An Ultrastructural Study of Melanoma in *Xiphophorus*

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SUMMARY

Melanotic melanoma (MM) of *Xiphophorus* (Teleostei: Poeciliidae) was studied by conventional preparations and freeze-etch preparations for electron microscopy.

MM of *Xiphophorus* exhibits tightly packed pigment cells with prominent dendritic processes and interdigitations of their plasma membranes. The most impressive feature of MM cells is the occurrence of large lobulated nuclei with numerous nuclear pores and some nuclear pockets. Abundant spheroidal or ellipsoidal melanosomes (diameter 200–650 nm) and vesicular structures are distributed throughout the cellular dendrites, whereas the perinuclear cytoplasm is free of melanosomes.

A further characteristic feature of melanoma cells in fish is the occurrence of melanosome complexes (*i.e.*, "compound melanosomes"). These melanosome complexes consist of a few to numerous melanosomes, which are enveloped by a separate membrane. Pinocytotic vesicles could be demonstrated with distinct differences in frequency and distribution patterns, indicating differences in the metabolic activities of the cells in the same melanoma. Intercellular junctions are lacking in the MM cells.

The conventional TEM technique showed clear advantages in the demonstration of internal architecture of organelles, whereas FE had considerable potential in respect to the visualization of membrane surface specializations.

INTRODUCTION

During the past 20 years the fish *Xiphophorus* (Teleostei: Poeciliidae) has been proven to be a useful model for studies of melanoma formation.[1] The morphology of fish melanoma has been successfully investigated by light microscopy and transmission electron microscopy.[2–4] Freeze-etch studies, which have been very useful for investigating the ultrastructure of normal and neoplastically transformed pigment cells in humans,[5–8] were carried out recently in fish melanoma.[8]

The present study was carried out to obtain a better understanding of the ultrastructural changes during tumor formation by comparing conventional preparations and freeze-etch preparations of melanoma in fish. It is part of a broad-scale comparative study of tumor development in the model system *Xiphophorus* and in humans.

MATERIALS AND METHODS

Materials

Melanotic-melanoma-bearing *Xiphophorus* hybrids were kindly provided by Dr. A. Anders, Giessen.

They were produced by crossing *Xiphophorus maculatus* (population Rio Jamapa), carrying the gene complex *Sd-Tu,* with *Xiphophorus helleri* (population Belize River), lacking this gene complex; the resulting F_1-hybrids were backcrossed to *X. helleri.* For a detailed description of this cross-breeding and the underlying principles of melanoma formation in *Xiphophorus,* see Ref. 1. Only fish carrying malignant melanoma (for classification see Ref. 9) were used in this study.

Methods

Light microscopy. All specimens were fixed in Bouin's solution, dehydrated in ascending ethanol, and embedded in paraffin. Then 5-μm sections were cut with a Leitz base sledge microtome and stained with acid alizarine blue and anilin orange G in a modification of azan staining (see Ref. 10).

Transmission electron microscopy. Small pieces of melanoma tissue were fixed in 4% glutaraldehyde at room temperature for 3 hr. After washing in phosphate buffered saline the specimens were postfixed in 2% OsO_4. The tissue blocks were prestained with 2% uranyl acetate in 20% ethanol at 60°C for 3 hr. Then they were dehydrated through a series of ascending ethanol and embedded in ERL-4206. Ultrathin sections were cut with a diamond knife using a Reichert ultramicrotome Om U3 and were examined in a Zeiss EM 10A electron microscope.

Freeze-etching. For FE, small pieces of melanoma tissue were fixed in 3% glutaraldehyde in sodium veronal acetate buffer at 4°C (1–3 hr). Freezing, fracturing, shadowing, and cleaning of the specimens were carried out as described in detail elsewhere.[7] The cleaned replicas were placed on standard 300-mesh copper grids and viewed in a Zeiss EM 10A electron microscope. The terminology of fracture faces is according to Ref. 11.

