한국전자현미경학회지 제 27 권 제 1 호 1997
Korean J. Electron Microscopy 27(1), 71~86(1997)

Redifferentiation of the Cutaneous Pigment System during the Wound Healing Process in the Goldfish, *Carassius auratus*

Moon, Myung-Jin and Moon-Jin Jeong
Division of Biological Sciences, Dankook University, Cheonan 330-714, KOREA

금붕어 (*Carassius auratus* L.) 상처치유과정중 피부색소체계의 재분화에 관한 연구

문 명 진 · 정 문 진
단국대학교 자연과학대학 생물과학부
(Received February 10, 1997)

요 약

상처치유과정중 관상어류 피부 색소체계의 재분화경로를 규명하기 위하여 외부 색채가 화려한 담수산 금붕어 (*Carassius auratus* L.)를 실험재료로 하여 피부의 일정부위에 인위적인 상처를 유도한 후, 시간 경과에 따른 조직과 색소 체계의 치유과정을 고배율의 투과 전자현미경으로 관찰하였다. 금붕어 피부 색소세포는 정상조직에서 황색소세포, 백색소세포 그리고 흑색소세포 등 세 종류의 진피성 색소세포로 이루어져 있었다. 황색소세포에는 pterinosome과 carotenoid vesicle 등 두 종류의 색소과립이 분포되어 있었고, 백색소세포와 흑색소세포에는 무정형 색소결정인 leucosome과 전자밀도가 높은 구형의 색소과립인 melamosome이 각각 함유되어 있었다.

초기 상처치유반응은 상처 유도직 후에 표면 손상부위로 전이되는 상피세포와 혈구세포에 의하여 수행되었다. 상처 유도후 5~7일이 경과된 조직의 표본에서는 조면소포체가 특이하게 발달되어 진피성 색소세포의 공통 원기로 추정되는 세포의 출현이 확인되었다. 또한 재생된 조직내에서 재분화된 색소세포는 상처유도 후 3주가 경과된 표본에서 처음 관찰되었다. 색소과립의 재분화 경로는 세포 내에서의 색소과립 형성 과정과 마찬가지로 색소세포내에 잘 발달된 조면소포체와 골지체를 경유한 후, 분비소포의 형태로 생성됨이 확인되었다. 그리고 재분화된 색소세포로 유입되는 색소 원기물질은 음세포과립을 통하여 수송되었는데, 특히 조면소포체가 풍부한 원기세포와 연접된 색소세포의 원형질막에서 매우 활발한 물질의 수송이 관찰되었다.

한편, 각 색소세포의 일차적인 재분화과정은 상처 유도후 4주가 경과되어 피부 상처가 치유되는 시점을 전후하여 완료되었으나, 재분화된 색소세포의 수나 분포밀도는 충분한 체색발현을 위한 상태에 비해 크게 미달되는 것으로 분석되었다. 따라서 특별한 환경의 변화가 없는 한, 상처유도 이전의 색소체계와 동일한 상태로의 복구에는 적어도 3개월 이상이 소요되는 것으로 확인되었다.

Key words : Differentiation, Wound Healing, Chromatophores, Goldfish, *Carassius auratus*

INTRODUCTION

The changes of the color patterns of fishes are due to the presence and cellular activity of pigment-containing cells located in the integument (Bagnara and Hadley, 1973; Schliwa, 1986). These highly specialized chromatophores are capable of changing body color of the animal by two different mechanisms-a rapid "physiological color change" being mediated by nerve endings, and a much slower "morphological color change" that involves either a decrease or an increase in the total number of pigment cells or the amount of pigment they contain (Fujii and Novales, 1969; Bagnara and Hadley, 1969, '73; Schliwa and Euteneuer, 1983).

As a rule, the primary role of the animal integument is that of a barrier preventing the entry of foreign substances from the environment into the body (Christophers, 1973; Honda *et al.*, 1982). Thus the basic structure must be repaired if the animal is to survive injury. Normal repair follows an orderly sequnece of cellular and biochemical events, initiated by injury and resulting in formation of new tissue (Bereiter-Hahn, 1986). General events during wound healing has been a subject of study for many years, therefore numerous experimental models have been introduced to explain the complex microenvironment of a healing wound (Christophers, 1973; Radice, 1980; Honda *et al.*, 1982; Donaldson and Mahan, 1983; Wong and Gotlieb, 1984).

Studies conducted to date have been concerned almost entirely with healing in higher animals, in which reorganization of the dermal connective tissue plays a leading role in wound healing (Wong and Gotlieb, 1984; Bereiter-Hahn, 1986).

However, little information is available on the wound healing of the cutaneous pigmentary system in ornamental fishes. So this paper presents a detailed study of the morphological differentiation of the cutaneous pigmentary system and discusses the wound healing responses of the chromatophores in bright-colored ornamental fishes.

MATERIALS AND METHODS

Adult goldfish *Carassius auratus* L., one of the most common ornamental fishes, were used in this experiment. One week after caring, wounds were made on the integument by two direct ways either burning with sharp needles or damaging with Laser beam. After wounding, the fishes were incubated (25±2°C) in groups of 4~6 heads in the medium sized aquariums and fed normal food 2~3 times a day.

At days 1, 3, 5, at weeks 1, 2, 3 and at months 1, 2, 3 post-wounding, the integument (apporximately 5 mm×5 mm squares) surrounding the wound was gently cut out and fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde buffered with Cacodylate buffer (Karnovsky, 1965). Postfixation was performed with 1% osmium tetroxide in the same buffer. Subsequently, the tissue pieces were dehydrated in ascending concentrations of ethanol, and embedded in Epon-Araldite mixture via propy-lene oxide. Polymerization was carried out at 60°C for 28 hours.

For routine histology, semithin (1 μm) sections were cut out on glass knives, mounted on glass slides, and stained with 1% toluidine blue in 1% borax. Ultrathin sections (silver interference colors) were obrained from a LKB ultramicrotome using glass or diamond knives. The sections were mounted on Formvar-coated, 200-mesh nickel grids, and double stained with a saturat-

ed aqueous solution of uranyl acetate and lead citrate. The sections were examined with a JEM 100 CX II electron microscope (JEOL Ltd, Japan) at 80 kV.

RESULTS

1. Normal Chromatophores

The goldfish, *Carassius auratus*, contains only dermal chromatophores which composed of three kinds of pigment cells-xanthophores, leucophores and melanophores. The epidermis and the dermal chromatophores are separated by a thick sheath of subepidermal collagenous layer. Dermal xanthophores are always located just underneath the epidermis in close to the basal lamina (Fig. 1). Pigment granules of the xanthophores are carotenoid vesicles and pterinosomes. Carotenoid vesicles have no limiting membrane and marginal electron densities of these vesicles are higher than those of internal region (Fig. 2).

