

IMMUNOBIOLOGY OF MELANOMA

Gross and Ultrastructural Studies in a New Melanoma Model: The Sinclair Swine

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Work with the Sinclair swine began about 5 years ago when certain animals were noted to have an unusually large number of pigmented tumors (1) (Fig. 1). Initial analysis of these animals was done in a 2-year study of the evolution of these tumors, (2) which characterized the tumors in several stages that paralleled the life cycle of human melanocyte nevi (3). It appeared then that all lesions progressed through all these stages, from flat macules—Stage I, to smooth nodules—Stage II, to florid exophytic tumors—Stage III, and then slowly regressed in Stages IV and V (Fig. 2). A significant finding of this study was the striking generalized depigmentation seen with tumor regression in some animals (Fig. 3). It was apparent in the preliminary studies that not all lesions progressed from Types I–V and that more than one type of lesion was present in these animals. About 2 years ago we began to study these animals with the following objectives: (1) To characterize the types of pigmented lesions and determine their human correlates; (2) to determine the chronology of the lesions with particular reference to the processes of depigmentation and malignant degeneration; (3) to begin selective breeding to increase the incidence of tumors; (4) to examine these lesions at the ultrastructural level. Ultrastructural study was necessary since on initial light-microscopy studies melanin was so abundant that even after bleaching the exact separation of melanocyte and melanophage was not possible (Fig. 4).

Many of these lesions have now been evaluated and characterized. One example of malignant degeneration resulting in death of the animal was seen during the study. The types of pigmentary disorders seen in animals include: vitiligo, blue nevus, junctional nevus, compound nevus, congenital or bathing trunk nevus, Sutton's halo nevus, and melanoma (5).

Selective breeding has dramatically increased the number of pigmented lesions present at birth and early life (Fig. 5). The rate of regression and incidence of depigmentation is currently being evaluated.

From initial studies it appeared that the rate of malignant degeneration was less than 10%. This needs to be reevaluated. With the new genetic makeup from in-



FIG. 1. Large exophytic tumors on a young pig. In the lower abdomen in the region of the umbilicus is a large tumor from which a biopsy specimen has been taken. On the left thigh is another small lesions that later became much more elevated.

breeding the rate of malignant degeneration may change just as has the incidence of congenital tumors.

#### **MATERIALS AND METHODS**

Materials used for the study are: (1) large pigmented lesions prior to undergoing regression clinically comparable to large compound nevi in human tissue, (2) regressing lesions from animals which were beginning to have initial stages of perilesional depigmentation, (3) sections from the mediastinal tumor in a pig that had died because of respiratory obstruction from the tumor; and (4) cutaneous tumors



FIG. 2. Early involution. In many of these animals the first sign of regression is the appearance of depigmented hair around the lesions. These changes in the hair coat allow one to look deep into the hair to find an involuting lesion. Here we can see some whitening and keratosis of this lesion.

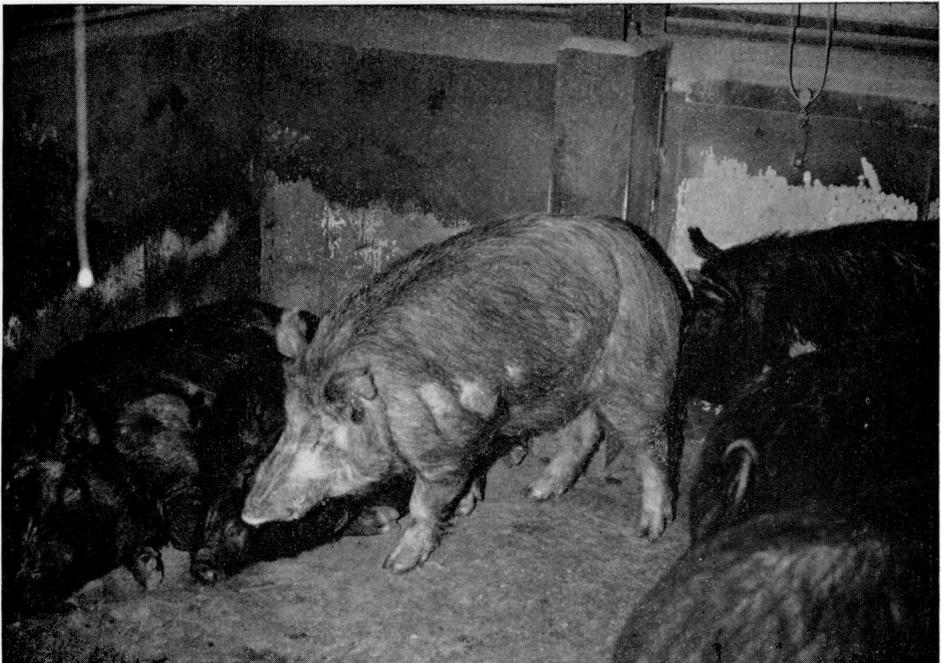


FIG. 3. Depigmentation of the swine. With involution, depigmentation often begins in symmetrical sites much like vitiligo. The animal in the center has undergone near-complete depigmentation. Accentuation is seen around the eyes and proximal to the hooves. The normally pigmented swine surround this animal.