RESULTS

Anatomical and light microscopical observations

All melanomas of fish used for this study were heavily pigmented nodular cell masses with smooth regular surfaces and had a lobulated appearance. The melanoma was composed of abnormal melanocytes and abnormal melanophores. The tumor spreads and finally covers almost one-third of the fish surface (Fig. 1). Because of the three-dimensional growth of the melanoma, the fin tissue is replaced by the growing tumor tissue as neoplasia development proceeds.

In histological sections the tumor appears as a well-vascularized pigmented thickening of the corium with pigmented cells infiltrating the thickened epidermis and the muscular tissue by invading the myosepta (Fig. 2).

Following the complete infiltration of the connective tissue, the muscle fibers degenerate and are replaced by transformed melanocytes, which may differentiate to melanophores. The transformed pigment cells of fish malignant melanoma are larger than the normal ones. They are round or polygonal, and some cells show dendritic processes that

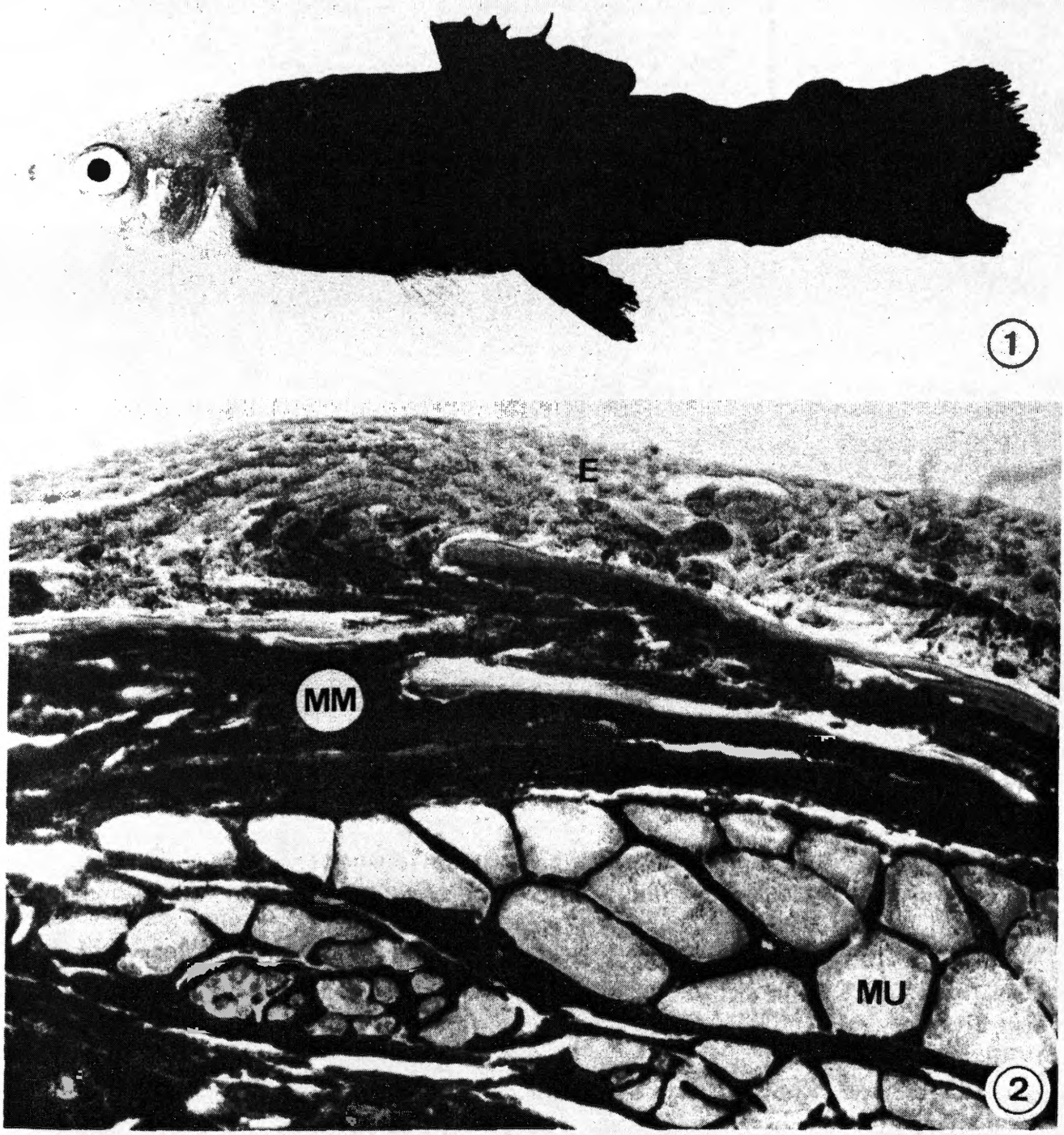

FIG. 1. Pigmented platyfish-swordtail hybrid bearing a melanotic malignant melanoma.
FIG. 2. Histological section through melanomatous skin of *Xiphophorus* showing hyperplastic epidermis (E), pigmented thickening of the corium (MM), and infiltration of muscular tissue (MU) by melanoma cells. (× 1100)

interlace with each other. The nuclei are polymorphic. All other subcellular structures are obscured by the dense melanin pigmentation.

Ultrastructural observations

In both TEM and FE the ultrastructure of the malignant melanoma of fish shows that