The cutaneous leucophores have oval shaped nucleus and long cytoplasmic processes. Pigment granules of the leucophores-leucosomes-have amorphous limiting membrane and membranous or fibrous inner materials (Fig. 3). Among the three kinds of dermal chromatophores, melanophores are located at the lowest portion. The melanophores have electron dense melanin pigment granules. In the cytoplasm of the melanophores well developed microtubules are observed. Motility of pigment granules are performed by these structures (Fig. 4).

2. At 1, 3 and 5 Days Post-Wounding

Integumental wounds are made by burning with sharp needles or damaging with Laser beam. By this treatment whole parts of epidermis and dermis containing basement membrane are profoundly affected (Figs. 5, 6). After injury, the wound cavity is filled by an mucous material containing components from damaged tissue, cytoplasm, and numerous hemocytes. Some of epidermal cells which have been firmly attached to the basement membrane become detached and mobilized during this phase. This process occurs within one day after injury (Fig. 7).

At 3 days post-wounding, epidermal cells spread over the wound surface by migration. These migrating cells participating in primary wound closure are originated from the adjacent epidermis. It has been observed that all kinds of cells near the wounds seem to have the ability to participate in migration. The lower epidermal cells are separated from each other, and germinative cells from normal epidermis migrate into the dermis (Fig. 8). Particularly numerous hemocytes, mostly composed of leukocytes, are condensely aggregated at the wounded area (Fig. 9).

At the time of primary wound closure, 5 to 7 days after wounding, rER rich cells- presumably common precursors of dermal chromatophores-immigrated into the wound area. The rER rich cells have long and irregular shaped nuclei and its electron densities are relatively higher than those of the chromatophores observed at normal tissues (Fig. 10). In addition to this, free ribosomes, mitochondria and Golgi complexes, which also seen at the normal chromatophores appear at the cytoplasms of this cells (Fig. 11). Most of all this cell is characterized by their long cytoplasmic processes distributed parallel to the basement membrane and well developed rough endoplasmic reticulum within the cytoplasm (Fig. 12).

3. At 1, 2 and 3 Weeks Post-Wounding

Intercellular collagenous fibers appear along

the cellular boundaries during these phases. After primary wound closure, a growth and differentiation phase being started. The moving sheets of tissues contact each other, and high mitotic activity at the newly formed epithelium during this period-1 to 2 weeks post-wounding -is observerd (Fig. 13).

First redifferentiated chromatophores appear 3 weeks after wounding. At the vicinity of this chromatophores, rER rich cells are also observed (Fig. 14). Cellular boundaries between chromatophores and rER rich cells are smooth and regular, showing no special relationships (Fig. 15). However the plasma membrane of the chromatophores dose show numerous pinocytotic vesicles adjacent to the rER rich cells. These pinocytotic vesicles, associated with accumulation of pigment material within chromatophores, appear only at the inner surface of the chromatophores adhering to the rER rich cells, characteristically (Figs. 16, 17).

Multivesicular bodies, which presumed to be the intermediate pigment granules are formed by the vesicular fusion of the Golgi bodies (Fig. 18). And the mosaic pigment cells containing more than one type of pigment also appear at this period(Fig. 19).

4. At 1, 2 and 3 Months Post-Wounding

Cell proliferation and remodelling are balanced by death of cells damaged from burns (Fig. 20). The differentiation of each chromatophore in addition to integumental wound repair is accomplished within 4 weeks after wounding at most cases, however the total numbers and densities of these repaired chromatophores still primitive state (Fig. 21). Moreover, It has been revealed that complete repair of chromatophores at wounded tissues requirs more than 3 months in normal environment.

During this period, differentiation of the pigment granules, especially pterinosomes in xanthophores are clearly distinguished. After the pigment precursors being transferred to chromatophores as the form of pinocytotic vesicles, small vesicles are synthesized from Golgi complex and forming large pigment granules by vesicular fusion (Fig. 22).

By the fine structure of the granular differentiation, pterinosomes are subdivided into 3 types. Most primitive, Type I pternosomes have clear limiting membranes and contain some amorphous fine fibrous structure (Fig. 23). While intermediate Type II pternosomes have thick and densely aggregated fibrous materials, mature Type III pterinosomes have concentric lamellar structure within the granules (Fig. 24).

DISCUSSION

The previous study has indicated most fishes were covered by a stratified squamous epithelium (Merrilees, 1974; Eastman and Hikida, 1991; Hertwig *et al.*, 1992), and contain dermal and epidermal chromatophores (Bagnara and Hadley, 1969; Taylor and Bagnara, 1972). The pigments found in the four major classes of chromatophores are melanins, carotenoids, pteridines, and purines (Baker *et al.*, 1960; Ortiz, *et al.*, 1963; Denton, 1971). And four different types of chromatophores (melanophores, erythrophores, xanthophores, and leucophores or iridophores) were identified in fishes (Bagnara and Hadley, 1969; Taylor and Bagnara, 1972).

In the goldfish, *Carassius auratus*, no epidermal chromatophores were found, and three kinds of pigment cells excluding erytrophores were observed in dermis of the integument. Schliwa (1986) reported that the dermal chromatophores of fishes were far more abundant in

most species, and Moon *et al.*(1986, '87) also reported same observations in ornamental goldfishes.

Basically dermal chromatophores were classified according to the color of the pigment they contain (Bagnara and Hadley, 1973). Since the red or yellow colors of xanthophores and erythrophores were commonly composed of carotenoids and pteridines (Ortiz *et al.*, 1963; Kamei-Takeuchi and Hama, 1971; Byers and Porter, 1977), sometimes it is very difficult to distinguish each other. However by electron microscopical observation, some morphological criteria were already established by several workers (Kamei-Takeuchi and Hama, 1971; Yasutomi and Hama, 1972; Menter *et al.*, 1978). According to these criteria, red color of the goldfish was due to the presence of xanthophores rather than erythrophores.

It has been reported that most animal species are unable to synthesize carotenoids, they have to be supplied with the animal's diet (Fox and Vevers, 1960; Bagnara and Hadley, 1973; Schliwa, 1986). Since xanthophores of the goldfish also contain the insoluble carotenoid pigments (Yasutomi and Hama, 1972; Moon *et al.*, 1986), pigment precursors must be supplied during the period of wound healing. By this reason, the colors of some ornamental fishes can be controled by artificial diet commercially.

Experimental investigation reveals mechanical injury to animals is preferred to wounds made by burning, or by local application of toxic substances (Christophers, 1973; Phromsuthirak, 1977; Pickering *et al.*, 1982). However the influence of very weak impact was reported in fish skin (Finn and Nielson, 1971; Pickering *et al.*, 1982). In this experiment, integumental wounds were made by damaging with Laser beam or burning with sharp needles according to Pickering *et al.*(1982). By this treatment whole parts of epidermis and dermis were affected, so redifferentiation of dermal chromatophores after wounding was easily induced.

