The Structure of the Pigment Cells in the Turtle
*Trionyx sinensis*

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Summary. A light and transmission electron microscopic study of the pigment cells (chromatophores) revealed the presence of three types of cells, namely: melanophores, xanthophores or erythrophores, and iridophores. The melanophores contained eumelanin containing organelles, the melanosomes and iridophores showed reflecting platelets, whereas, the xanthophores contained pterinosomes. Quantitatively melanophores appeared in large numbers and were widely distributed, ranging from the skin to many internal viscera, whereas iridophores were few and xanthophores very rare.

Pigment cells in the vertebrate skin and their pattern of distribution have been long investigated by developmental biologists and geneticists (Bagnara and Hadley, 1969; Bagnara, Frost and Matsumoto, 1978; Bagnara, 1983). Nevertheless, there have been only few studies on the ultrastructural analysis of these pigment cells (Bagnara, personal communications; Berns and Narayan, 1970; Frost, Epp and Robinson, 1984a, b). In the course of an embryological study of the development of the fresh water turtle *Trionyx sinensis* (to be published), the pigment cells in many parts of the embryo including the intestines, blood vessels and in skeletal muscles were observed. Since these pigment cells were visible even without staining, the possibility of using these as marker cells to study the migration of cells and also to understand the function of these pigment cells was realized, and a detailed study of their structure was undertaken.

MATERIAL AND METHODS

The eggs of the fresh water turtle *Trionyx sinensis* were collected from a commercial farm in Singapore, were washed repeatedly in tap water till all the sand and mucus in the surface of the shell were removed and finally washed in 70% alcohol. The eggs were incubated at 30°C. The incubation period was about 60 days. The eggs were daily examined and early embryos were collected and fixed in Bouin's fixative. Late embryos were fixed in 10% formalin for light microscopic study; the very late embryos (50–55 days), young hatchlings and adults were anaesthetized using thiopentone sodium and perfused with 10% formalin through the ventricle. Serial sections were cut longitudinally and transversely and were stained by the hematoxylin eosin method. Certain sections were examined without staining.

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For transmission electron microscopy, the fixative used was a mixed aldehyde solution made up of 2% paraformaldehyde and 3% glutaraldehyde in 0.1M cacodylate buffer at pH 7.3.

Blocks of tissues were removed from the limb buds in the case of embryos and from the limb musculature in the case of young hatchlings and adults. The blocks were trimmed into small pieces (about 1 mm) which were further fixed in a freshly mixed aldehyde solution for 3 hrs at 4°C. After overnight rinsing in cacodylate buffer the tissues were postfixed in 1% osmium tetroxide containing 1.5% potassium ferrocyanide at 4°C for 2 hrs.

Fig. 1. a. Light micrograph of a section of the skin area of the hind limb of a young hatchling of the *Trionyx sinensis*. The pigment cells are seen just beneath the epidermis. ×420. b. Light micrograph of an unstained section of the hind limb of a young hatchling of the *Trionyx sinensis*. The pigment cells are clearly seen as dark cells just beneath the epidermis, dermis and in the skeletal muscle below. ×45
The tissue samples were then dehydrated in an ascending series of ethanol and embedded in Araldite. Semithin sections were cut with a Porter Blum MT2 ultramicrotome and stained with 1% methylene blue in 1% borax. Selected areas of blocks were trimmed for ultrathin sections which were then stained with uranyl acetate and lead citrate and examined with a Philips 400T electron microscope.

Fig. 1.  