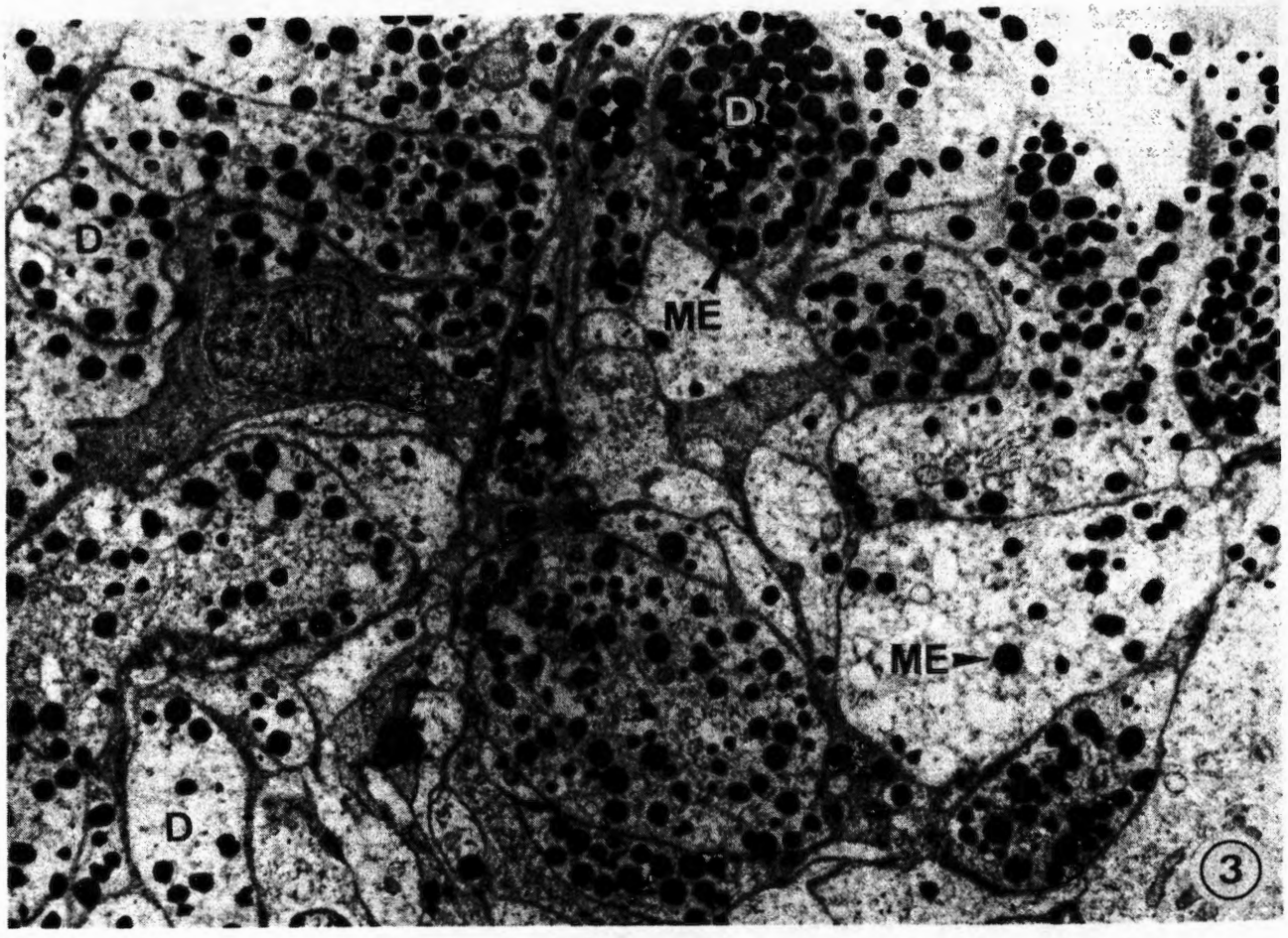

FIG. 3. Low-power electron micrograph of melanoma cells, which show many interdigitating dendritic (D) processes. The cytoplasm is filled with numerous melanosomes (ME). N = nucleus. (× 4,800)

the tumor is composed of pigment cells, which are obviously transformed. The melanoma cells are tightly packed and exhibit dendritic processes (Figs. 3, 4). The plasma membranes show many interdigitations.

The malignant melanoma consists of a large number of incompletely differentiated cells and a smaller number of well-differentiated cells. The well-differentiated transformed pigment cell is highly dendritic. The interdigitating membranes form a meshwork of cells resembling nervous tissue cells. The poorly differentiated cells show less interdigitation or even none. In any case, intercellular junctions, *e.g.*, desmosomes, are lacking.

The nuclei of the melanoma cells are large and exhibit bizarre, lobulated surfaces. Protrusions, nuclear pockets, and numerous nuclear pores were found regularly (Figs. 3, 4). Strong pinocytotic activity is shown by abundant pinocytotic vesicles, which were mostly arranged in irregular patterns (Figs. 5, 6).

The spheroidal or ellipsoidal melanosomes (diameter 200–650 nm) are distributed throughout the cellular dendrites, whereas the perinuclear cytoplasm is free of melanosomes. The degree of melanization of melanosomes correlates positively with the degree of cell differentiation. Highly differentiated cells are heavily melanized, concealing the internal structure and occasionally the surrounding membrane (Fig. 7). In less well differentiated cells, the melanosomes are incompletely melanized (Fig. 8). In TEM there