According to Finn and Nielson (1971), Anderson and Roberts (1975), Phromsuthirak (1977), and Mittal *et al.*(1978), the inflammatory reaction is similar to that in mammals but less extensive and slower in fishes. Winter (1973) assumes that delayed healing of burn wounds is caused by a higher resistance of heated collagen against collagenase digestion. But integument of the ornamental fishes have not a mount of collagen, wound healing process were short and more easy than those of other animals.

In the case that the scales within integument were damaged by burning, the repair process was more complicated and was required additional healing periods.

The redifferentiation of the wounded scales was similar to that of the normal scale regeneration reported by Anderson and Roberts (1975), Sire (1984) in teleosts, and cellular events during regeneration of *Fundulus* scales by Frietsche and Bailey (1980).

There are two sequence of events during wound healing; one relates to the re-epithlialization of the wound, the other to degradation of injured tissue and reorganization of the dermal connective tissue (Viziam *et al.*, 1964; Christophers, 1973; Donaldson and Mahan, 1983). Simiar processes were deteched on the integument of the goldfish. After injury, wound cavity was filled by an mucous material containing components from damaged tissue, cytoplasm, and numerous hemocytes. Epidermal cells become detached and mobilized within one day after injury. And three days post-wounding, all kinds of cells near the wounds, mainly epidermal cells spread over the wound surface by migration.

The fundamental role of epidermal cell migration in wound closure is obvious (Marks *et al.*, 1972; Radice, 1980; Honda *et al.*, 1982). Phromsuthirak (1977) found macrophage, neutrophil granulocytes, lymphocytes and eosinophil granulocytes in dermal wounds of the teleost fish *Gasterosteus aculeatus*. Since these leukocytes were also present in normal skin, he concluded that most of them were immigrated from the blood into the dermis and also into the epidermis after wounding.

In small superficial wounds migration may be sufficient to restore epithelial continuity within less than one hour (Viziam *et al.*, 1964; Wong and Gotlieb, 1984).

However the exact mechanism of cell migration remains unclear. And the events taking places in the dermis after injury vary widely with the degree of tissue damage (Marks *et al.*, 1972; Bereiter-Hahn, 1986). In recent years, it has been reported that the closure of wounds were mediated by proliferation of undifferentiated mesenchymal cells and fibrocytes (Aho *et al.*, 1983), and differentiation of fibroblasts or myofibroblasts (Squier, 1981; Squier *et al.*, 1983).

The development of pigment cells and formation of pigment granules were investigated by several workers. Pigment cells of the vertebrate are derived from the neural crest, and migrate to their final location in the integument during later stages of development (Volpe, 1964; Weston, 1970). However, the mechanisms that control this migration were still not understood (Schliwa, 1986).

Recently it has been reported that while still in their migratory phase, cells may initiate the synthesis of their characteristic pigments. And it has been hypothesized that the various pigment cells are derived from a pluripotent stem cell capable of differentiation into any of the major chromatophore cell types (Bagnara *et al.*, 1979).

The important feature of this stem cell is a primordial organell derived from the endoplasmic reticulum that can differentiate into any of the known pigmentary organelles (Bagnara *et al.*, 1979; Schliwa, 1986).

During the wound repair in goldfish integument, we can observe undifferentiated rER (rough Endolasmic reticulum) rich cells, presumably the dermal stem cells, about three weeks after wounding. This stem cells appeared only at the dermal layer, have long cytoplasmic processes and well developed rough endoplasmic reticulum characteristically. Moreover numerous pinocytotic vesicles of the chromatophores adjacent to this stem cells represent close association of pigment accumulation. From these observations, such hypothesis was being possible that dermal chromatophores regenerated from wounding were also differentiated from this stem cells.

The molecular mechanism of granule movements in pigment cells is still not understood, however two possible models of the mass movement of pigment granules, which forms the cellular basis for color change are reported. While the first model holds that pigment granules are moved by direct interaction with microtubules, the second is the presence of the filamentous components of the contractile system (Byers and Porter, 1977; Schliwa and Eutereuer, 1983; Schliwa, 1986).

By these evidences observed at this study, it is confirmed that regeneration or redifferentiation of the pigment granules after wounding will be proceeded as follows; the pigment material is first synthesized from the rER rich cells, and is transferred to chromatophores as the form of pinocytotic vesicles. After that, small vesicles are synthesized from Golgi complex via rough

endoplasmic reticulum, and large pigment granules are formed by the vesicular fusion. At this experiment we could observe some fine structural evidences suggesting that the limiting membranes of the pigment granules are also originated from the Golgi complexes (Duden *et al.*, 1991), however further precise studies will have to be carried out confirming this observation.

ABSTRACT

The regeneration and differentiation of the cutaneous pigment system in the goldfish, *Carassius auratus* during the wound healing process were studied with high magnification electron microscope. The cutaneous pigment cells of the normal tissues were composed of three kinds of dermal chromatophores-xanthophores, leucoiphores and melanophores. While xanthophores contain two kinds of pigment granules-pterinosomes and carotenoid vesicles, leucophores and melanophores contain amorphous pigment granules (leucosomes) and oval shaped electron dense melanin pigment granules (melanosomes) respectively.

After injury, primary wound healing responses being carried out by migration of epidermal cells and hemocytes spreading over the wound surface at the day of wounding. And at the time of primary wound closure, 5 to 7 days after wounding, rER rich cells-presumably common precursors of dermal chromatophores-immigrated into the wound area. First redifferentiated chromatophores appeared 3 weeks after wounding. Pigment granules of the chromatophores were emerged from the cytoplasmic Golgi complex via rough endoplasmic reticulum. Pinocytotic vesicles which associated with accumulation of pigment material, appeared only at the inner surface of the chromatophores adhering to the rER rich cells, characteristically.

The differentiation of each chromatophore in addition to integumental wound repair were accomplished within 4 weeks after wounding at most cases, however the total numbers and densities of these repaired chromatophores still primitive state. Moreover, It has been revealed that complete repair of chromatophores at wounded tissues from burns requirs more than 3 months in normal environment.

ACKNOWLEDGEMENTS

The paper was supported by NON DIRECTED RESEARCH FUND, Korea Research Foundation, 1995, Project No. 01-D-0476.

REFERENCES

Aho HJ, Viljantom J, Raekallio J, Pelliniemi LJ, 1983. Ultrastructural characteristic of cells in human wound collected by cellstic device. J. Surg. Res. 35, 498-506.

Anderson CD, Roberts RJ, 1975. A comparison of the effects of temperature on wound healing in a tropical and a temperate teleost. J. Fish Biol. 7, 173-182.

Bagnara JT, Hadley ME, 1969. The control of bright colored pigment cells of fishes and amphibians. Amer. Zool. 9, 465-478.

Bagnara JT, Hadley ME, 1973. Chromatophores and color change. Prentice-Hall, pp. 58-68.

