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Light, Scanning and Transmission Electron Microscopic Studies on the Structure of the Pigment Cells of the Pisces

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When a piece of the dorsal skin(cat fish) was observed under a light microscope, melanophores were horizontally expanded and had a few processes filled with melanosomes. They were isolated from one another. Some melanophores looked black, while others were dark brown.

On observation with a transmission electron microscope, the epidermal melanophore of cat fish was highly branched and small segments of processes were found frequently in the vicinity of the interecellular spaces. The cross section of the processes of melanophore was almost circular, and often invested by a thin layer of epidermal cells. Some processes, however, occurred free in the wide interecellular spaces or at the cytoplasm of the superficial layers.

In the mature melanophore, the cell organelles including melanosomes, mitochondria and free ribosomes were prominent in the perinuclear portions. Many melanosomes were spherical or elipsoidal in shape. Each melanosomes was surrounded by a limiting membrane. The processes of the mature melanophore were well developed, but the processes of the immature melanophores were incompletely developed.

KEY WORDS: **Chromatophore, Melanophore, Melanosome, The processes of melanophore**

Structural coloration may result from the fraction and reflection of light from the surface of the skin, as in feather or in the tail of some lizards. The present interests will mainly concern itself with chromatophore pigments. The biochemistry and nature of animal pigmentation are introduced in detail by Fox and Vevers(1960).

Melanophores may be present in either the dermis or the epidermis. It is the special attribute of the epidermal melanophores that they not only synthesize melanin but that they may release (Bagnara *et al.*, 1968) this melanin into surrounding epidermal cells. This melanin may then play an important role as a sun screen or in the development of the specific patterns of epidermal melanuzation characteristic of most vertebrates.

In certain species, all chromatophore types may participate to some degree in response of color changes. Sumner (1934, 1935a, b) clearly demonstrated the protective value of adaptive color change in the mosquito fish, *Gambusia patruelis*.

Morphological changes involve changes in the amount of pigment present within the skin and may result from changes in the number of synthesis of the integumentary pigment cells (Hadley and Quevedo, 1967; Lamer and Chavin, 1975). As a rule, both morphological and physiological color changes occur simultaneously, with the former providing the baseline coloration upon which fine-turned responses of the animal to internal or external stimuli are superimposed (Fujii and Novales, 1969; Bagnara and Hadley, 1973; Schliwa, 1982; 1984; Schliwa and Euteneuer, 1983).

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There are probably many reasons for the existence of pigmentation and color changes in fishes, including protection of radiation, absorption of energy, deposition of waste materials, signal transmission to other animals, and camouflage. Of these, the latter probably is the best-known and most spectacular. In the words of Odiorne(1957), The pigmentation of fishes probably has a variety of advantages which vary in life of a single individual. The changes which occur confer the benefits of adaptability and thus perfect the relation of the animal to its environment.

Early morphological literature on fish chormatophore by means of light microscopy was refered to in the work by Franz(1940). In 1968, Hadley presented their electron microscopic observations of the vertebrate integumental chromatophore. They held that there were two cell membrane containing pigment granules. The space between the two was occupied by a meshwork of fibrils which they supposed to be contractiles. Author has studied particulaly about pigment cells of skin. In present report, the author selected the cat fish which has not been previously investigated, and observed the morphological aspects of melanophores using the light, scanning and transmission electron microscopes.

Materials and Methods

The drosal skin of cat sifh, *Parasiluru asotus,* was used as the experimental sample.

For the light microscopy, tissue blocks were fixed in 10% formalin, embedded in paraffin wax and serially sectioned at a thickness of 6 μm. These sections were stained with hematoxylin-eosin.

For the scanning electron mcriscopy, tissue blocks were prefixed in 1%-glutaraldehyde-1.5% paraformaldehyde solution, postfixed in 1% osmium tetroxide solution, dehydrated in graded alcohol, transferred to isoamyl acetate, and then dried in the critical point dryer (Polaron E 3000). These dried tissues were coated with gold and observed with a JSM-35C scanning electron microscope.