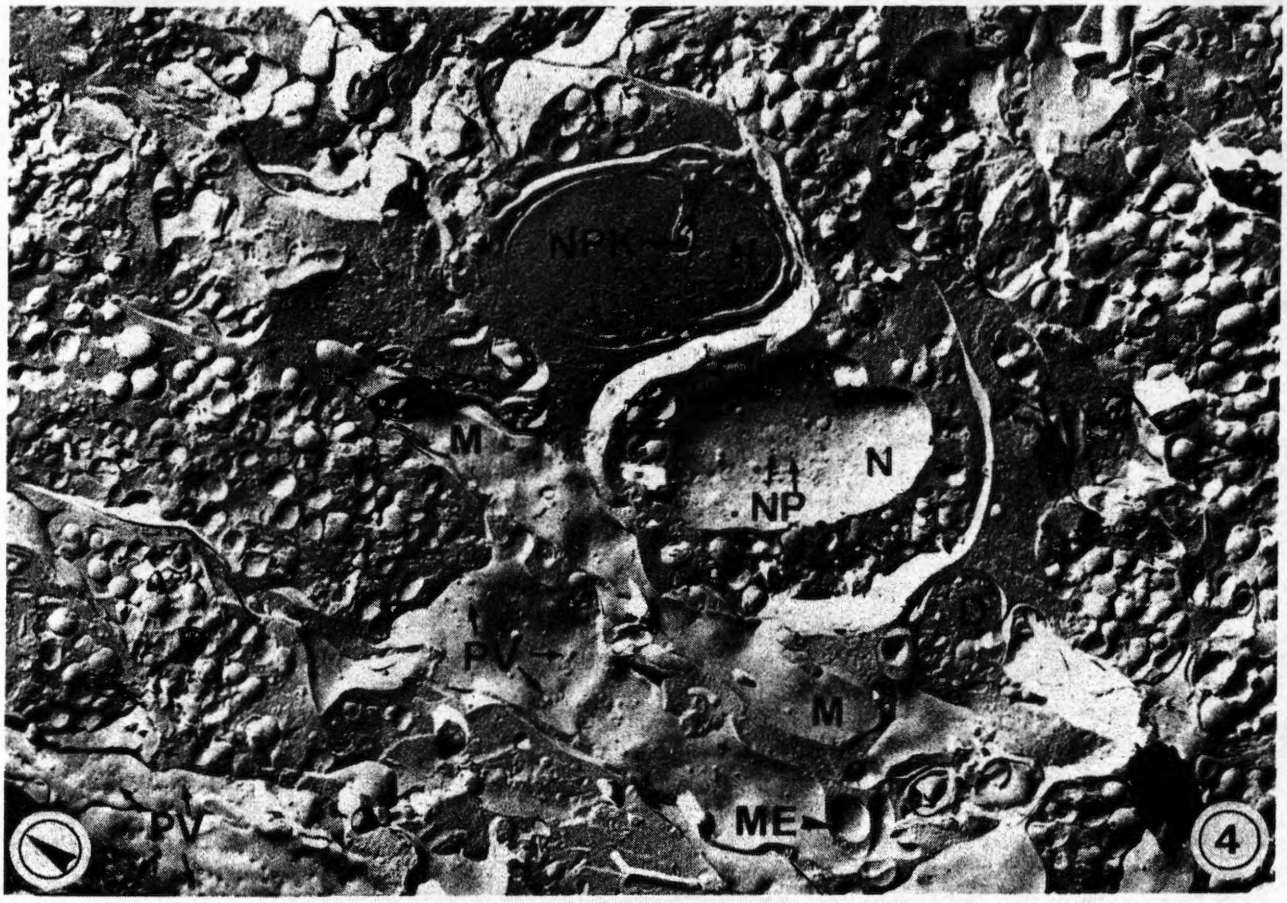

FIG. 4. Low-power FE micrograph of MM in *Xiphophorus*. Note the interdigitating dendrites (D) and the abundant melanosomes (ME). One nucleus (N) shows the outer surface with nuclear pores (NP); the other nucleus exhibits its interior resulting from a cross-fracture. M = membrane; NPK = nuclear pocket; PV = pinocytotic vesicles. Arrowhead indicates the shadowing direction. (× 8,250)

is no internal architecture (protein layer) or a higher order of organization of melanin granules detectable.

In TEM, melanosomes can be easily recognized by means of their inner architecture, whereas their identification is difficult in FE. In FE replicas, the melanosomes exhibit three types of fracture faces: types 1 and 2 represent complementary fracture faces of the limiting membrane (plasma and external face) (Fig. 9). Type 3 shows a cross-fracture of the melanosome without any internal structure. Types 1 and 2 are more frequent than type 3. The membrane surrounding the melanosomes carries particles with a random distribution, as seen by FE.

Occasionally, melanosome complexes (*i.e.*, "compound melanosomes") are found. They consist of a few to numerous melanosomes, which are all enveloped by a separate membrane (Fig. 10).