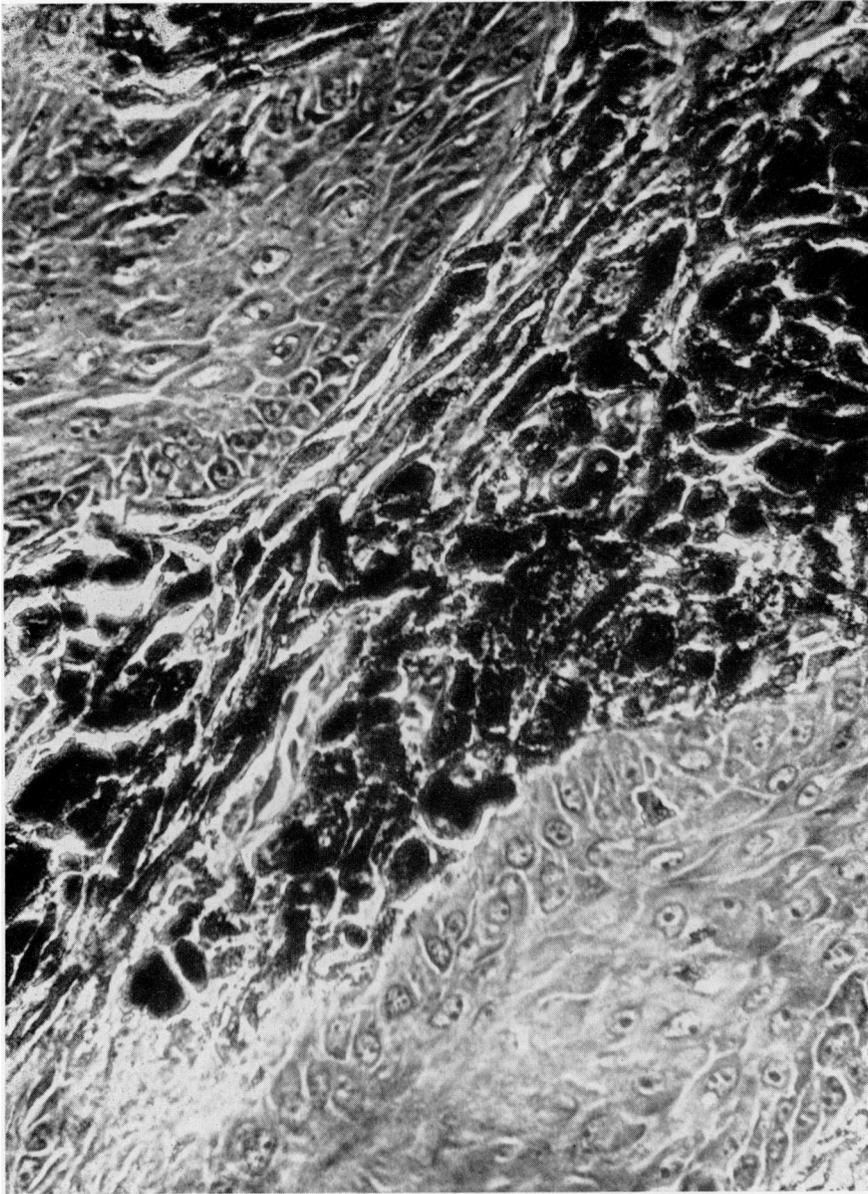


FIG. 4. Microscopic. The extreme abundance of melanin and melanocytes in the dermal papilla is seen on this high-power light-microscope picture. Nearly all the tumors contain this heavy deposition of melanocytes and melanin that obscures fine cellular structure.

and subcutaneous tumors in a weanling pig that were present at birth. These lesions were characterized, classified, and photographed prior to biopsy then excised and processed for both light and electron microscopy.

Lesions studied for light microscopy were first fixed in formalin, embedded in paraffin, and sectioned. These sections were then stained with hematoxylin and eosin. It was necessary in most cases to bleach the specimens with permanganate because of the heavy production of melanin in lesions.

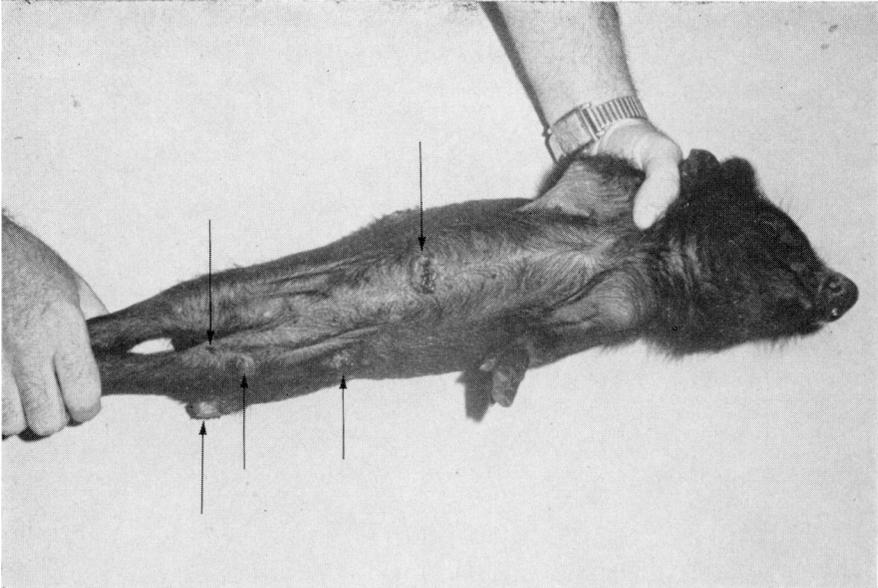


FIG. 5. Current breeding results have dramatically increased the number of pigmented tumors present at birth. In this 6-week-old pig we can easily make out (see arrows) the many tumors.