For the transmission electron microscopy, tissue blocks were prefixed in 2.5% glutaraldehyde-paraformaldehyde which had been adjusted to pH 7.2 with 0.1M phsophate buffer, and postfixed in a 1% osmium tetroxide solution. They were dehydrated and embedded in Epon 812 mixture. The ultrathin sections cut on a LKB-ultrotome with diamond kinves were stained with 2.% uranyl acetate solution and restained with lead citrate solution, and then observed under JEM-1200EX transmission electron microscope.

Results

When a piece of the dorsal skin was observed under a light microscope, melanophores were horizontally expanded and had a few processes filled with melanosomes. they were isolated from one another. Some melanophores looked black, while others were dark brown. Mucous cells were observed at or near the surface. They were releasing their contents to the body surface. The dermis of skin consisted of a superficial region with loosely arranged collagen fibers and a deep region composed of muscle fibers (Figs. 1, 2).

When the surface of the dorsal skin were observed under a scanning electron microscope, the ducts of mucous gland were opened on the epithelial surface and the mucous droplets were distributed on that (Fig. 3). And that droplets were ellipsoid and round in shape and some droplets showing different electron density were observed. In the laternal view, the rows of each bands of lateral view were constantly arranged in shape and size(Fig. 4)

On observation with a transmission electron microscope, the dorsal skin consisted of the epidermis and dermis. Melanophores in the epidermis had only a few processes filled with melanosomes. The epidermis consisted of 5 to 7 layers of oviod, polygonal, or flattened squamosal cells with numerous intercellular bridges. Mucous cells in the various stages of maturation were interposed between the squamous cells. The epidermis divided morphologically into the bases, intermediate and superficial regions (Figs. 5, 6).

The layer of the basal cells was a single row of ovoid cells adjacent to the upper surface of the basement membrane. The sueprficial layer was the single row of squamous cells at the outer surface of the epidemris. The squamous cells in all layers were typified by the presence of numerous intercellular bridges or desmosomes and by fine cytoplasmic filaments (Figs. 5, 6).

The cytoplasm of the squamous cells in all layers was divided into the perinuclear zone and the periphery zone. The perinuclear zone contained most of the cytoplasmix organelles including Golgi complex,mitochondria, rough and smooth endoplasmic reticulums, and ribosomes. The periphery zone of the cytoplasm contained numerous cytopoasmic filaments and a few melanosomes. The melanosomes were occasionally located in the cytoplasmic processes between intercellular spaces in the epidermis(Fig. 5).

In the cells of basal layer, the nucleus was located in the basal portion of the cell. The Golgi complex was especially well developed. Small vesicles filled with a faintly electron dense material was seen pinching off from the end of the Golgi membrane. The basement membrane proper was composed of very finely fibrillar material oriented in a plane parallel to the outer surface of the epidermis (Figs. 6, 7).

The epidermal melanophore of cat fish was highly branched and small segments of processes were found frequently in the vicinity of the intercellular spaces. The cross section of the process was almost always circular, often was invested by a thin layers of epidermal cells. Some processes, however, occured free in the wide intercellular spaces or at the cytoplasm of the superficial layer (Figs. 5, 6, 7).

The dermis consited mainly of collagen fibers of each lamella was oriented in the plane of the surface. The longitudinal axes of collagen fibers of each lamella were perpendicular to the same axis of the lamella immediately above and below (Fig. 8).

Melanophores were mainly found in the uppermost part of the dermis. Between the basal borders of the epidermis and the layer of melanophores there was a layer of rather uniform thickness consisting mainly of collageneous fibers Those fibers in such a layer were orderly arranged (Figs. 8, 9, 10, 11).

In a mature melanophore, the cell organelles including melanosomes, mitochondria and free ribosomes were prominent in the perinuclear portions. Many mlanosomes were spherical or ellipsoidal in shape. Each melanosome was surrounded by a limiting membrane (Fig. 8).

Cytoplasmic processes of the melanophores were observed in various parts of the dermis, located between collagen lamellae (Figs. 9, 10, 11, 12).

