of the offspring derived from No. 8 (W♀×SN–130♂) were used for examining the abnormality in chromatophores.

When adapted to a light environment for 48 hours, they were dull blue-green in dorsal ground color. The dorso-median stripe and dermal protuberances on the back were also dull blue-green. The dorso-lateral folds and irises were pale brown. The ventral surface of the body was white.

a. Light microscopy

All the three kinds of chromatophores are horizontally expanded as those of green-type frogs. Xanthophores are invisible by lighting with transmitted light. They are also invisible in most cases by lighting with reflected light, although some of them rarely look pale yellow. Iridophores were pale blue with metallic luster by lighting with reflected light and dull purple by lighting with transmitted light. Melanophores are always black when observed with reflected light as well as with transmitted one.

b. Electron microscopy

The three kinds of chromatophores are very similar to those of green-type frogs, except that the xanthophores contain no carotenoid vesicles. The blue color of the blue variants seems to be attributable to the absence of carotenoid vesicles (Plates II, 3 and V, 12).

2. Grayish-brown variant

This kind of variant is very similar to wild, brown-type frogs in color, except that they are darker than the latter. Grayish-brown variants were produced in 1970 by the method of diploid gynogenesis from the following five females obtained in 1967: female No. 1 from a spermatozoon exposed to 90 rads of X-rays, females Nos. 2 and 9 from spermatozoa exposed to 130 rads of neutrons and females Nos. 8 and 9 from spermatozoa exposed to 130 rads of neutrons. When the offspring of female No. 8 obtained from a spermatozoon exposed to 130 rads of neutrons were adapted to a light environment for 48 hours, there appeared two kinds of frogs, normal brown-type and grayish-brown. While the normal brown-type frogs are somewhat yellowish, the grayish-brown variants are not tinged with yellow.

a. Light microscopy

When a piece of the dorsal skin of a grayish-brown variant is observed under a light microscope, iridophores and melanophores are the same as those of brown-type frogs. In contrast with these two kinds of dermal chromatophores, xanthophores differ from those of brown-type frogs; they are invisible by lighting with reflected or transmitted light.

b. Electron microscopy

The three kinds of dermal chromatophores are the same as those of the brown-type frogs in shape and structure, except that the xanthophores contain no carotenoid vesicles. The xanthophores are filled with pterinosomes only. Type I and type II of the latter are distinguished from each other (Plate II, 4).

3. Greenish-olive variant

The greenish-olive variant is one of the four kinds of olive variants whose ventral surfaces are pale olive. They were first produced by the method of diploid gynogenesis in 1970 from female No. 1 which was derived from an egg exposed to 50 rads of neutrons in 1967. The frogs used for microscopical observation were obtained from a mating between a female and a sex-reversed phenotypic male of the above gynogenetic diploids which were greenish-olive variants. When they were adapted to a light environment for 48 hours, their backs were greenish-olive in ground color. They had a yellow-green dorso-median stripe, grayish-yellow dorso-lateral folds and irises, and dark brown or light grayish-brown protuberances on the back, while the ventral surface of the body was pale olive and semi-transparent.

a. Light microscopy

When a piece of the dorsal skin of a greenish-olive variant is observed under a light microscope, the three kinds of chromatophores are similar in shape to those of green-type frogs; they are horizontally expanded. The xanthophores and melanophores do not differ in color from those of green-type frogs when observed by lighting with reflected light as well as with transmitted one. In contrast with these two kinds of chromatophores, the iridophores differ distinctly from those of green-type frogs. When observed with reflected light, they are pale bluish-olive. When they are overlaid with xanthophores, they look greenish-olive. By lighting with transmitted light, the iridophores look light purple.

b. Electron microscopy

The three kinds of chromatophores in the skin of a greenish-olive variant are very similar to those of green-type frogs in shape and structure, except that the iridophores are abnormal in their inclusion. The reflecting platelets contained in each iridophore are distinctly small in size and nearly half the thickness of normal reflecting platelets. Moreover, they are remarkably few. The xanthophores and melanophores are normal and do not differ from those of green-type frogs in every respect (Plates III, 6 and V, 13).

4. Bluish-olive variant

Two bluish-olive variants were first produced by the method of diploid gynogenesis in 1970 from female No. 8 which was derived from a spermatozoon exposed to 130 rads of neutrons in 1967. The frogs used for microscopical observation were those which produced from one of these two variants by repeating the method of diploid gynogenesis in 1973. When adapted to a light environ-

ment for 48 hours, they were bluish-olive in the ground color of the back. The dorso-median stripe was light bluish-olive, the dorso-lateral folds and irises were grayish-yellow and the dermal protuberances on the back were dark or pale brown. The ventral surface of the body was pale olive and semi-transparent.

a. Light microscopy

When a piece of the dorsal skin is observed under a light microscope, xanthophores are invisible by lighting with reflected or transmitted light. In this respect, the bluish-olive variant is distinguishable from the greenish-olive one. The three kinds of chromatophores are horizontally expanded. The iridophores and melanophores are very similar to those of greenish-olive variants.

b. Electron microscopy

Bluish-olive variants differ from greenish-olive ones in that the xanthophores are deprived of carotenoid vesicles. In the other respects, these two kinds of color variants are very similar to each other (Plates II, 5 and V, 14).

5. Yellowish-olive variant

This kind of color variant was produced in 1970 by the method of diploid gynogenesis from the following nine females obtained in 1967: female No. 9 from a spermatozoon exposed to 170 rads of X-rays, females Nos. 1 and 4 from eggs exposed to 145 rads of X-rays, and females Nos. 1, 3, 8 and 9 from spermatozoa exposed to 130 rads of neutrons, and females Nos. 1 and 2 from eggs exposed to 50 rads of neutrons. Two color variants produced from female No. 8 of these nine females were adapted to a light environment for 48 hours. As a result, it was found that one of them was a yellowish-olive variant, while the other was a grayish-olive one.