Bagnara JT, Matsumoto J, Ferris W, Frost SK, Turner Jr. WA, Tchen TT, Taylor JD, 1979. Common origin of pigment cells. Science 203, 410-415.

Baker RV, Birbeck MSC, Blaschko H, Fitzpatrick TB, Seiji M, 1960. Melanin granules and mitochondria. Nature 187, 392.

Bereiter-Hahn J, 1986. Epidermal cell migration and wound repair. In Biology of the Integument, Eds. J. Bereiter-Hahn *et al.*, Springer-

Verlag, Berlin, pp. 443-471.

Byers HR, Porter KR, 1977, Transformation in the structure of the cytoplasmic ground substance in erythrophores during pigment aggregation and dispersion. J. Cell Biol. 75, 541.

Christophers E, 1973. Kinetic aspects of epidermal healing. In Epidermal Wound Healing, Eds. J.I. Maibach and D.T. Rovee, Year Book Med. Publ., Chicago, pp. 53-69.

Denton E, 1971. Reflectors in fishes. Sci. Amer. 224, 65.

Donaldson DJ, Mahan JT, 1983. Fibrinogen and fibronectin as substrates for epidermal cell migration during wound closure. J. Cell Sci. 62, 117-127.

Duden R, Allan V, Kreis T, 1991. Involvement of β-COP in membrane traffic through the Golgi complex. Trend Cell Biol. 1, 14-19.

Eastman JT, Hikida RS, 1991. Skin structure and vascularization in the antartic notothenoid fish *Gymnodraco acuticeps*. J. Morphol. 208, 347-365.

Finn JP, Nielson NO, 1971. The inflammatory response of rainbow trout. J. Fish Biol. 3, 463-478.

Fox HM, Vevers G, 1960. The Nature of Animal Colours. Sidgwick & Jackson Ltd., London, pp. 1-21.

Frietsche RA, Bailey CF, 1980. The histology and calcification of regenerating scales in the black-spottet topminnow, *Fundulus olivaceus* (Storer). J. Fish Biol. 16, 693-700.

Fujii R, Novales RR, 1969. Cellular aspects of the control of physiological color changes in fishes. Am. Zool. 9, 453-463.

Hertwig I, Eichelberg H, Hentschel J, 1992. Light and electron microscopic studies of the skin of the Palembang puffer, *Tetraodon steindachneri* (Teleostei: Tetraodontidae). Zoomorphology 111, 193-205.

Honda, H, Higuchi Y, Kani K, 1982. Cell movements in a living mammalian tissue: Long-term observation of individual cells in wounded corneal endothelial of cats. J. Morphol. 174, 25-39.

Kamei-Takeuchi I, Hama T, 1971. Structurual change of pterinosome (pteridine pigment granule) during the xanthophore differention of Oryzias fish. J. Ultrastruct. Res. 34, 452.

Karnovsky MJ, 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. 27, 137A.

Marks R, Bhogal B, Dawber RP, 1972. The migratory property of epidermis in vitro. Arch. Dermatol. Forsch. 243, 209-220.

Menter DG, Obika M, Tchen TT, Taylor JD, 1978. Leucophores and iridophores of *Fundulus heteroclitus*: biophsical and ultrastructural properties. J. Morphol. 160, 103-120.

Merrilees MJ, 1974. Epidermal fine structure of the teleost *Esox americanus* (Esocidae: Salmoniformes). J. Ultrastruct. Res. 47, 272-283.

Mittal AK, Rai AK, Banerjee TK, 1978. Studies on the pattern of healing of wounds in the skin of a cat-fish *Heteropneustes ossilis* (Bloch) (Heteropneustidae: Pisces). Z. Mikrosk. Anat. Forsch. 91, 270-286.

Moon MJ, Kim WK, Kim CW, 1986. Ultrastructures of the cutaneous chromatophores and pigment granule formation in the goldfish, *Carassius auratus* L. (I) Xanthophore. Korean J. Electron Microsc. 16, 35-48.

Moon MJ, Kim CW, Kim WK. 1987. Ultrastructures of the cutaneous chromatophores and pigment granule formation in the goldfish, *Carassius auratus* L. (II) Leucophore and melanophore. Korean J. Electron Microsc. 17, 65-75.

Ortiz E, Bachli E, Price D, Williams-Ashman HG, 1963. Red pteridine pigments in the dewlaps of some anoles. Physiol. Zool. 36, 97-103.

Phromsuthirak P, 1977. Electron microscopy of wound healing in the skin of *Gasterosteus aculeatus*. J. Fish Biol. 11, 193-206.

Pickering AD, Pottinger TG, Christie P, 1982. Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a timecourse study.

J. Fish Biol. 20, 229-244.

Radice GP, 1980. The spreading of epithelial cells during wound closure in *Xenopus larvae*. Dev. Biol. 76, 26-46.

Schliwa M, Euteneuer U, 1983. Comparative ultrastructure and physiology of chromatophores, with emphasis on changes associated with intracellular transport. Am. Zool. 23, 479-494.

Schliwa M, 1986. Pigment cells. In Biology of the Integument. 2. Vertebrates, Eds., J. Bereiter-Hahn *et al*., Springer-Verlag, New York, pp. 65-77.

Sire JY, 1984. Fine structure of regenerating scales and their associated cells in the cichlid *Hemiochromis bimaculatus* (Gill). Cell Tissue Res. 237, 537-547.

Squier CA, 1981. The effect of stretching on formation of myofibroblasts in mouse skin. Cell Tissue Res. 220, 325-335.

Squier CA, Leranth CS, Ghoneim S, Kremenak CR, 1983. Electron microscopic immunochemical localization of actin in fibroblasts in healing skin and plate wounds of beagle dog. Histochemistry 78, 513-522.

Taylor, JD, Bagnara JT, 1972. Dermal chromatophores. Am. Zool. 12, 43-62.

Viziam CB, Matoltsy AG, Mescon H, 1964. Epithelialization of small wounds. J. Invest Dermatol. 43, 499-567.

Volpe EP, 1964. Fate of neural crest homotransplants in pattern mutants of the leopard frog. J. Exp. Zool. 157, 179-196.

Weston, JA, 1970. The migration and differentiation of neural crest cells. Advan. Morphol. 8, 41-114.

Winter, DG, 1973. Epidemal regeneration studied in the domestic pig. In Epidermal Wound Healing, Eds., HI Maibach and DT Rovee, Year Book Med. Publ. Inc., Chicago, pp. 71-112.

Wong, MK, Gotlieb AI, 1984. In vitro reendothelialization of a single-cell wound: Role of microfilaments bundles in rapid lamellipodia-mediated wound closure. Lab. Invest. 51, 75-112.

Yasutomi M, Hama T, 1972. Electron microscopic study on the xanthophore differentiation in *Xenopus laevis*, with special reference to their pterinosomes. J. Ultrastruct. Res. 38, 421-432.