The processes of that melanophore were well developed, but the processes of a immature melanophore were incompletely developed (Figs. 9, 11). Those processes were mainly surrounded by an extracellular matrix, usually containing collageneous fibers(Figs. 9, 11, 12). A few long processes of some melanophores had the limiting arborization and those processes containing the melanosomes in the dermis extended upward from the plane of cells(Figs. 10, 11, 12).

Discussion

In the integument of fish, the rapid changes of color patterns in some species are due to the presence and cellular activity of pigment-containing cells located in the integument. Since the pigmentary movements precede the morphological modification, it is not surprising that numerous investigation have assumed that morphological color changes are dependent upon the physiological state of the pigment cells. This theory was first suggested by Bagnara *et al.* (1968) who had explained how the color pattern of the vertebrates might be determined by environment.

It is clear that cat fish is without morphologically thick keratinized layers in the apical surface, and in this regard differs from the skin of amphibians, bird and mammals (Henrikson and Matoltsy, 1968a; McArn, 1968; Wellings *et al.*, 1967; Spitzer *et al.*, 1979; Lethbridge and Potter, 1981).

In other respects, however, similarities exist. The epidermal cells of cat fish, as described by Farquhar and Palade(1963), possess typical desmosomes, complex interdigitation between adjacent cells, terminal bars between cells at the free surface, and cytoplasmic filaments.

The number of cytoplasmic filaments increases as the cells progress from the germinal layer toward the surface where eventually exfoliate. Cytoplasmic filaments are apparently much more numerous in the epidermal cells of the marine species studied by Brown and Wellings (1970) than in the present cat fish. Moreover, in the fresh-water cat fish described herein, the cytoplasmic filaments are more concentrated at the periphery of the cells, leaving a relatively large perinuclear zone containing other cellular organelles, including golgi complex, mitochondria, and smooth endoplasmic reticulum. The possibility exists that these morphological differences of the epidermal cells of the marine and fresh-water species studied so far are adaptations related to differences in salinity of the surrounding water.

As in vertebrates a subcorneal space seperates the keratinized layer from the replacement cells below, which are united by tight junctions at the rim. Keratinized cells have a dense envelope below the plasma membrane on the outer side only (Mittal and Whitear, 1979; Sato, 1982). Under a keratinized area the epidermis has numerous desmosomes and a convoluted base but lacks secretory elements. The desmosomes described in the present study appear morphologically identical to those observed in different species. There is no evidence of morphological variations in the desmosomes at different levels in the epdiermis.

The mucous cells line the epidermal surface. They are relatively small, quite numerous vesicles containing a faintly electron-dense material. Mucous droplets in apical cytoplasm of the cells result in the release of mucous into the epidermal surface. They may be particulary important in lubricating the epidermal surface as it burrow into the sandy bottom of the rivers and stream that it inhabits.

Morphologically, the dermal collagen in this fish is similar to collagen in other phyla(Gross, 1956). The fibers have the typical major periods with microscoping, and are gouped into multilamellar arrangements. The fibers within a particular lamella are parallel, and the fiber axis of each lamella is approximately 90 out phase with the lamellae immediately above and below, thus giving the dermal collagen an overall structure similar to plywood. Although the melanophores of cat fish exhibit basically the same structureal characterietics as those of other vertebrates, some of their figures deserve comment.

Cat fish posseses the epidermal melanin unit, as defined by Hadley and Quvedo(1967), although it does not occur as extensively as that Neoceratodus of terrestrial vertebrates.

As noted in Neoceratodus, the lose association between melanophore dendrites and neighboring epidermal cells observed in cat fish appears to play a crucial role in the transfer of melanosomes. However, unlike the epidermal melanin unit of Neoceratodus in which melanosome deposition is direct, may melanosome complexes, although some are free in the cytoplasm of the epidermal cells. In addition, free melanosomes are frequently found in the interecellular spaces in the cat fish epidermis, while some dendrites are found at the outermost layer of the epidermis. It is possible, then that this mode of dendrite disposition may serve as a melanosome elimination mechanism in the epidermis of cat fish.