The yellowish-olive variant was yellowish-olive in the ground color of the back. The dorso-median stripe, dorso-lateral folds and irises were also yellowish-olive, while the dermal protuberances on the back were grayish-olive. The ventral surface of the body was pale olive and semi-transparent.

a. Light microscopy

When a piece of the dorsal skin is observed under a light microscope, all the three kinds of chromatophores are contracted. The xanthophores and melanophores are normal; they do not differ from those of brown-type frogs in shape and structure. Differing from these two kinds of chromatophores, the iridophores are abnormal. They look pale brownish-olive by lighting with reflected light, while purplish-blue by lighting with transmitted light.

b. Electron microscopy

The yellowish-olive variant is very similar to brown-type frogs in that the three kinds of chromatophores are stocky in shape. The xanthophores and melanophores do not differ from those of brown-type frogs in shape and structure. How-

ever, the iridophores of the yellowish-olive variant are abnormal; the reflecting platelets contained in each iridophore are extremely few and, moreover, they are only about half the thickness of those of brown-type frogs (Plates III, 7 and IV, 10). The iridophores themselves are somewhat thinner than those of the latter, too.

6. Brownish-olive variant

Brownish-olive variants were produced in 1970 by the method of diploid gynogenesis from three females, that is, female No. 9 from a spermatozoon exposed to 170 rads of X-rays and females Nos. 8 and 9 from spermatozoa exposed to 130 rads of neutrons in 1967. A brownish-olive variant which was produced from female No. 8 and became a sex-reversed phenotypic male was adapted to a light environment for 48 hours. As a result, the ground color of the back as well as the irises, dorso-median stripe, dorso-lateral folds and dermal protuberances on the back were brownish-olive. The ventral surface of the body was pale olive and semi-transparent.

a. Light microscopy

Xanthophores are invisible by lighting with reflected and transmitted lights. Iridophores are very similar to those of the yellowish-olive variant stated above under a light microscope; pale brownish-olive by lighting with reflected light and pale purplish-blue by lighting with transmitted light. Melanophores are normal and do not differ from those of brown-type frogs.

b. Electron microscopy

The three kinds of chromatophores are stocky in shape as those of brown-type frogs. However, both xanthophores and iridophores are abnormal. The xanthophores are deprived of carotenoid vesicles, while they contain pterinosomes that are normal in structure and amount. The iridophores are the same as those of the yellowish-olive variant. The reflecting platelets contained in each iridophore are extremely few and, moreover, they are distinctly reduced in thickness. The melanophores do not differ from those of brown-type frogs (Plate III, 8).

DISCUSSION

The majority of amphibians has three kinds of dermal chromatophores, xanthophores, iridophores (guanophores) and melanophores (PARKER, 1948). The behaviors of these chromatophores in the rapid color changes of the European tree-frog, *Hyla arborea*, have already been described in detail by SCHMIDT (1920). However, the fine structure of each kind of chromatophore was not clarified at the level of a light microscope. KAWAGUCHI, KAMISHIMA and SATO (1965) examined the green and brown skins of the Japanese tree-frog, *Hyla arborea japonica*, under an electron microscope and described the arrangement and fine structure of each of the three kinds of chromatophores. According to them,

melanin granules are concentrated under the basal portion or at most along the side wall of the iridophores in the green skin, while these granules are more densely distributed over the iridophores than below and mask the effect of the iridophores, resulting in brown coloration in the brown skin. In two American tree-frog species and two other anurans including *Rana pipiens*, BAGNARA, TAYLOR and HADLEY (1968) have found that the three kinds of dermal chromatophores comprise a morphologically and physiologically distinct unit and described details of chromatophore structure in the state adapted to a white or dark background. Pale coloration ranging from tan to green was provided by the xanthophores and iridophores overlying the melanophores.

BERNS and NARAYAN (1970) compared a blue part of the skin of a blue variant *Rana clamitans* with a green part under light and electron microscopes. The blue skin had no xanthophore layer above the iridophores. There was an unusual cell type which contained neither pterinosomes nor carotenoid vesicles in place of xanthophores. In the green skin, there was a contiguous xanthophore layer above the iridophores which lay directly above the melanophores. The xanthophores contained comparatively large carotenoid vesicles intermingled with clear pterinosomes.

The dorsal skin of the green-type *Rana nigromaculata* is very similar in gross and fine structures to the green skin described by BERNS and NARAYAN. The dorsal skin of the brown-type frog differs from the green one in that all the three kinds of dermal chromatophores are contracted. While the iridophores of the green skin reflect a pale blue light, those of the brown skin reflect a pale brown. This difference in reflected light seems to be attributable to a difference in the arrangement of reflecting platelets in each iridophore. Moreover, the green skin differs from the brown one in the arrangement of carotenoid vesicles in each xanthophore. Accordingly, the green color seems to be produced from the collaboration of a yellow layer of contiguous xanthophores, a pale blue layer of contiguous iridophores and a dark background formed by many long arms of expanded melanophores. In contrast with this, the brown color seems to occur mainly from the layer of iridophores which reflect a pale brown light. The latter is more or less modified by a xanthophore layer with minute yellow spots and a dark layer of contracted melanophores.