DISCUSSION

In this study, the ultrastructure of malignant melanoma in *Xiphophorus* is described as investigated by analysis of FE replicas. To complete our data, we also reinvestigated the morphology of malignant melanoma by light microscopy and conventional TEM

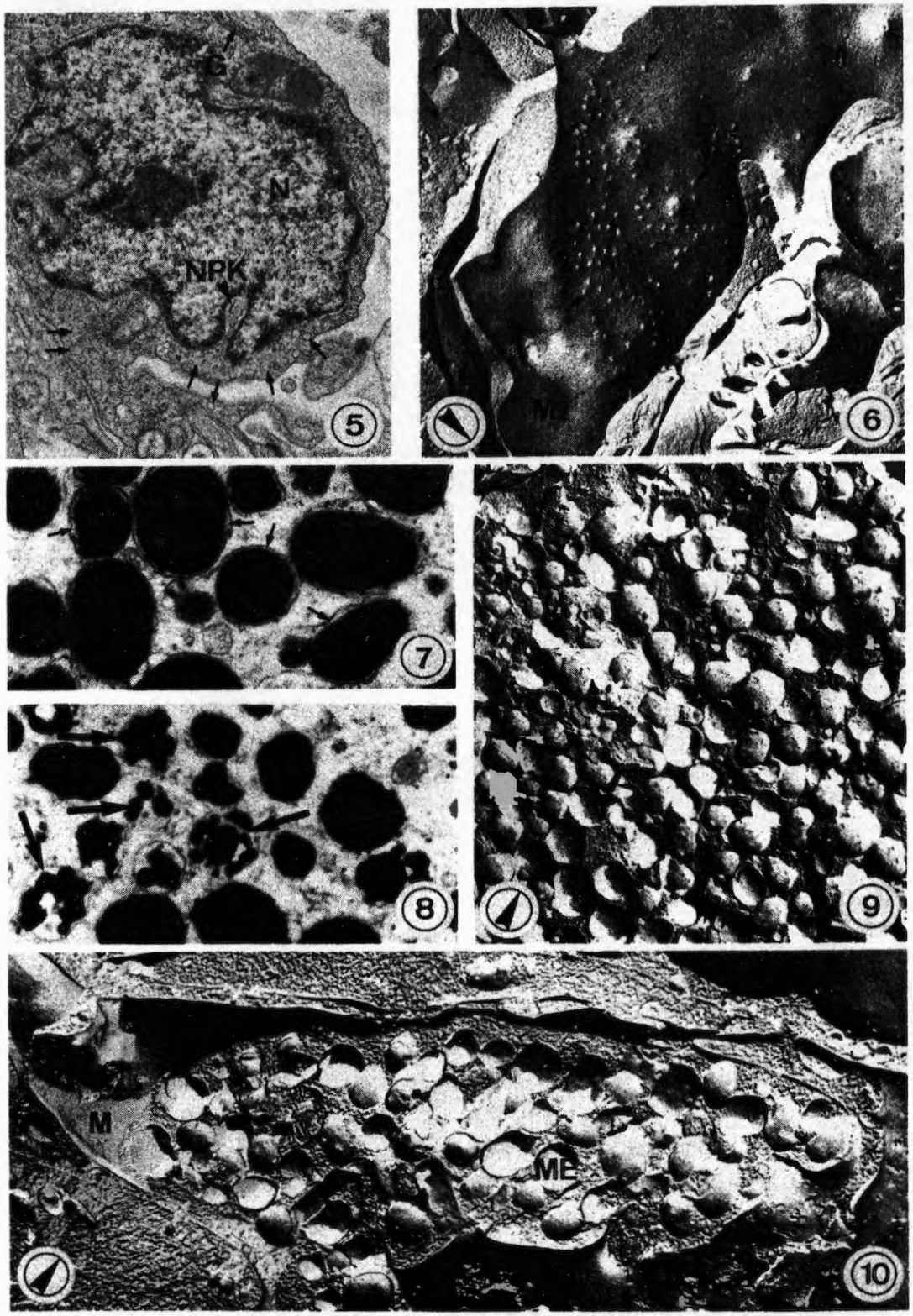

FIG. 5. TEM micrograph of a lobulated MM cell nucleus (N) with a nuclear pocket (NPK). Small arrows indicate pinocytotic vesicles. G = Golgi apparatus. (× 10,000)