The dermal melanophores of cat fish usually possess a large number of premelanosomes in the perinulcear region suggestive of active melanogenesis. Judging from the internal structure of these organelles, it appears that the melanosome development in cat fish follows as established in higher vertebrates (Seiji *et al.*, 1963; Jimbow and Kukita, 1971; Lane and Whitear, 1980; McNiven and Porter, 1984; Chakraborty *et al.*, 1986).

Thus, author thinks that in the integument of cat fish, the morphological changes of color patterns are due to the presence of cellular activity of pigment-containing cells located in the integument and this survey of morphological color changes, though brief, is important to demonstrate the existence of a significant relationship between the morphological and physiological changes.

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어류 피부 색소세포에 관한 광학, 주사 및 전자현미경적 연구

지영득(가톨릭 의과대학 생물학교실)

광학 현미경하에서 메기(cat fish)배부 피부의 멜라닌 색소포(melanophore)는 피부의 표면층에 수평으로 배열되어 있었으며, melanosome으로 채워진 소수의 돌기들이 관찰되었다. 각각의 멜라닌 색소포들은 흑색 또는 어두운 갈색으로 보였다.

전자현미경하에서 표피 멜라닌 색소포들은 양측면으로 크게 분지되었고 멜라닌색소포의 작은 돌기들은 세포간극(intercellular space)의 부근에서 자주 발견되었다. 멜라닌 색소포돌기의 횡단면은 거의 환상형이며, 가끔 얇은 층의 표피세포로 둘러사여 있었다. 몇몇 돌기들은 넓은 세포간극이나 표면층 상피세포의 세포질 속에서 구분되었다.

성숙한 멜라닌 색소포에서는 mealnosome, mitochondria, free ribosome이 핵 주변부에 현저하게 발달되었고, melanosom은 구형 또는 난원형이었으며 각 melanosome은 한계막으로 둘러싸여 있었다. 성숙한 멜라닌 색소포의 돌기들은 잘 발달되었지만 미성숙한 멜라닌 색소포의 돌기들은 불완전하게 발달되었다.