The blue variant in *Rana nigromaculata* differs from *Rana clamitans* reported by BERNS and NARAYAN in the existence of a xanthophore layer. While the American blue frog had no xanthophore layer, the Japanese one had this layer, although each xanthophore is devoid of carotenoid vesicles. The variant affecting xanthophores occurs by the presence of a recessive gene x in the homozygous condition (NISHIOKA, 1977). The other two kinds of dermal chromatophores, iridophores and melanophores, in the blue variant do not differ from those of the green-type frog. The skin of the grayish-brown variant is also very similar to that of the brown-type frog except that the xanthophores contain no carotenoid vesicles.

The four-types of olive variants, the greenish-olive, bluish-olive, yellowish-olive and brownish-olive, are common with respect to the abnormality of irido-

phores. The variation affecting iridophores occurs by the presence of a recessive gene *i* in the homozygous condition (NISHIOKA, 1977). In the greenish-olive variant the iridophores are distinctly small and contain fewer, smaller and thinner reflecting platelets than those in the iridophores of the green-type frog. The xanthophores and melanophores of the greenish-olive variant do not differ from

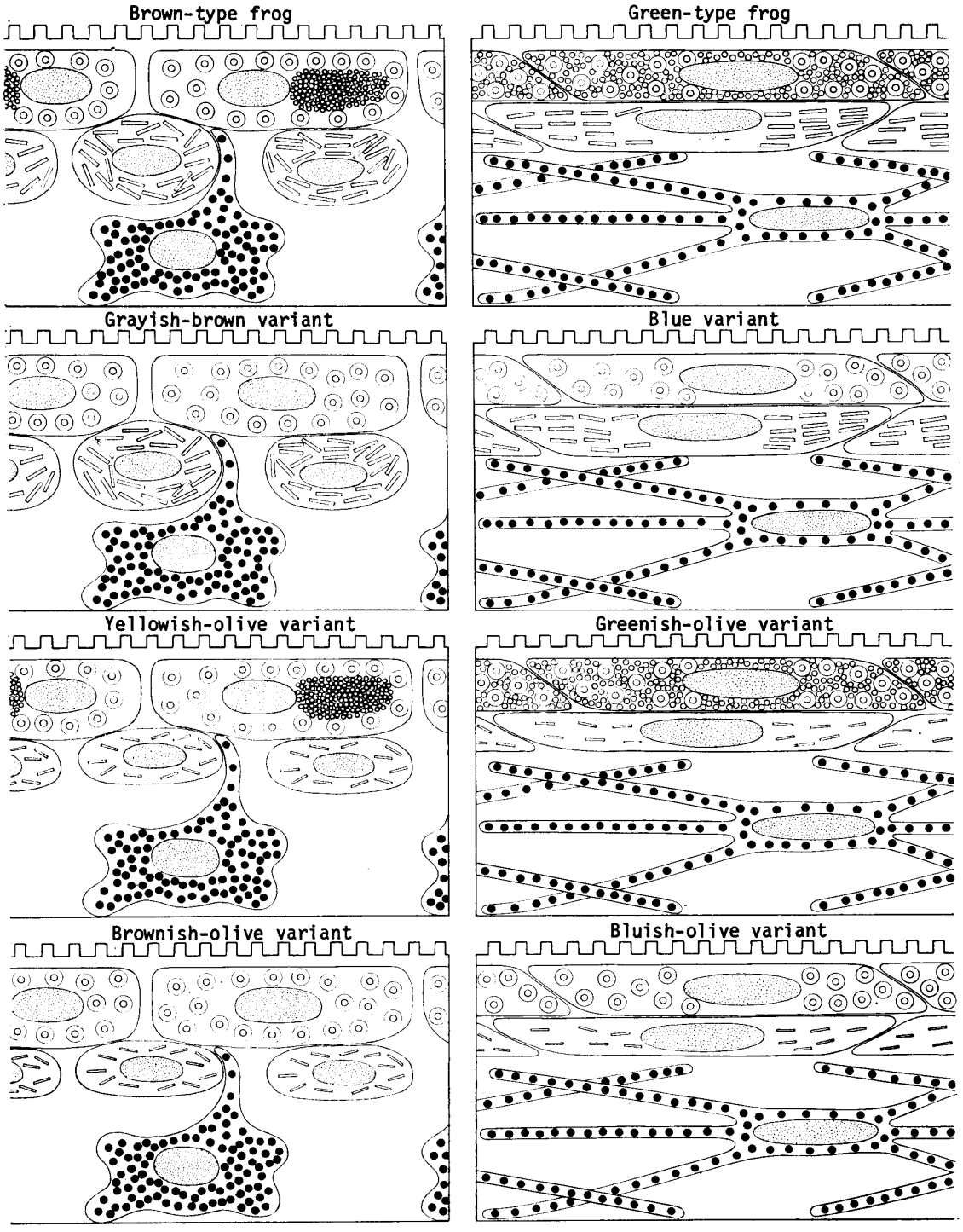

Fig. 1. Schematic representation of the three kinds of dermal chromatophores, xanthophores (upper), iridophores (middle) and melanophores (lower), in two types of normal frogs and six color variants.

those of the green-type frog in arrangement, size, shape and structure. The skin of the yellowish-olive variant is likewise very similar to that of the brown-type frog except that the iridophores are very abnormal as in the greenish-olive variant. The bluish-olive variant is the same as the brownish-olive one in that both xanthophores and iridophores are abnormal. However, all the three kinds of dermal chromatophores are expanded in the bluish-olive variant as in the green-type frog, while they are all contracted in the brownish-olive variant as in the brown-type frog (Fig. 1).

It seems rather curious that there was only one kind of abnormality in xanthophores or iridophores among different color variants. This situation is similar to that in albino *Rana nigromaculata* induced by irradiation of gametes with X-rays or neutrons (NISHIOKA, 1977), and differs from those in albino *Hyla arborea japonica* (NISHIOKA and UEDA, 1977) and *Rana nigromaculata* (NISHIOKA and UEDA, unpublished) collected from the field. Accordingly, it is assumed that there are more than one kind of abnormality in xanthophores or iridophores among color variants found occasionally in the field. A variant *Rana nigromaculata* similar to a blue one described by BERNS and NARAYAN in *Rana clamitans* may be found before long.