Because of the difficulty in evaluating even bleached hematoxylin and eosin specimens subsequent tissues were all studied by preparing for electron microscopy and then evaluating the thick sections under the light microscope.

For electron microscopy specimens were prefixed in buffered 2.5% glutaraldehyde, postfixed in osmic acid, and dehydrated in graded solutions of alcohol. The tissue was then embedded in epon 812, thick sections were evaluated under light microscopy, and thin sections of appropriate areas for study were then sectioned on a Porter-Blum MF2 ultramicrotome and stained with uranyl acetate and lead citrate. Sections were then studied in the electron microscope Hitachi models HS-7 and HS-8.

## RESULTS

Tumor tissue was evaluated to determine whether the predominant cells were melanocytes or, the predominance of melanosomes within lysosomes without early melanosome production. The melanocytes were compared with benign and malignant human counterparts. Criteria of benignity or malignancy (those of Clarke (6), Hirone (7), and Mishima (8)) were (1) degree of organization of organelles; (2) presence of disarrayed melanosome, granular organelles, and lamellar organelles; (3) nuclear aberration; (4) evidence of increased ribosomal and mitochondrial elements; (5) completeness of melanization of the organelle; and (6) abnormalities in melanosome transfer to keratinocyte and the presence of compound melanosomes.

The typical exophytic benign tumor (Figs. 1, 4) showed a network of melanocytes, with dendrites extending in every direction (Fig. 6) among the collagen bundles just beneath the basal lamina.

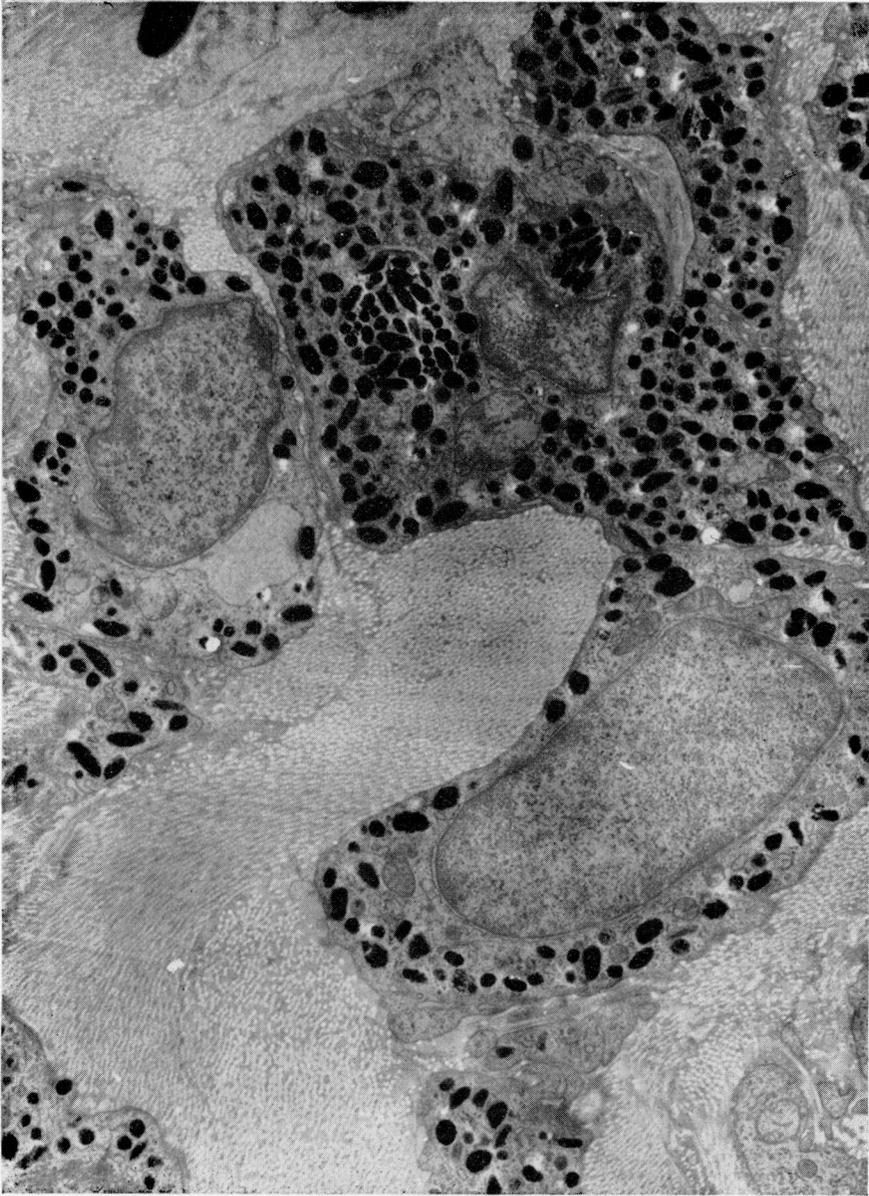


FIG. 6. Low-power view of an exophytic abdominal tumor. Melanocytes here are in the papillary dermis forming an extensive network just below the basal lamina.  $\times 10,000$ .