Explanation of Figures

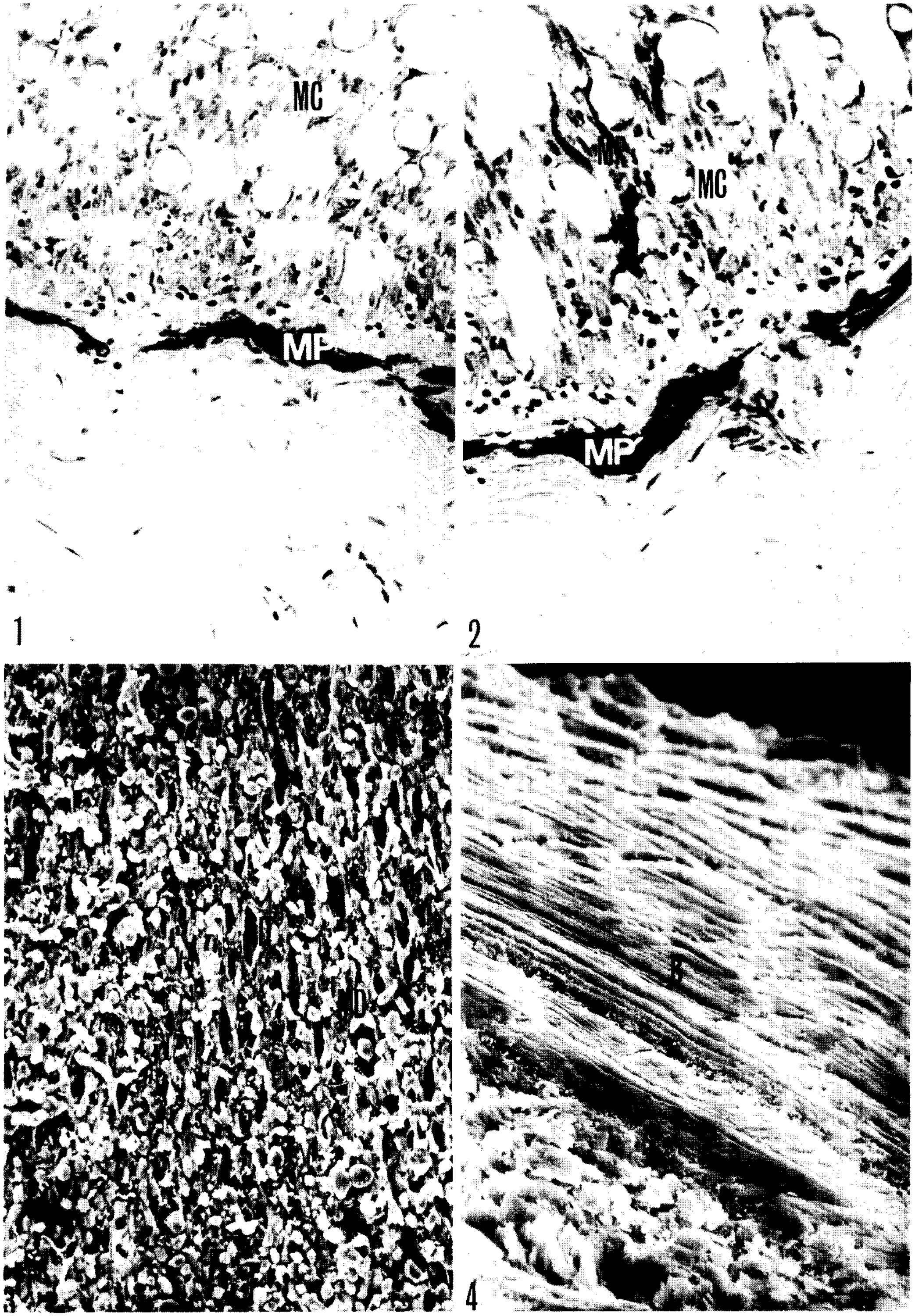
Figs. 1, 2. Cross section of cat fish integument. The epidermis is approximately 5 to 7 cell layers thick and contains many muous cells. Numerous mucous cells(MC) are located at the superficial portion in the epidermis. Beneath the basement membrane is a single layer of melanophores(MP). Several melanophores are visible between the epithelial cells in the epidermis. x 200, x 300.

Figs. 3, 4. The surface and lateral view of the dorsal skin. the ducts(D) of mucous glands are opened on the peithelial surface. Mucous droplets(MD) are covered on that. The rows of each band(B) of the lateral view are constantly arranged. x 640, x 1,440.

Figs. 5, 6, 7. Three kinds of epithelial cells. Characteristic features including a prominent nucleus(N), Golgi complex(G), numerous free ribosomes(R), desmosomes(D), isolated patches of rough endoplasmic reticulum-(RER), smooth enoplasmic reticulum(SER) and several mitochondria(MI) are osberved in those cells. The cell periphery is composed of a dense and of closely aggregated microfilaments(MF). The isolated melanosomes(M) occurring in the cytoplasm and melanoomes which appear to discharge its contents into the intervellular space is well seen. The mucous cell between epithelial cells is filled with mucous-containing vesicles(MV). x 20,000, x 20,000, x 20,000.

Fig. 8. The melanophore located in the dermis. The melanophore has melanosomes(M) of the verious stages. Golgi complex(G), mitochondria(MI), and free ribosomes(R) are well developed in the perinuclear cytoplasm. x 20,000.