SUMMARY

1. The dorsal ground colors of normal, green-type (*XIE*) and brown-type (*XIee*) frogs and six color variants, blue, grayish-brown, greenish-olive, bluish-olive, yellowish-olive and brownish-olive, derived from irradiated gametes in *Rana nigromaculata* were examined under light and electron microscopes. The fine structures of the xanthophores, iridophores and melanophores in the dorsal integument of each of these eight kinds of frogs are described.

2. In the presence of *E*, all the three kinds of dermal chromatophores, xanthophores, iridophores and melanophores, are expanded. The carotenoid vesicles are mingled with pterinosomes, without being gathered into a mass in each xanthophore. Reflecting platelets are mostly arranged in parallel with the surface of skin in each iridophore.

In the presence of *ee*, all the three kinds of dermal chromatophores, xanthophores, iridophores and melanophores, are contracted. The carotenoid vesicles are mostly gathered to form a mass near the central area of each xanthophore. The reflecting platelets are mostly arranged irregularly.

3. In the presence of *xx*, no carotenoid vesicles are produced in each xanthophore. In the presence of *ii*, the reflecting platelets become remarkably fewer, smaller and thinner in each iridophore.

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EXPLANATION OF PLATES

PLATE I

Electron microphotographs of dermal chromatophores in the dorsal skin of *Rana nigromaculata*, I. ×4000.

1. Three kinds of chromatophores in a green-type frog.
2. Three kinds of chromatophores in a brown-type frog.

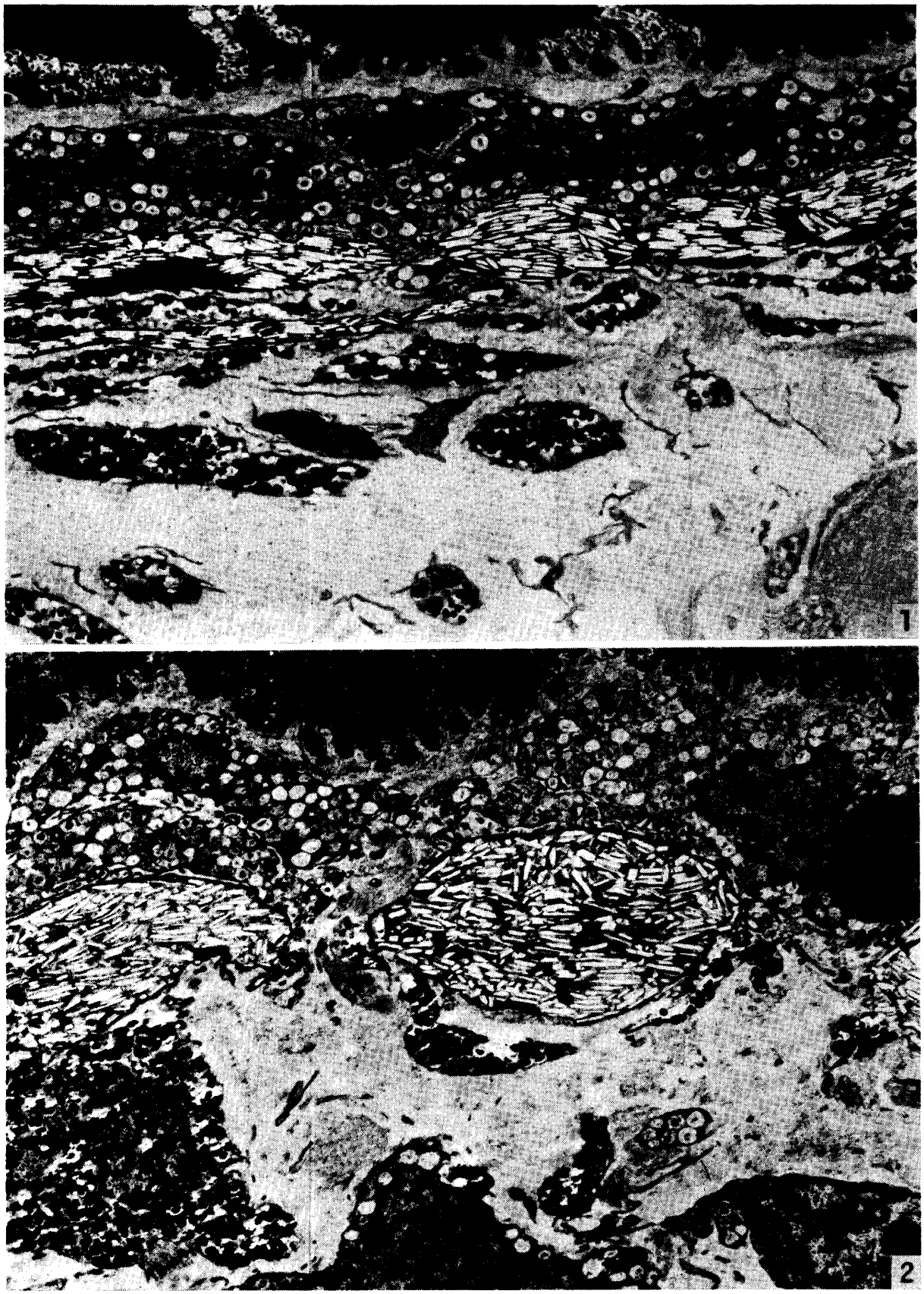
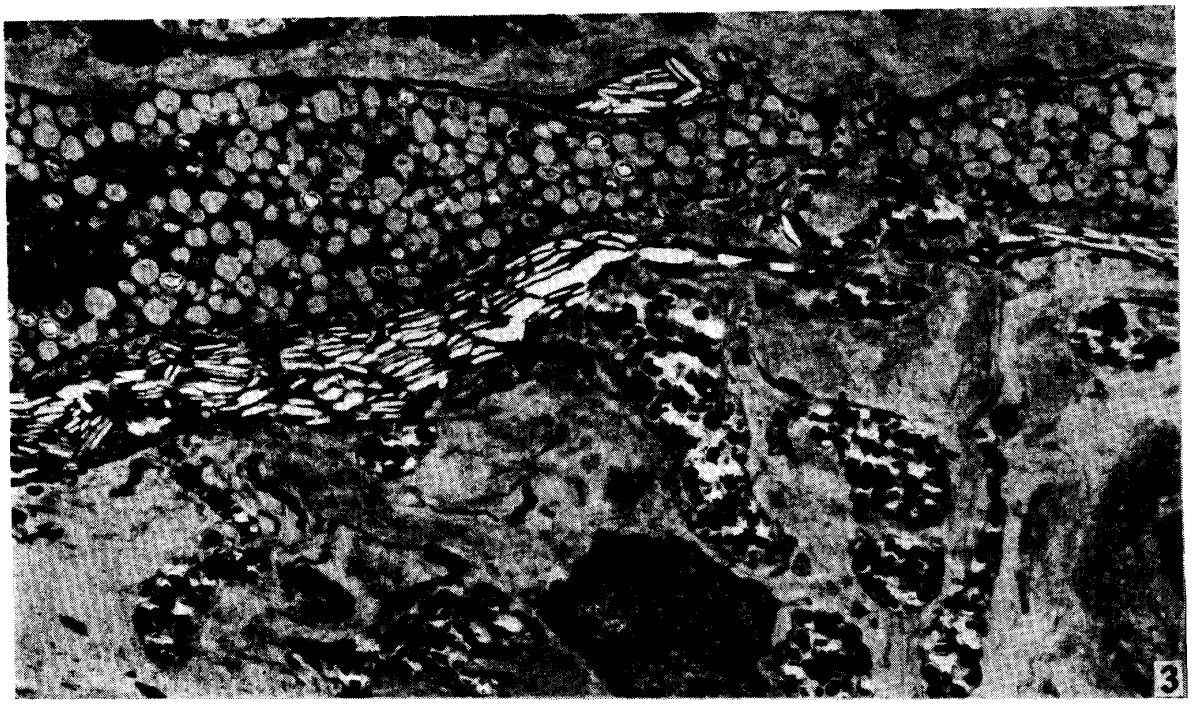