FIGURE LEGENDS

Fig. 1. Electron micrograph of the normal integument of the goldfish, Carassius auratus. Red skin of the goldfish contains only one type of dermal chromatophores. The cutaneous xanthophores (XP) just distributed beneath the basement membrane have oval shaped nucleus (N) and long cytoplasmic processes.

Fig. 2. Pigment granules of the dermal xanthophores are carotenoid vesicles (C) and pterinosomes (P). Carotenoid vesicles have no limiting membrane and marginal electron densities of these vesicles are higher than those of internal region.

Fig. 3. Luminous white skin of the goldfish is expressed by dermal leucophores (LP) which composed of amorphous pigment granules, leucosomes.

Fig. 4. Normal black spot of the fish is expressed by multilayered dermal chromatophores composed of xanthophores, leucophores and melanophores. Melanophores (MP) have electron dense melanin pigment granules, known as melanosomes (M).

Fig. 5. Low magnification electron micrograph of normal integument in the goldfish before damaging. Three layers-uppermost epidermis (E), intermediate dermis (D) and underneath scales (S) are identified.

Fig. 6. After treating integumental wounds by burning with sharp needles, whole parts of epidermis and dermis containing basement membrane (arrows) are profoundly affected.

Fig. 7. Within one day after injury, the wound cavity (arrows) is filled by an mucous material containing components from damaged tissue, cytoplasm, and numerous hemocytes (H). Some of epidermal cells become detached and mobilized during this phase.

Fig. 8. At 3 days post-wounding, epidermal cells spread over the wound surface (arrows) by migration. These migrating cells participating in primary wound closure are originated from the adjacent epidermis.

Fig. 9. Migtrated hemocytes (H), mostly composed of leukocytes, are condensely aggregated near wounded area.

Fig. 10. At 7 days after wounding, dermal stem cells (SC)-presumably common precursors of dermal chromatophores-immigrate into the wound area. Electron density of this cell is relatively higher than that of other normal chromatophores.

Fig. 11. In the cytoplasm of the stem cells, well developed rough endoplasmic reticulum (ER), mitochondria (M) and Golgi complex (G) are observed.

Fig. 12. The stem cell is characterized by its long cytoplasmic processes (CP) distributed parallel to the basement membrane (BM).

Fig. 13. After primary wound closure, high mitotic activities at the newly formed epithelial cells are observered. Intercellular collagenous fibers (CB) re-arranged along the cellular boundaries during 1 to 2 weeks post-wounding.

Fig. 14. At 3 weeks after wounding, the first redifferentiated chromatophores (arrows) appear in dermal layer. At the vicinity of this chromatophores, undifferentiated stem cells (SC) also appeared.

Fig. 15. Electron micrograph reveals that the formation of pigment granules in the primitive chromatophores initiated after 3 weeks after wounding. These primitive pigment granules (arrows) are accumulated along the marginal region of the cytoplasm.

Figs. 16, 17. High magnification electron micrograph of the pinocytotic vesicles (PV) at the plasma membrane of the primitive chromatophores. These vesicles, associated with accumulation of pigment material within chromatophores, appear only at the inner surface of the chromatophores adhering to the stem cells.

Fig. 18. Multivesicular bodies (MB), which presumed to be the intermediate pigment granules are also observed within this primitive chromatophores. These bodies being formed by vesicular fusion of Golgi bodies.

Fig. 19. During this period, the mosaic pigment cells containing more than one type of pigment granules (arrows) were also seen at dermis.

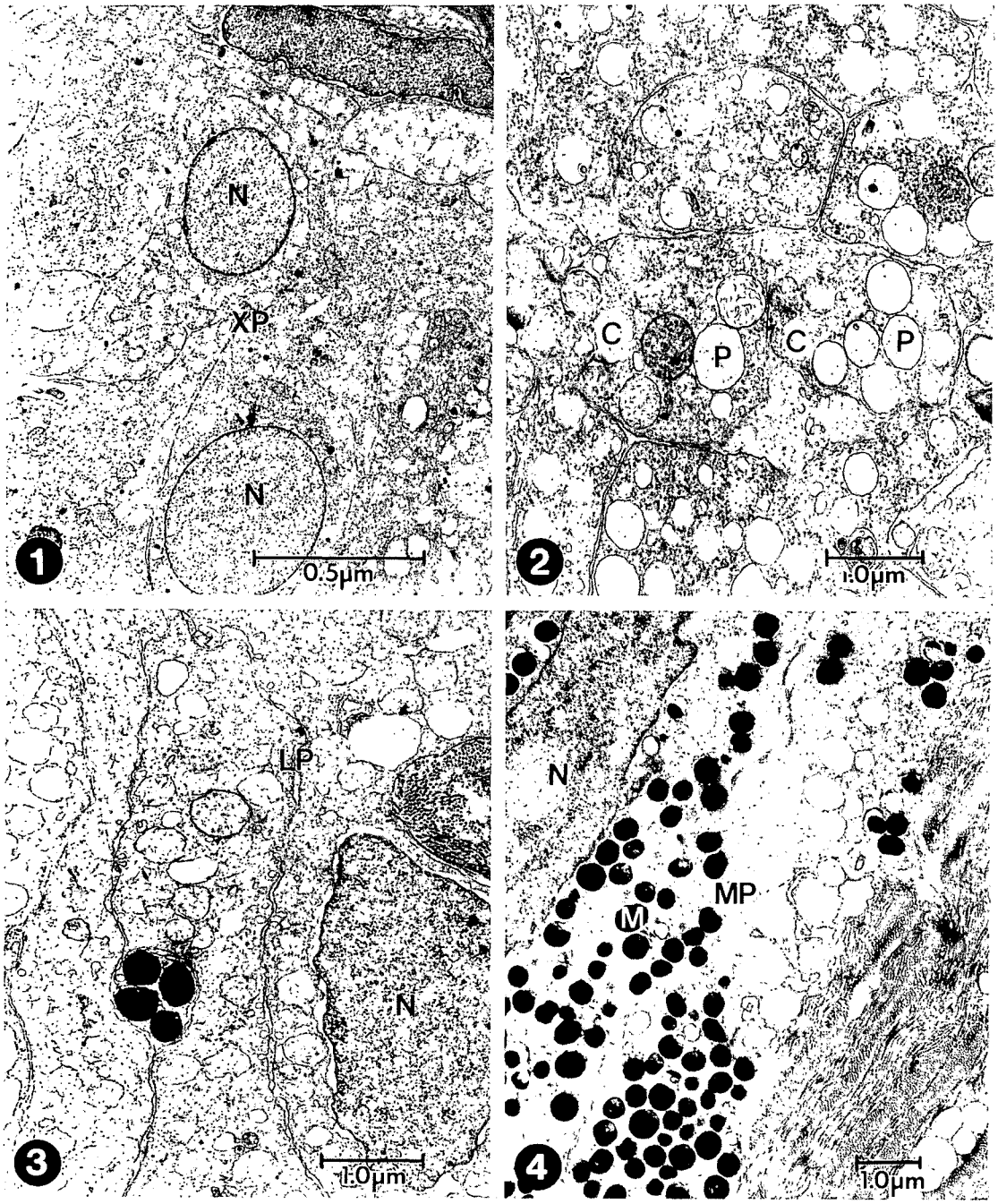
Fig. 20. Integumental wound repair (arrows) is accomplished within 4 weeks after wounding at most cases. Cell proliferation and remodelling are balanced by death of cells damaged from burns.