Figs. 9, 10, 11, 12. Melanophore processes(P) in the dermis. The various styles of processes in the dermal melanophore showing a beading effect produced by the presence of melanosomes extend between collagen masses(C). The fiber axes of adjacent lamellae are approximately perpendicular to each other, so that the fibers of each lammela are cut tangentially, or in cross section. x 16,000, x 20,000, x 16,000.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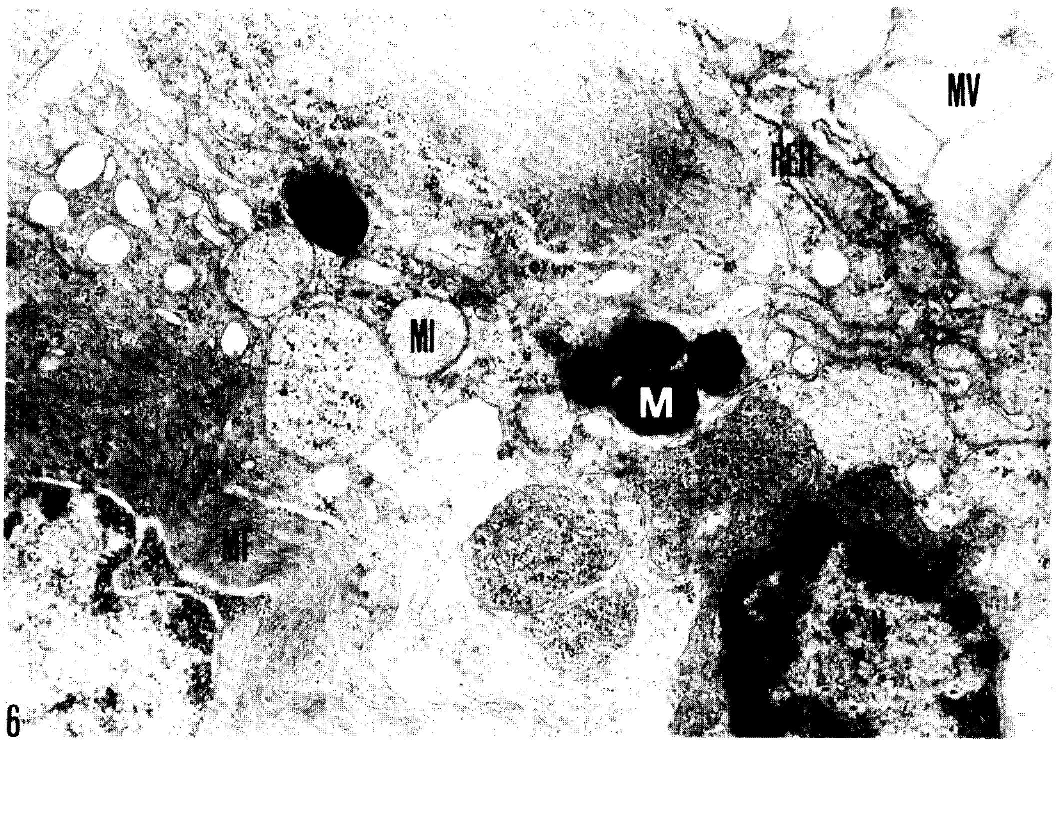