PLATE II

Electron microphotographs of dermal chromatophores in the dorsal skin of *Rana nigromaculata*, II. ×4000.

3. Three kinds of chromatophores in a blue variant.
4. Three kinds of chromatophores in a grayish-brown variant.
5. Three kinds of chromatophores in a bluish-olive variant.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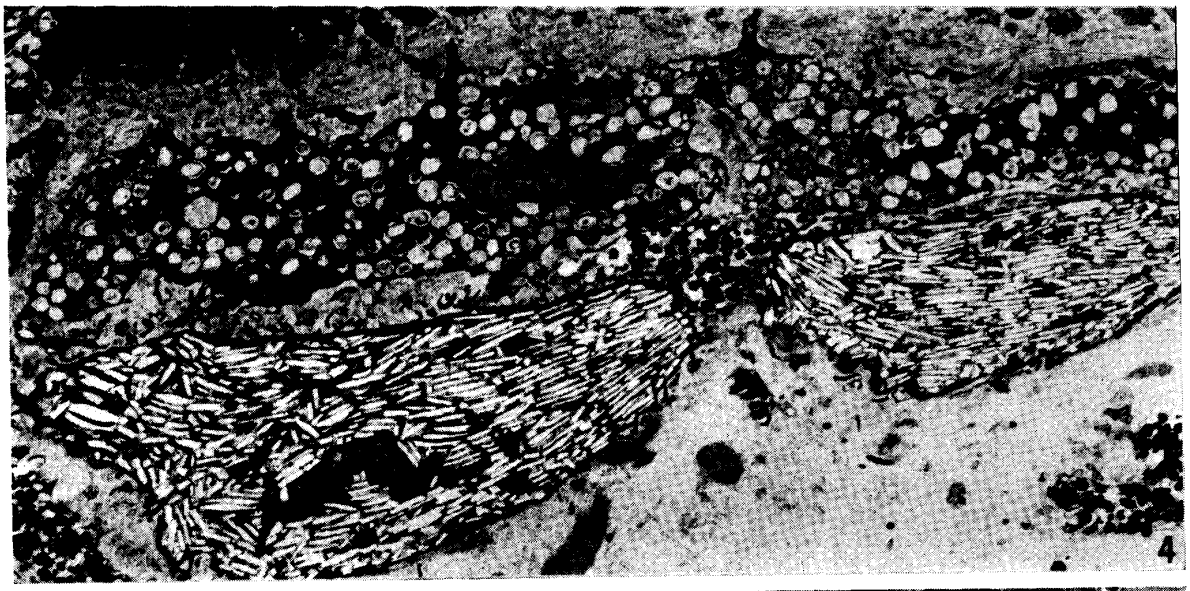