- **c.** An area of the bone marrow in the femur of a young hatchling clearly showing the pigment cells. ×110.  
- **d.** The appearance of the skeletal muscle showing the fibers with central nuclei. Note the arrangement of pigment cells (arrow) around a blood vessel. ×420
Fig. 2. Legend on the opposite page.
OBSERVATIONS

The pigment cells were visible under the light microscope even in unstained sections. The embryos at about 30 days (the halfway point before hatching) showed pigment cells with many processes. These were seen around the internal viscera, in mesenteries, in the connective tissue around blood vessels, in the epineurium of nerves as well as in the bone marrow and between skeletal muscle fibers. The blood vessels were so finely covered, in dissected specimens under the dissection microscope, that the center blood vasculature could be traced without any difficulty. In the skin, they were seen just beneath the epidermis, in the dermis, in the subcutaneous connective tissue and in the blood vessels (Fig. 1).

The skeletal muscles were blackish in nature due to these pigment cells; these were seen between muscle fibers (Fig. 1b, d) in the intramuscular connective tissue (Fig. 6, 7) and in relation to the intramuscular nerves (Fig. 5) and blood capillaries (Fig. 2, 4). In the viscera, especially in the gastro-intestinal tract, they were seen on the surface of the structures covered with the peritoneum.

Electron microscopy

For description of the pigment cells the classification of Bagnara (1983) was taken. The three basic types of pigment cells of chromatophores were the melanophores, xanthophores or erythrophores and iridophores.

In the present study, the melanophores were observed most frequently in comparison to the other two types both in embryos as well as in adults. The melanophores appeared as irregular cells with many cytoplasmic processes and with amoeboid features. These cytoplasmic processes radiating from the cell body also contained melanosomes. The cells showed a large number of pigment (granules) covered by a membrane called the melanosomes (Fig. 3). The melanosomes were usually round but occasionally ovoid or ellipsoid in shape and their diameters ranged from 0.5-0.9 μm. In some cells the melanosomes packed the entire cytoplasm of the melanophage, but in others there were only few melanosomes. The nuclei of the melanophores were well developed and usually centrally located. The nuclei showed prominent nucleoli and peripheral chromatin. A nuclear membrane with the nucleopores could also be observed (Fig. 8a).

The cytoplasm showed a well developed Golgi apparatus with numerous translucent vesicles associated with it. Mitochondria were seen between the melanosomes; some mitochondria showed electron dense granules in their cristae (Fig. 8b). In the cytoplasm there were also free ribosomes, and some filamentous and tubular structures.

The second type of chromatophore observed was the iridophore. In comparison to the melanophores, the iridophores were few in number and when seen usually in close proximity to a melanophage. The iridophores were characterized by the presence of the reflecting platelets in the cytoplasm (Fig. 9).

These reflecting platelets were usually rectangular in shape measuring about 0.6-0.9 μm in length, appearing as empty spaces or holes surrounded by a membrane. They also showed a regular pattern of arrangement in the cytoplasm as parallel rows.

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Fig. 2.  

- a. Light micrograph of a transverse section of a blood vessel showing blood cells in the lumen \((L)\) and pigment cells (arrows) forming a continuous layer around the wall. \(\times100\).  
- b. Electron micrograph of a blood vessel showing a blood cell in the lumen \((L)\) and many pigment cells arranged around the wall. \(\times7,400\)
Electron micrographs of two melanosomes in the subcutaneous tissue, showing an electron-dense and membrane-bound melanosome (arrows). The cytoplasm also shows mitochondria and rough endoplasmic reticulum. a. The indented nucleus with prominent nucleolus. b. Crescentic nucleus. a, b: ×17,500
Fig. 4. Two melanophores (M) are seen in relation to the wall of a blood vessel. × 4,400

Fig. 5. Melanophores (M) are seen in relation to a nerve bundle. × 5,000
or as stacks (Fig. 9). Unlike the melanophores, the iridophores did not show a well developed Golgi apparatus, though the cytoplasm did contain mitochondria, vesicles and a few melanosome-like structures.