Several melanocytes are in juxtaposition, many with their dendrites abutting each other. The overall architecture typically resembles a human compound nevus. The extensive melanin production characterizes these lesions in pigs. Normal benign melanocytes showing several stages of melanosomes are visible at higher power (Fig. 7). At the tip of the dendrites most of the melanocytes are Stage IV, while in other areas of dendrite are Stage II and III melanosomes. Melanophages are common in these deeply pigmented tumors (Fig. 8). These are easily differentiated from melanocytes at the ultrastructural level.

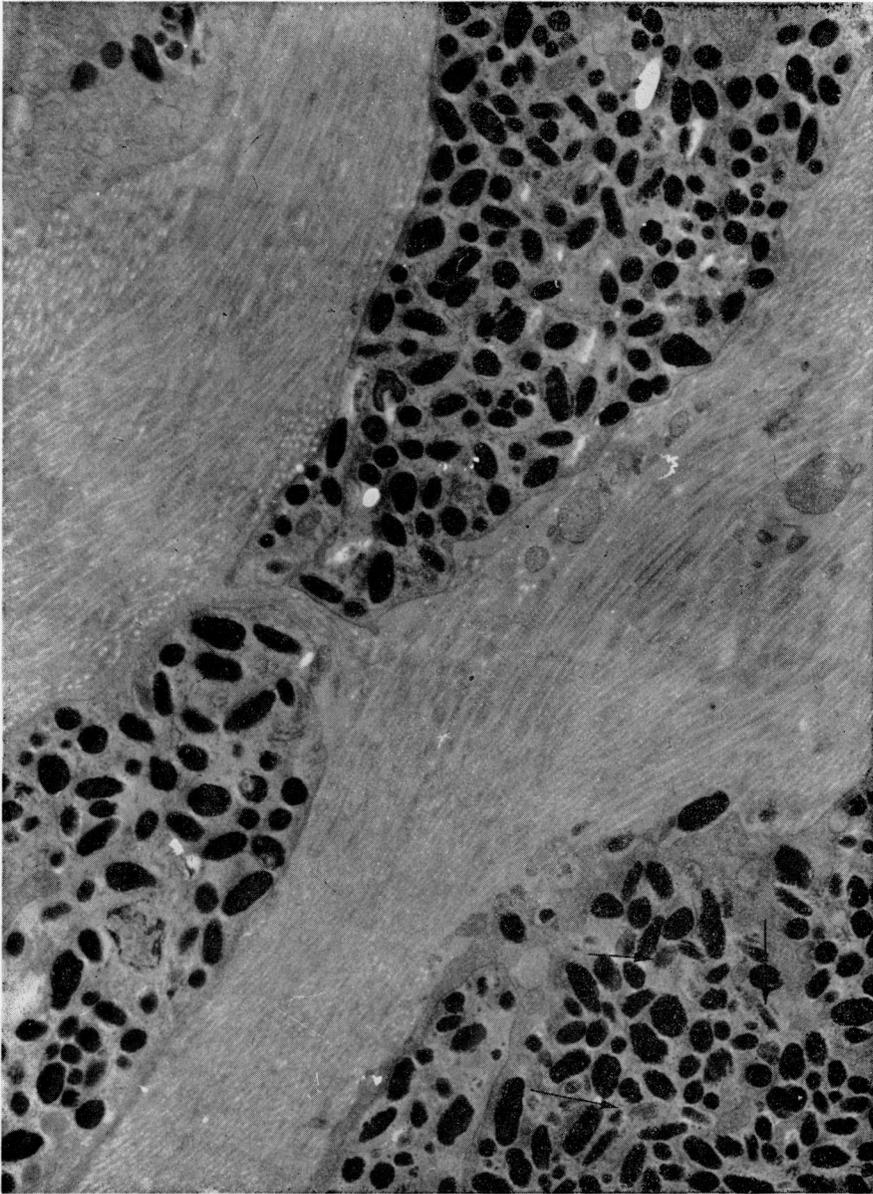


FIG. 7. High-power view of the dendrites of two melanocytes. In this distal region most of the melanosomes are Stage IV. In the corner is another cell in which are visible melanosomes of early stages (see arrows).  $\times 16,200$ .

Many of these animals have had subcutaneous masses we considered to be tumor metastases. Biopsy confirmed this on many but others were either adenopathy or dermal melanosis. The benign subcutaneous masses are characterized by large numbers of melanophages. Melanocytes are also present in these tumors (Fig. 9) and are easily differentiated ultrastructurally.