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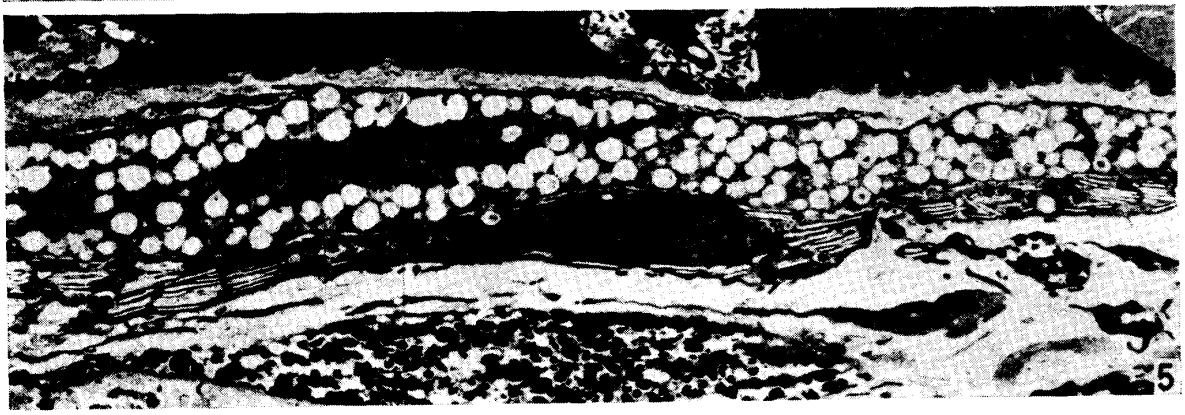
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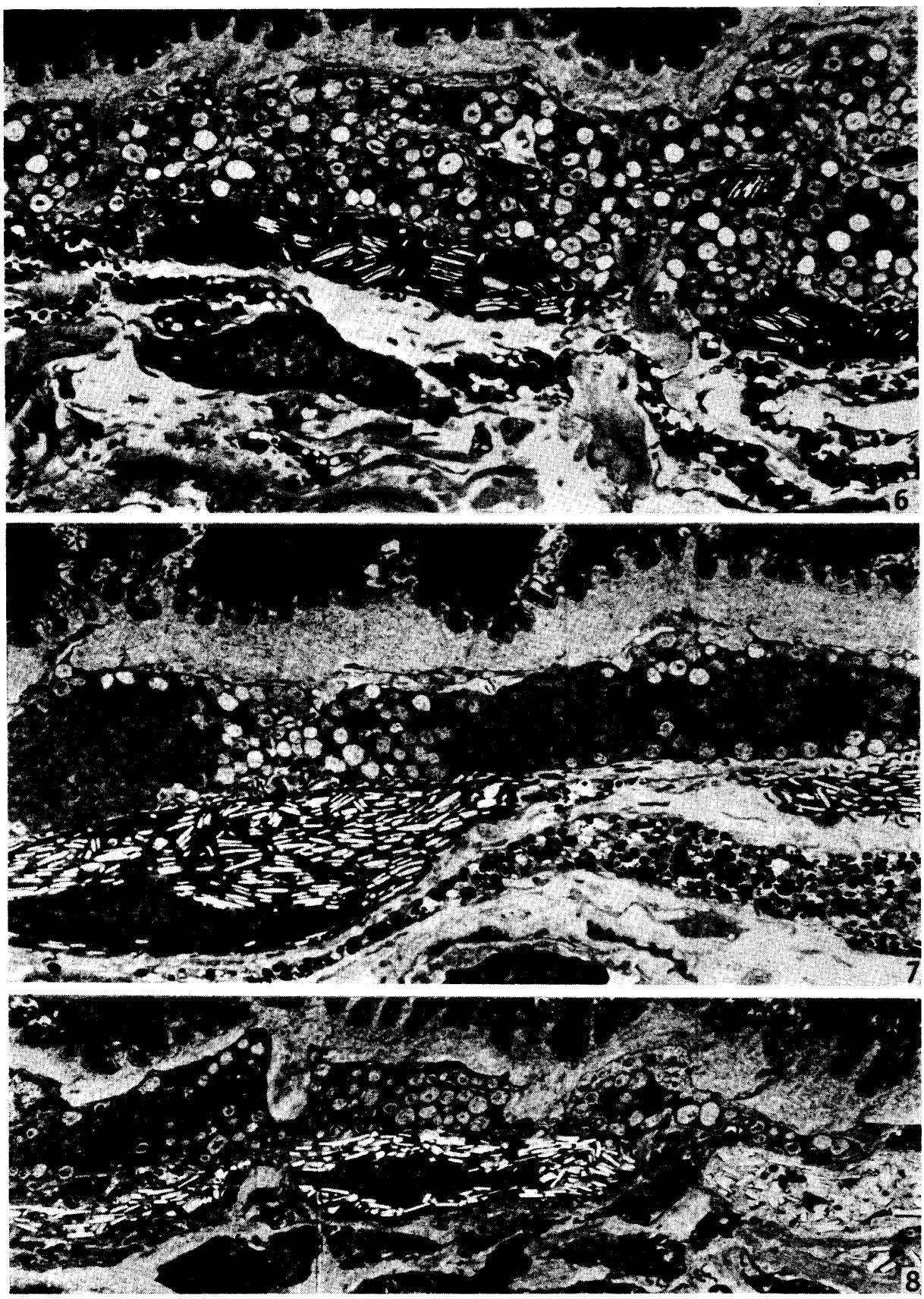
PLATE III

Electron microphotographs of dermal chromatophores in the dorsal skin of *Rana nigromaculata*, III. ×4000.