The third type of chromatophore, the xanthophore, was observed very rarely. The

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**Fig. 6.** A melanophore is seen between muscle fibers. Note that the cytoplasm is filled with a large number of melanosomes with very minimal cytoplasmic organelles. \( \times 14,000 \)

**Fig. 7.** A melanophore is seen in close proximity to skeletal muscle, but note that the cytoplasm and the cell wall are disorganized. \( \times 17,500 \)
Fig. 8. a and b. High power electron micrographs of melanophore showing melanosomes of varying sizes, Golgi apparatus (G), mitochondria (M) and endoplasmic reticulum. The nucleus (N) shows a well defined nuclear membrane. a: ×27,000, b: ×35,000
xanthophores (Fig. 12) were seen mainly in relation to skin and not in the internal viscera. The characteristic feature of the xanthophore appeared to be the large number of “empty” looking round vesicles which occupied the cytoplasm. They showed a prominent nucleus, Golgi vesicles and some melanosomes. They were sometimes seen in relation to collagen fibers.

Embryonic chromatophores or chromatoblasts were observed in embryos which had passed half the period of incubation. These were large prominent cells with a prominent nucleus. The cytoplasm showed numerous mitochondria, a Golgi apparatus associated with numerous vesicles, rough endoplasmic reticulum, free ribosomes and melanosomes at various stages of development and pigment formation (Fig. 10, 11).

**DISCUSSIONS**

The present observations on the various types of chromatophores in the reptile *Trionyx sinensis* such as the melanophores, iridophores and xanthophores coincide well with previous observations on the amphibians and fishes (Bagnara and Hadley, 1969; Bagnara and Taylor, 1970; Bagnara, Ferris and Taylor, 1976; Bagnara, Frost and
In the turtle, melanophores appeared in large numbers whereas the iridophores were few and xanthophores rare. The distribution of the melanophores such as in the viscera, in the mesenteries, around the nerves and the blood vessels in the skeletal muscle, and even in the bone marrow, indicated that they are a "dermal"

**Fig. 10.** a and b. Electron micrographs showing the early stages of melanosome formation. The premelanosomes (arrows) show different stages of melanization. ×15,500
type of chromatophore and respond to hormones like those pigment cells normally found in the dermis (Bagnara—personal communication).

The melanosomes which were observed in the melanophores of the turtle compared similarly to previously described ones in amphibians and fish. The well developed Golgi apparatus and the abundance of rough endoplasmic reticulum with numerous

Fig. 11. a and b. Electron micrographs showing later stages of melanosome formation. ×15,500
vesicles related to the Golgi apparatus were indicative of the functional status of an actively secreting cell (MENTER et al., 1979).

The development of the different types of chromatophores from their precursors or propigment cells or chromatoplasts and the formation of pigments or melanosome formations which is also referred to as melanogenesis have been described (BAGNARA et al., 1979b) in fish (TURNER, TAYLOR and TChEN, 1975) and in the red-spotted newt (FORBES, ZACCARIA and DENT, 1973), and have also been recently reviewed (BAGNARA, 1983). BAGNARA et al. (1979a) has postulated a common origin for pigment cells from a stem cell containing a primordial organelle with the potential of becoming any of the three pigments described, such as the melanosomes of the melanophores, reflecting platelets of the iridophores and pterinosomes of the xanthophores. It has also been shown that vertebrate pigment cells are formed by the migration of neural crest cells (Du SHANE, 1935; LE DOURAIN and TEILLET, 1974).

What are the functions of these melanophores? Why are they distributed in so
many tissues? These two questions cannot be answered by morphological studies alone, as biochemical analysis of the pigments as well as physiological studies are needed. Biochemical studies have shown (see review by Bagnara, 1983) that melanosomes from melanophores contained brown or black eumelanin and yellow or red phaeomelanin. The reflecting platelets of the iridophores have crystalline deposits of purines whereas the xanthophores have pteridines and/or carotenoid as pigments deposited in granules called pterinosomes or in vesicles. In the present study, most of the cells observed were dermal melanophores and possibly contained eumelanin. As for the function of these melanophores which were distributed in so many regions of the body of the turtle, a possible protective or thermal regulating function could only be speculated (Bagnara, personal communication).

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