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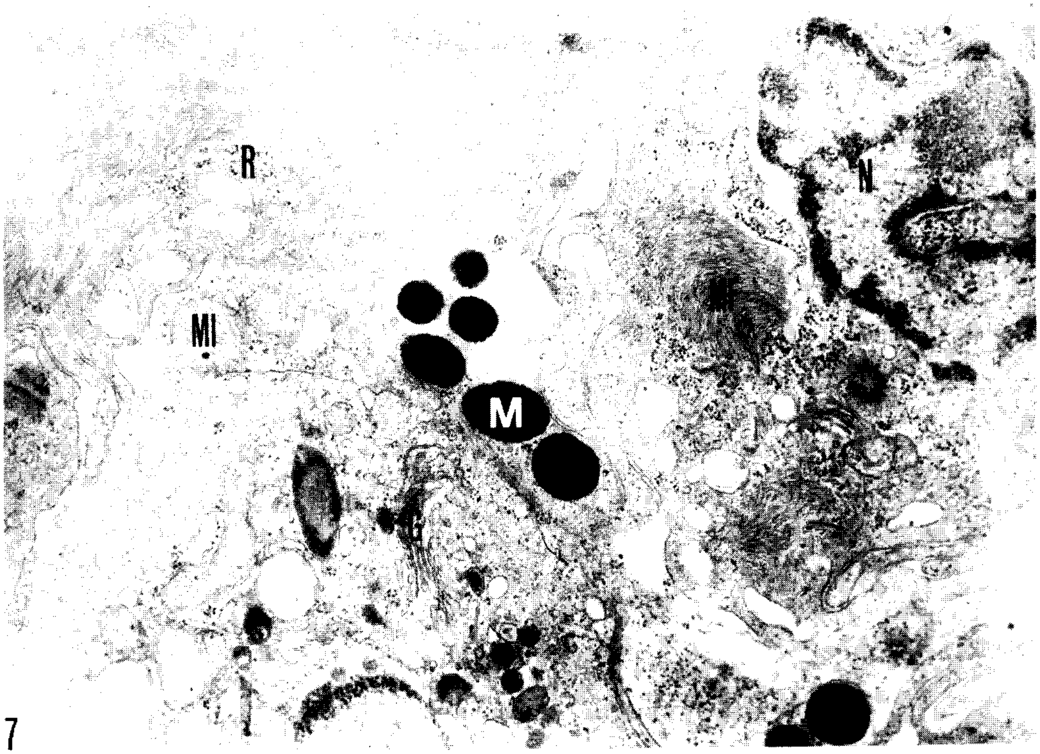

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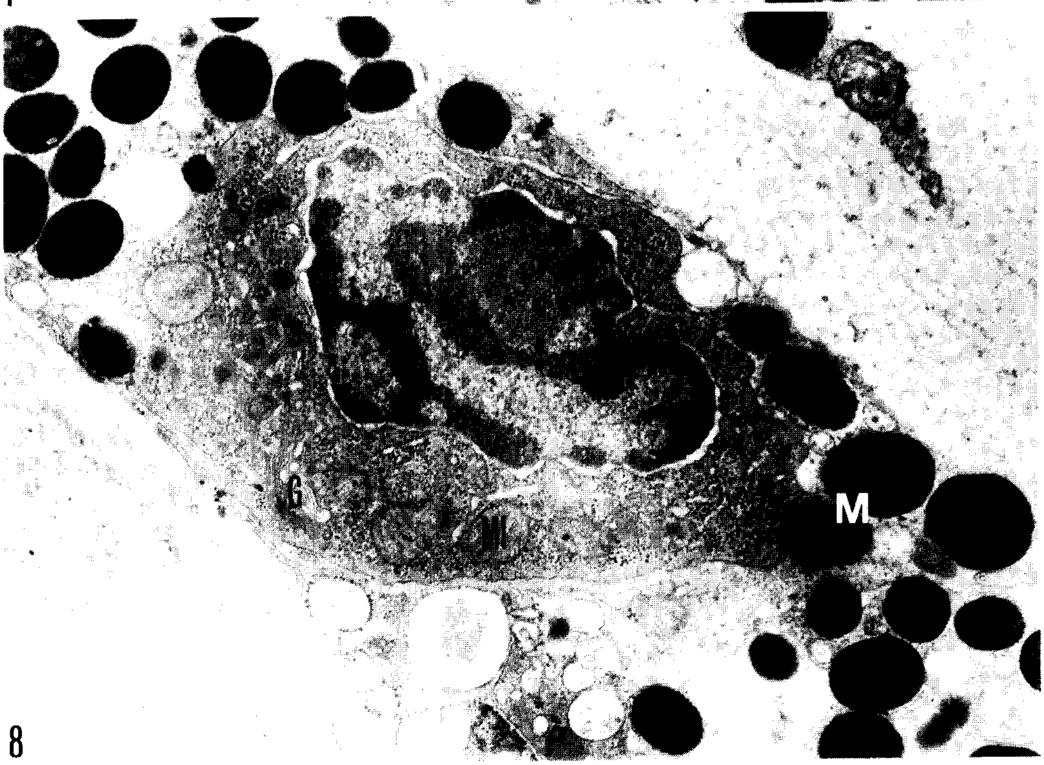