6. Three kinds of chromatophores in a greenish-olive variant.
7. Three kinds of chromatophores in a yellowish-olive variant.
8. Three-kinds of chromatophores in a brownish-olive variant.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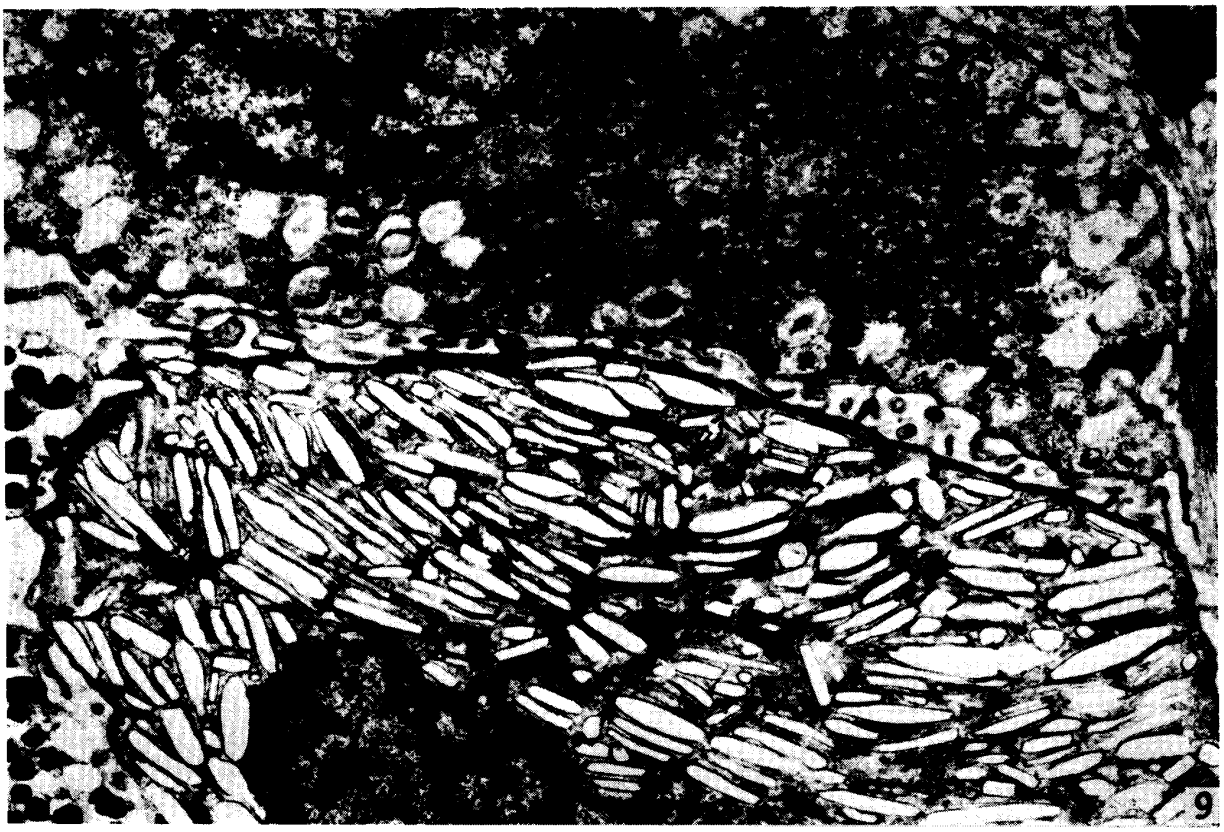
8

PLATE IV

Electron microphotographs of dermal chromatophores in the dorsal skin of *Rana nigromaculata*, IV. ×8200.

9. Portion of a xanthophore and an iridophore in a brown-type frog.
10. Portion of a xanthophore and an iridophore in a yellowish-olive variant.