Some benign tumors showed cells with bizarre changes suggesting malignancy. Melanosome production is intense and unusual vacuolization is present in some cells as well as nuclear bodies (Fig. 10).

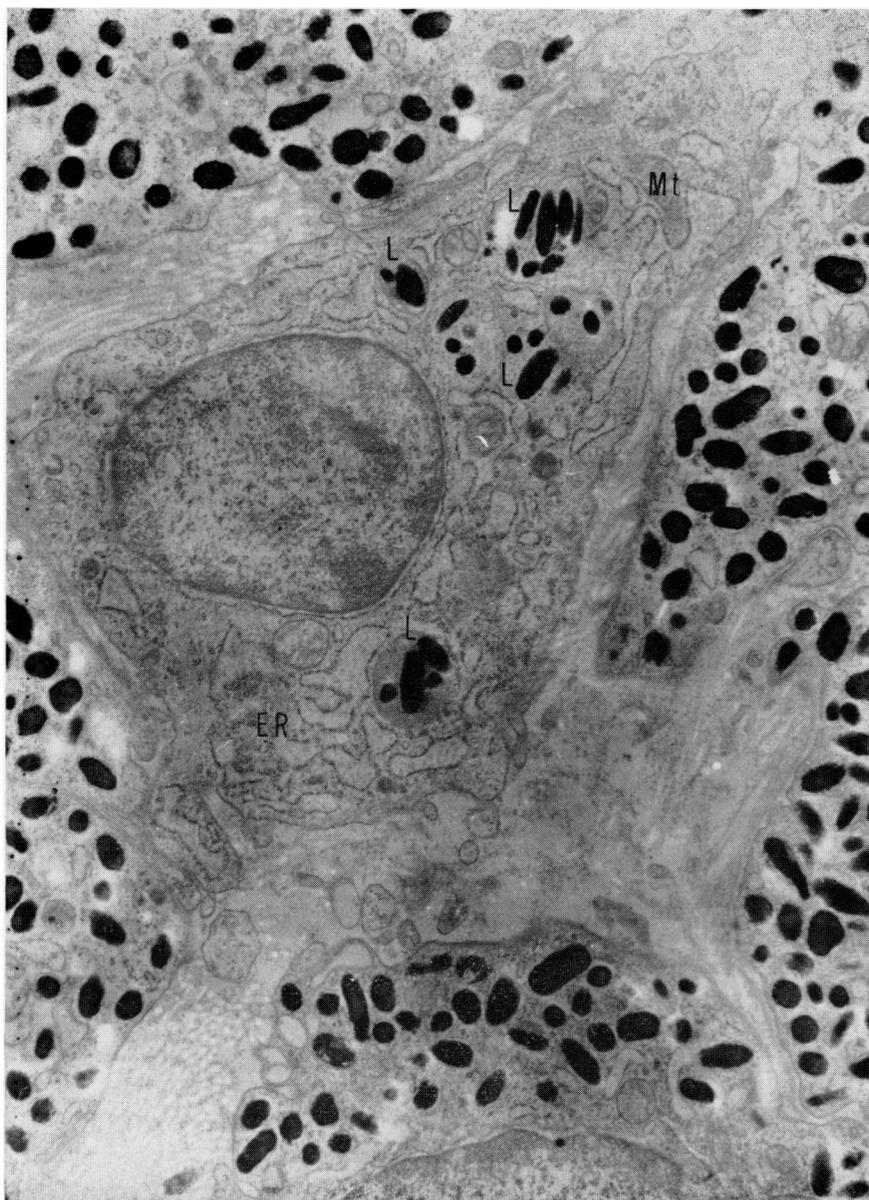


FIG. 8. A melanophage in the tumor. Particularly prominent is the endoplasmic reticulum (er) and mitochondria (mt) which helped differ them from melanocytes. The lysosomes (l) contain phagocytized melanosomes.  $\times 11,000$ .

The ultrastructure of a tumor in the lung of a pig (Fig. 11) that died from obstruction caused by a large mediastinal tumor shows many melanosomes. Most of these cells are early forms showing irregular pigmentation of the melanosomes. In a dendrite are early melanosomes (Fig. 12) with disordered development of the melanosome stages, and unusual mitochondria. These tumors were highly productive of melanin and numerous melanophages found within the pulmonary tissue of this pig (Fig. 13). Here the striking and very large phagolysosomes are easily