FIG. 6. Area of a fractured membrane (M) with numerous pinocytotic vesicles (arrows). Arrowhead = shadowing direction. (× 19,800)

FIG. 7. TEM high-power micrograph of mature melanosomes, which are mostly enveloped by a membrane (arrows). (× 40,000)

FIG. 8. TEM high-power micrograph of mature melanosomes and premelanosomes (arrows). (× 50,000)

techniques. The results thus gained provide us with basic information with which to compare the pathomorphological changes during melanoma formation in fish and humans.

In both TEM and FE, the MM cells of fish showed a marked cellular and nuclear polymorphism. There were many interdigitations among the cell membranes of highly differentiated cells, whereas they occurred sparsely in poorly differentiated cells. The nuclei were hyperplastic and lobulated.

Another feature of MM cells is the occurrence of heavy pigmentation. In TEM, mature melanosomes of fish melanoma cells showed no internal structure. In the immature premelanosomes, a coarse-grained arrangement of melanin was found. In this case, melanosomes in fish differ from those in humans, where the majority of melanosomes show a mostly granular internal architecture, or sometimes a lamellar one.[12]

There are different observations as to the presence of cell junctions in fish and human melanoma. While a lack of intercellular junctions has been reported from human melanoma[7,8,13,14] and from fish melanoma,[3,8] Sobel *et al.*,[4] who have already compared the ultrastructure of fish and human melanoma on the TEM level, reported that junctional changes and desmosomes occur in human melanoma, but not in fish melanoma. Hitherto, intercellular junctions have neither been found in normal human melanocytes, melanocytes of nevi or melanomas, nor in fish melanomas.

FE provides additional information in respect to surface specializations. In FE replicas, the plasma membranes of *Xiphophorus* melanoma cells show numerous pinocytotic vesicles. This finding is in good agreement with TEM studies,[2,4] which frequently describe pinocytotic vesicles at the membranes of melanoma cells in fish. The abundance of pinocytotic vesicles points to strong transport activity, which is believed to reflect the exocytosis of "basement-membrane-like" material in these areas.[4]

In FE replicas it is difficult in many cases to distinguish melanosomes from other vesicular elements like mitochondria or lysosomes. Corresponding to the observations on B16 melanoma[6] and human melanoma,[7] this can only be performed by parallel studies of ultrathin sections of the same melanoma.

Melanosome complexes (*i.e.*, "compound melanosomes"), which have been shown by FE to occur regularly in human melanoma,[7] were also demonstrated by FE to occur in fish without any structural deviation. They are interpreted as autophagic melanosome degrading vacuoles.[3,4]

The only crucial difference between fish and human melanoma is the presence of an additional stage of pigment-cell differentiation, the transformed melanophore. These cells represent the terminal stage of differentation in fish, whereas in mammals pigment-cell differentation ends in the non-tumorous as well as in the tumorous condition at the preceding stage, the melanocyte.

FE supplements TEM of ultrathin sections, which has clear advantages in the demonstration of the internal architecture of cytoplasmic organelles and nuclei. FE, how-

FIG. 9. FE micrograph of numerous melanosomes. Arrowhead = shadowing direction. (× 14,000)

FIG. 10. FE micrograph of a melanosome complex ("compound melanosome"). This complex consists of numerous melanosomes (ME), which are enveloped by a separate membrane (M). Arrowhead = shadowing direction. (× 13,000)

ever, provides a new dimension for the examination of general ultrastructural topography with respect to the nuclear pores, pinocytotic vesicles, and junctions.

We have shown in a recent study[8] that, especially in FE replicas, fish melanomas cannot be distinguished from those of humans. There are no crucial differences in architecture of inner organization. Minor deviations are—as pointed out here—due to fish-specific features, especially to the presence of the melanophores, which do not occur in higher vertebrates.

Acknowledgments

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