9

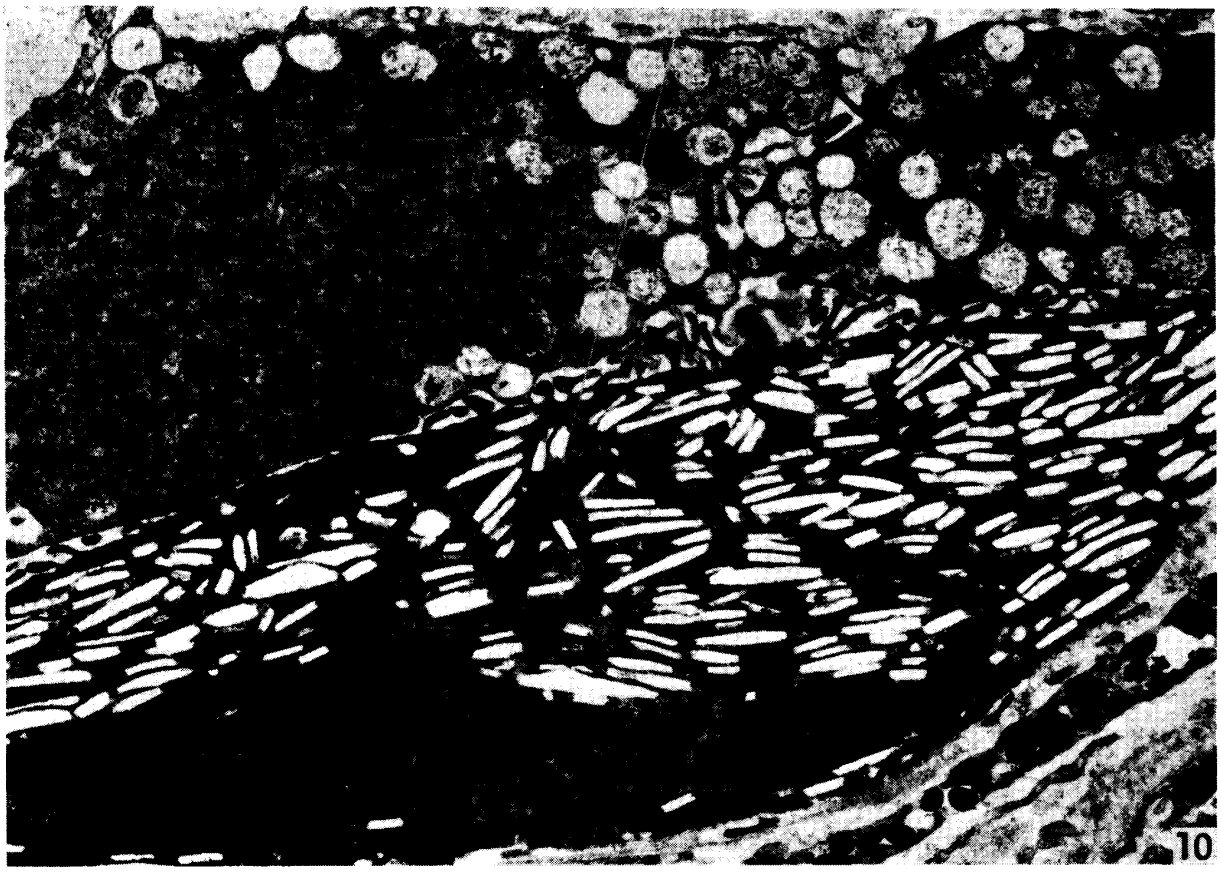
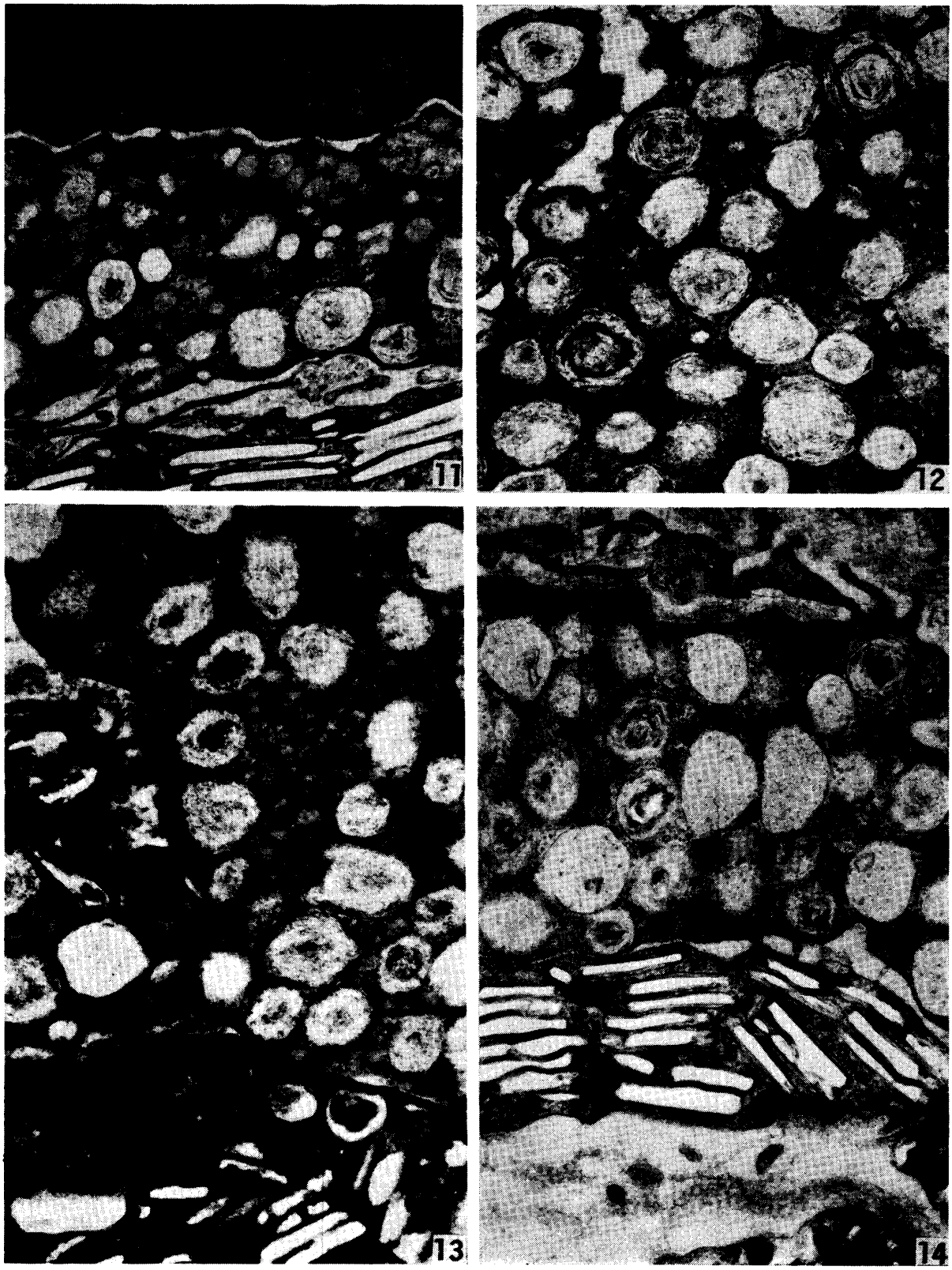

10

PLATE V

Electron microphotographs of dermal chromatophores in the dorsal skin of *Rana nigromaculata*, V. $\times 16500$.

11. Portion of a xanthophore in a green-type frog.
12. Portion of a xanthophore in a blue variant.
13. Portion of a xanthophore in a greenish-olive variant.
14. Portion of a xanthophore in a bluish-olive variant.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