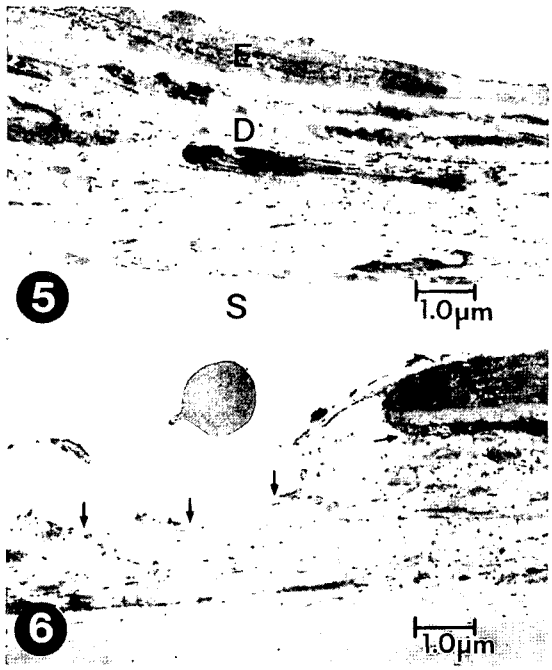
Fig. 21. Electron micrograph of the repaired xanthophore in the goldfish at 4 weeks after wounding. Electron densities of the pigment granules (PG) are still low, representing low level of pigment differentiation.

Fig. 22. During 1 to 3 months post-wounding, precursors of the pterinosomes in xanthophores are are vesiculated through Golgi complex (G), and large pigment granules were formed by the vesicular fusion (arrow).

Fig. 23. While type I pterinosomes (P1) have amorphous fine fibrous materials and clear limiting membrane, type II pterinosomes (P2) have thick and aggregated fibrous structures in addition to clear limiting membrane.

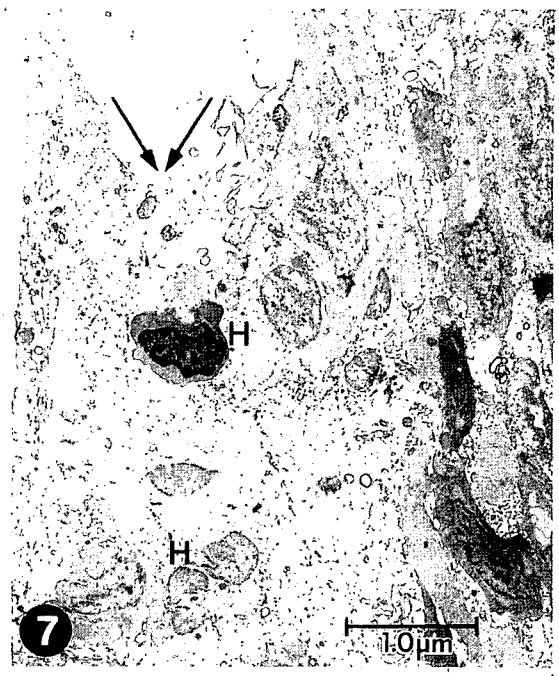
Fig. 24. Type III pterinosomes (P3) have lamellar structures arranged concentrically. The outer most lamella is difficult to be distinguished from the limiting membrane. Bar indicates 5 μm, 5 μm, 5 μm, 5 μm, 5 μm, respectively.