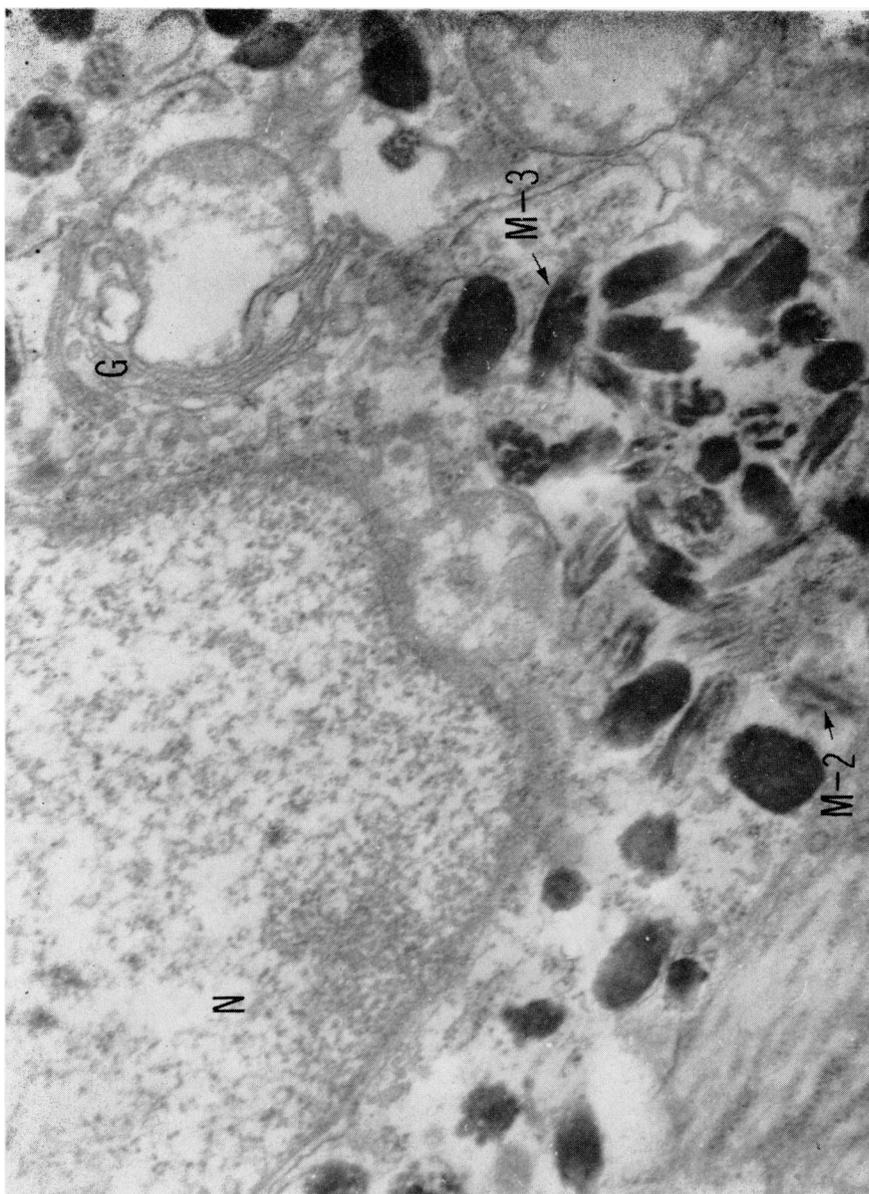


FIG. 9. High-power view of a typical melanocytes within these tumors showing several stages of melanogenesis. Stage II melanosomes m2) and Stage III (m3) are seen as well as fully melanized forms. (n-nucleus, g-Golgi apparatus).  $\times 33,000$ .

visible. There are several lysosomes without apparent melanosomes within them, and varying degrees of degradation of the melanosomes within the phagolysosomes. In this same animal within mediastinal lymph nodes (Fig. 14) are both melanocytes and lymphocytes.

## DISCUSSION

Electron microscopy has allowed a better look at ultrastructure confirming similarities of our animal model to human tissue. The impressive exophytic tumors