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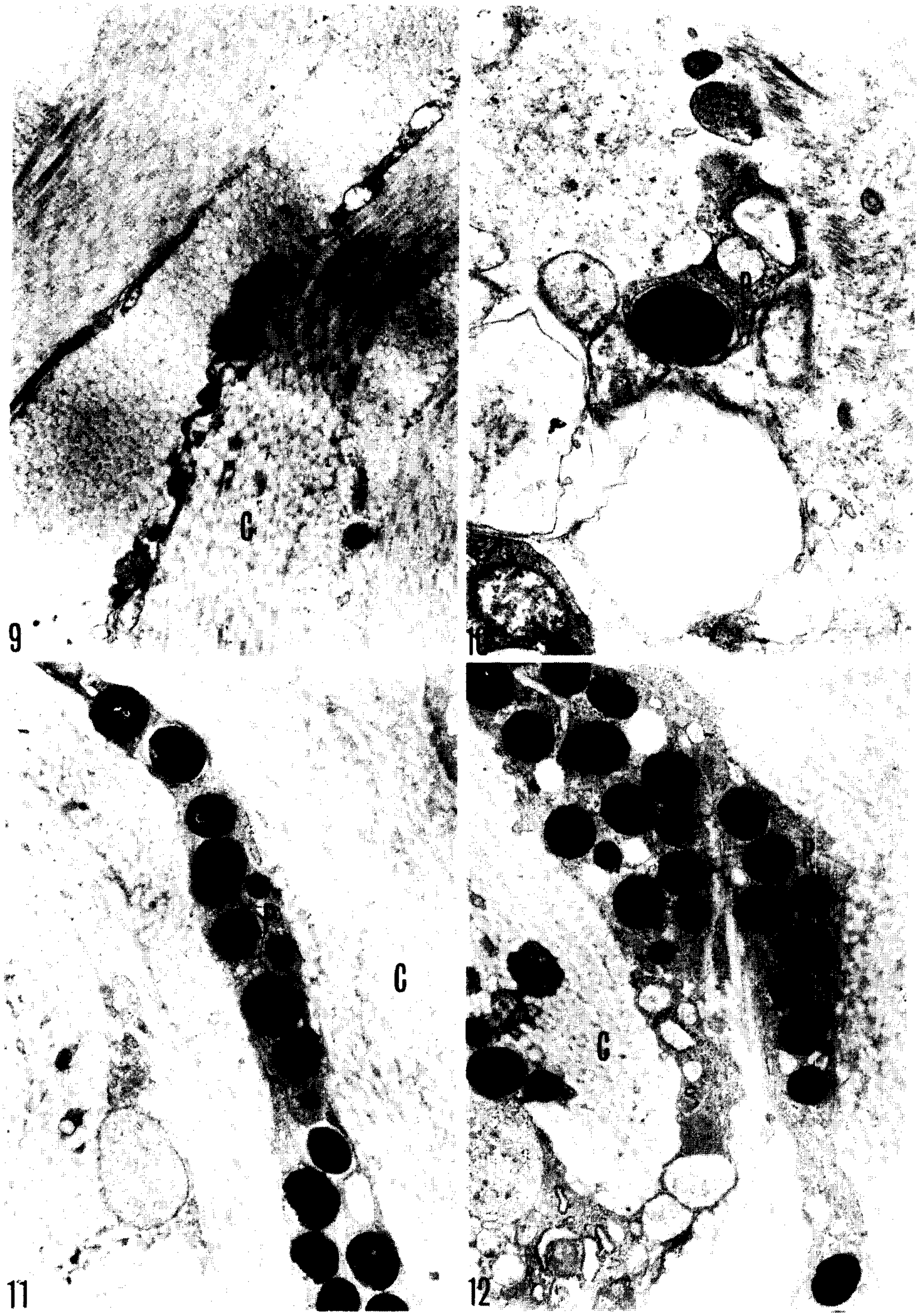
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