N
XP
N
0.5μm
C
P
C
P
1.0μm
LP
N
1.0μm
N
MP
M
1.0μm

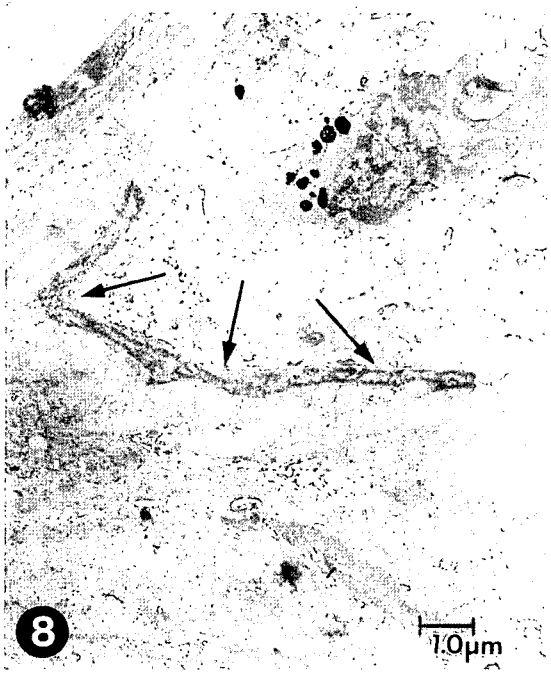


5

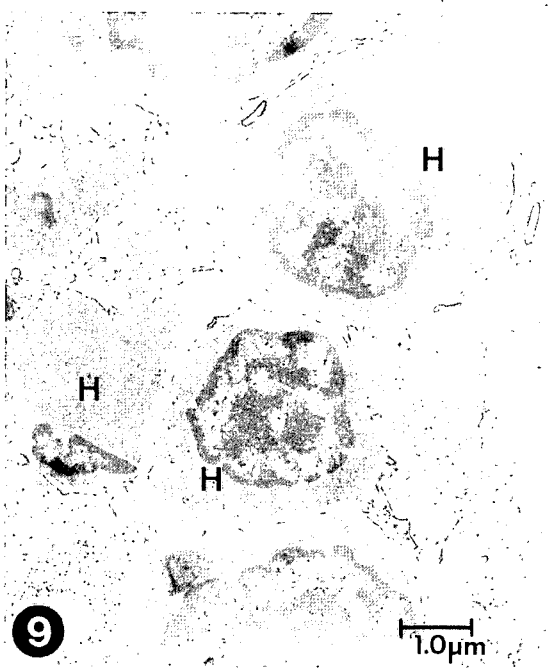
6



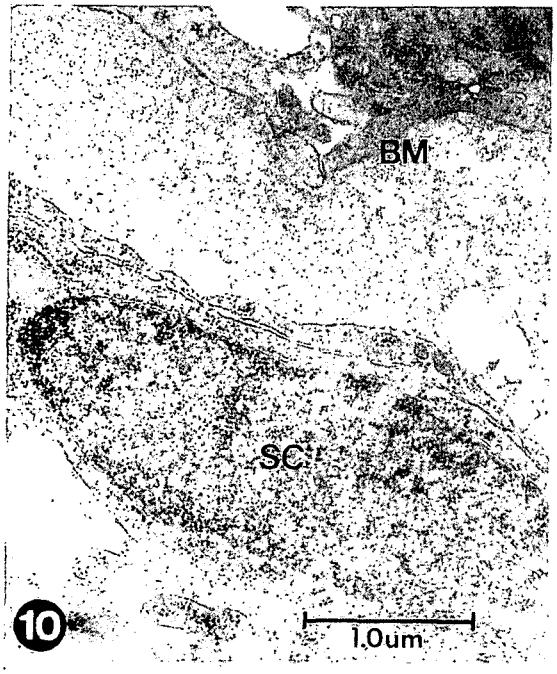
7

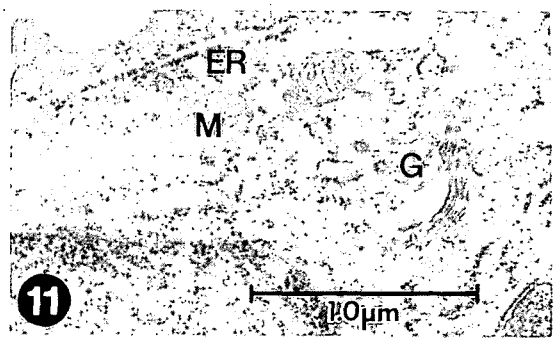


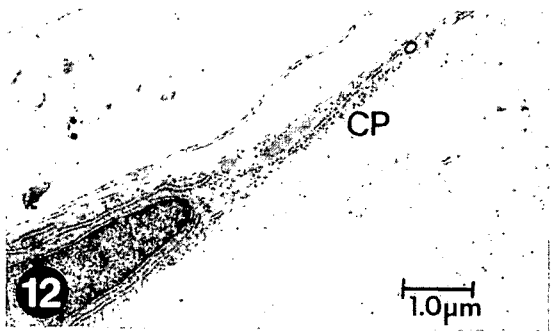
8

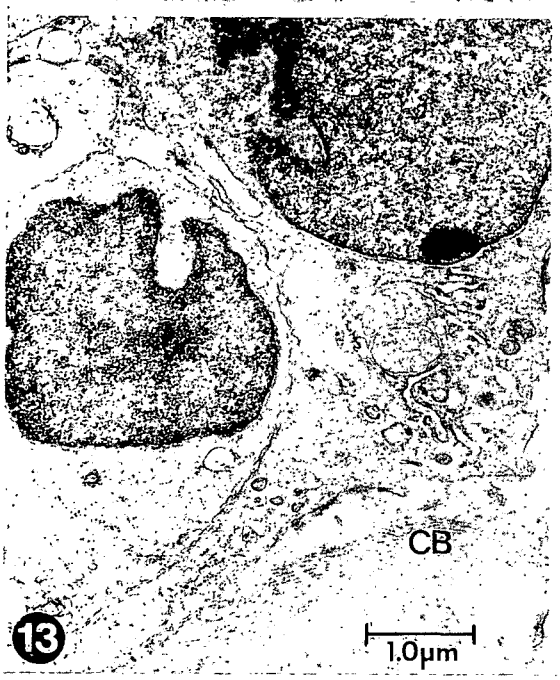


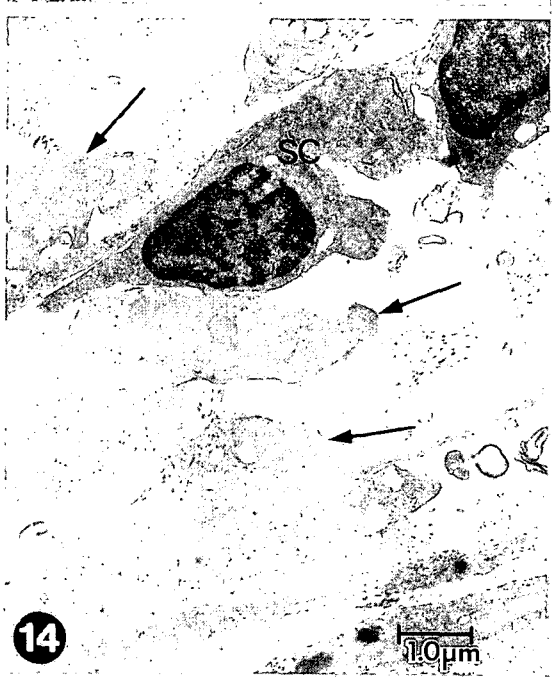
9

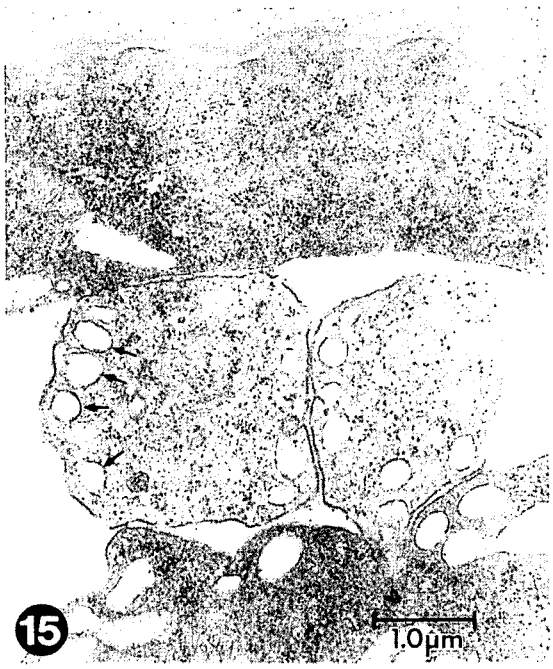