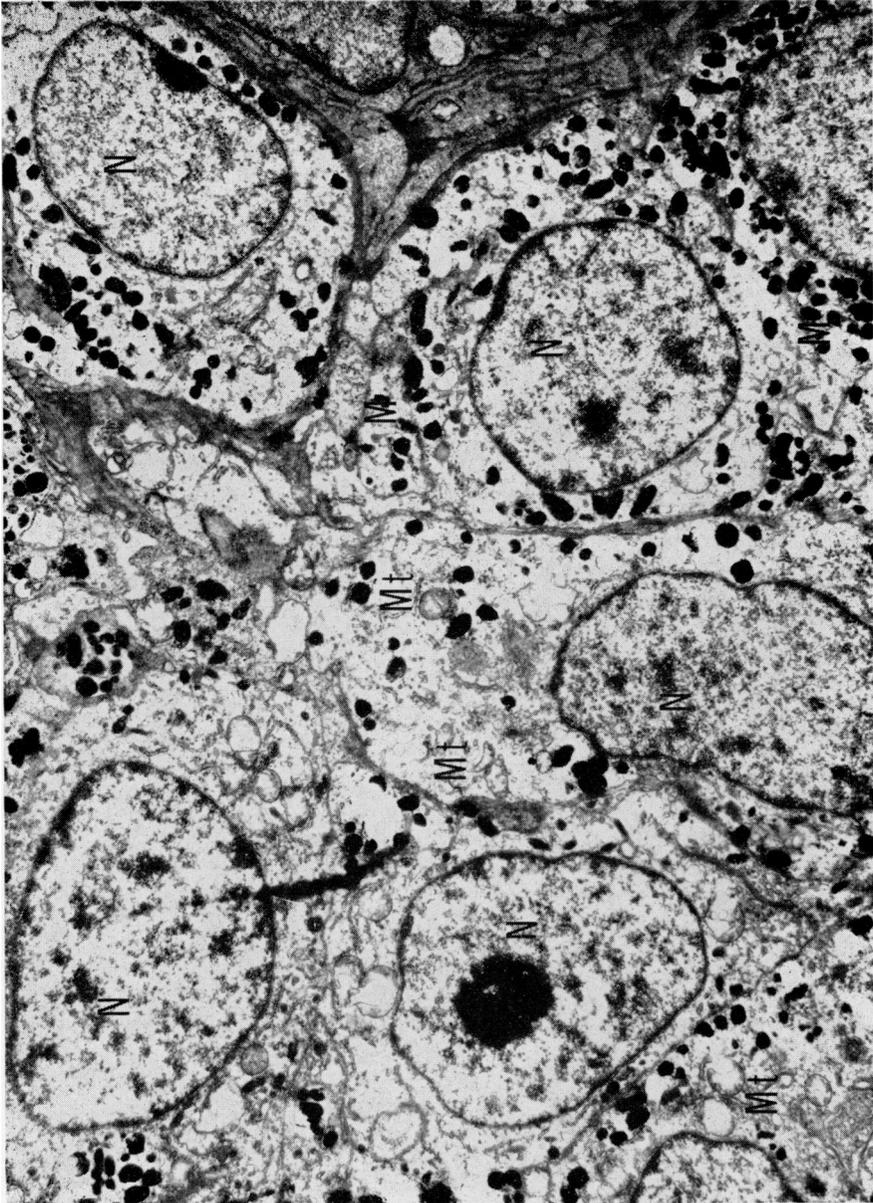


FIG. 10. In some tumors the cells demonstrate bizarre nucleoli and clumped heterochromatin in the nucleus (n). Some changes are also seen within the mitochondria (mt) and many of the melanosomes (m) are in Stage IV.  $\times 7500$ .

contain normal melanocytes. The only exception in this category of clinically benign exophytic tumors are those demonstrated in Fig. 9. The vacuolization in the cell and the mitochondrial changes approach Klug's type-B melanoma (8). These tumors were in some cases rejected shortly afterward, suggesting that these changes could be degenerative. Regressed lesions show a nearly total lack of melanocytes similar to human vitiligo.

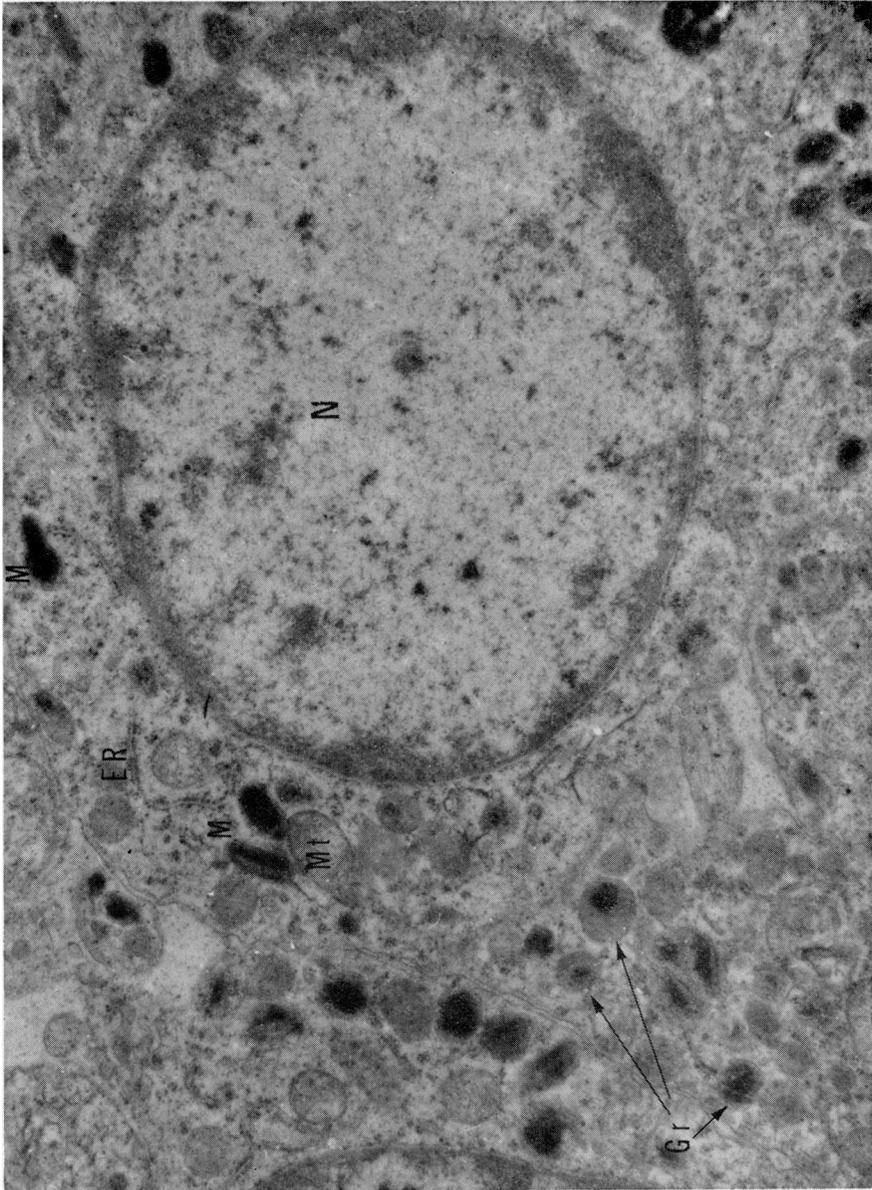


FIG. 11. High-power view of lung tumor in pig. This cell shows evidence of abnormal melanin synthesis with some early granular organelles (gr) and incomplete melanization of other melanosomes. The Stage III and IV melanosomes show variation in shape (m). (n-nucleus, er-endoplasmic reticulum, mt-mitochondria).  $\times 18,000$ .