BM
SC
10
1.0um

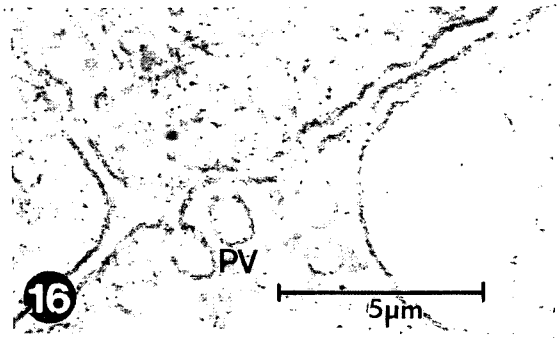

ER
M
G
11
1.0μm

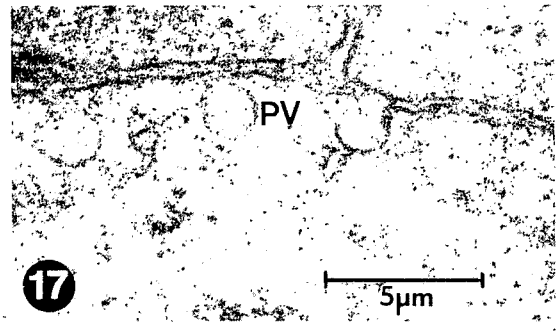

CP
12
1.0μm

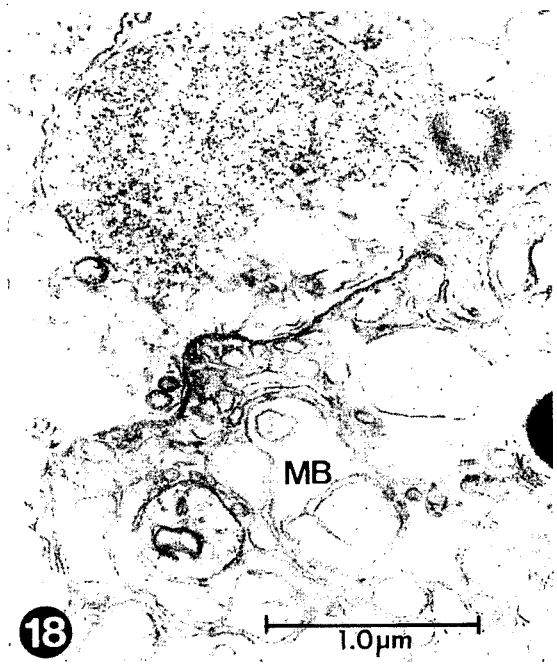

CB
13
1.0μm

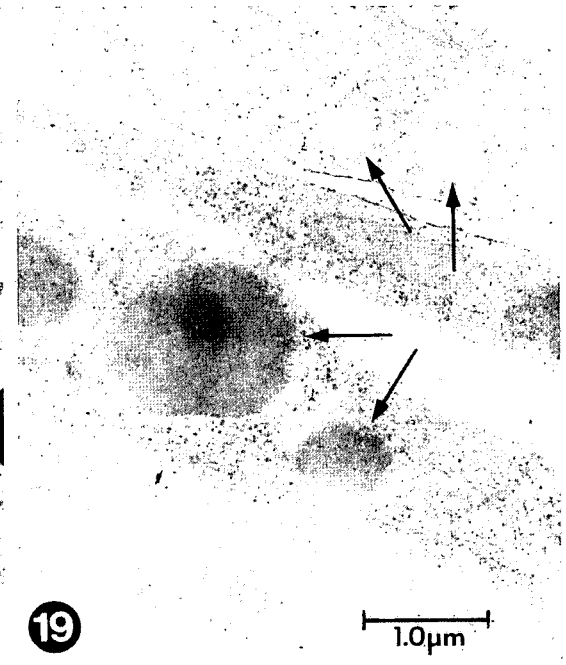

SC
14
1.0μm

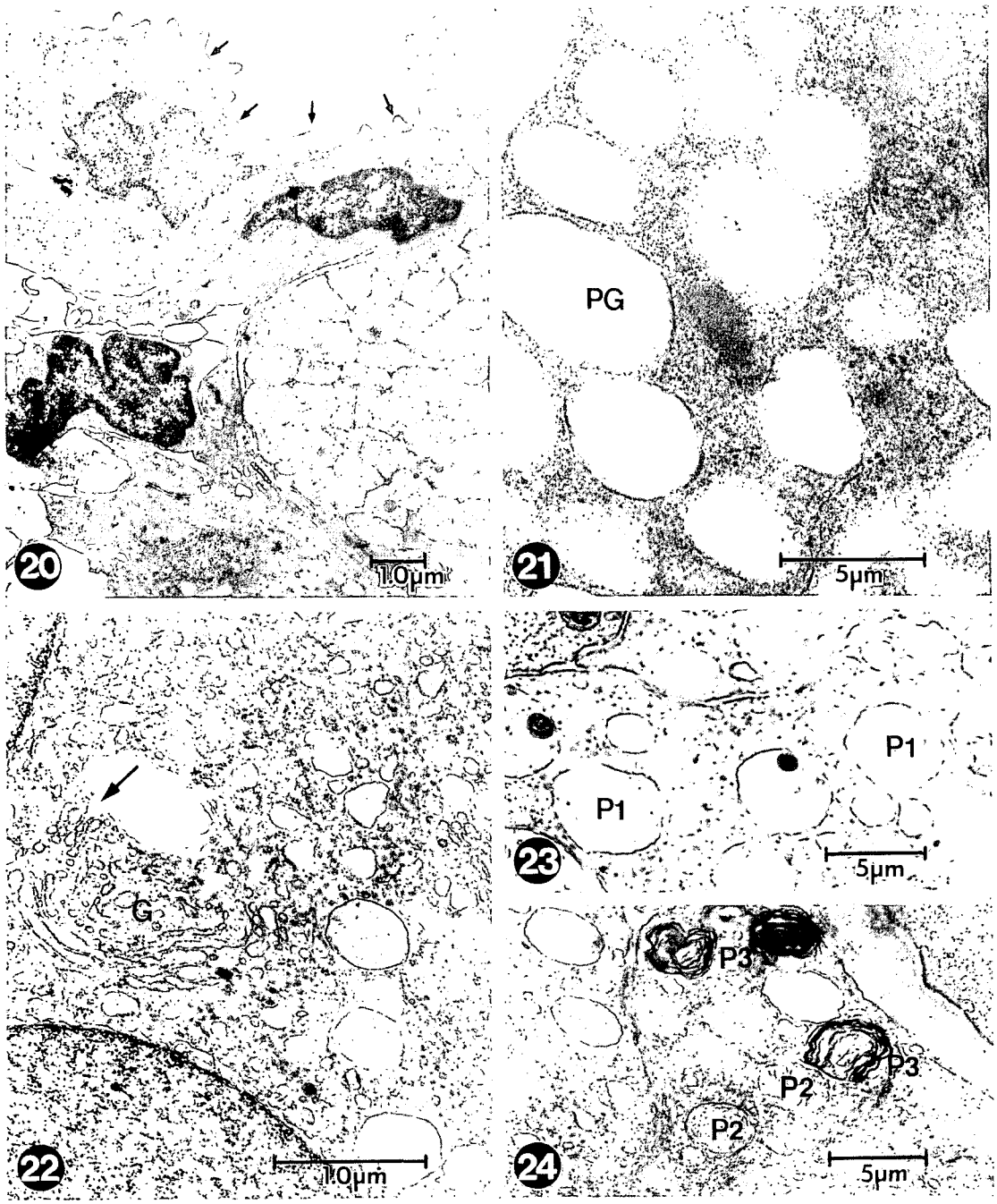

15
1.0μm


PV
16
5μm


PV
17
5μm


MB
18
1.0μm


19
1.0μm


20
1.0μm
21
PG
5μm
22
G
1.0μm
23
P1
P1
5μm
24
P3
P3
P2
P2
5μm