The malignant tumors are similar to the type-A melanoma described by Klug and some of our specimens of superficial spreading melanoma. The abnormal mitochondria have been reported previously by Mishima and Hirone. The type-A melanoma of Klug has nuclear changes most similar to those seen in our animals including occasional nuclear bodies, large nucleoli, and prominent heterochromatin. Melanization is abnormal and parallels human tumors. Incomplete melanization

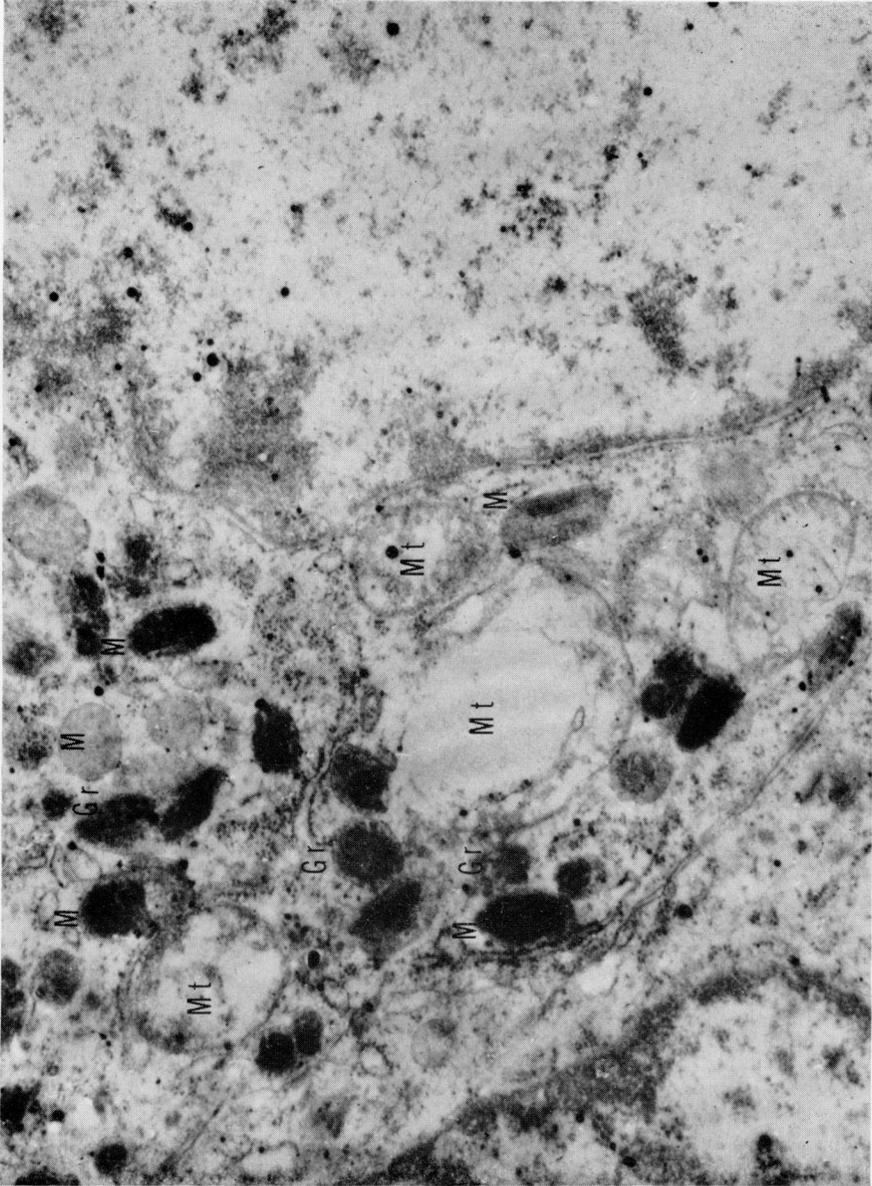


FIG. 12. High-power view of a dendrite in the lung tumor. Considerable variation is seen in mitochondria (mt) similar to that described in many human melanomas. Melanosomes of considerable variation in shape (m) are seen, as well as many that appear to have the granular variation (gr).  $\times 22,000$ .

is common in the tumors and both granular and fibrillar patterns in the melanosomes are seen. This is easily contrasted with the progressive, orderly, overabundant melanosome production in benign tumors. Variations in shape of melanosomes have previously been described (9) and in our experience are common both in metastatic human melanoma and in our animals.

The newer litters with the high incidence of congenital tumors are particularly

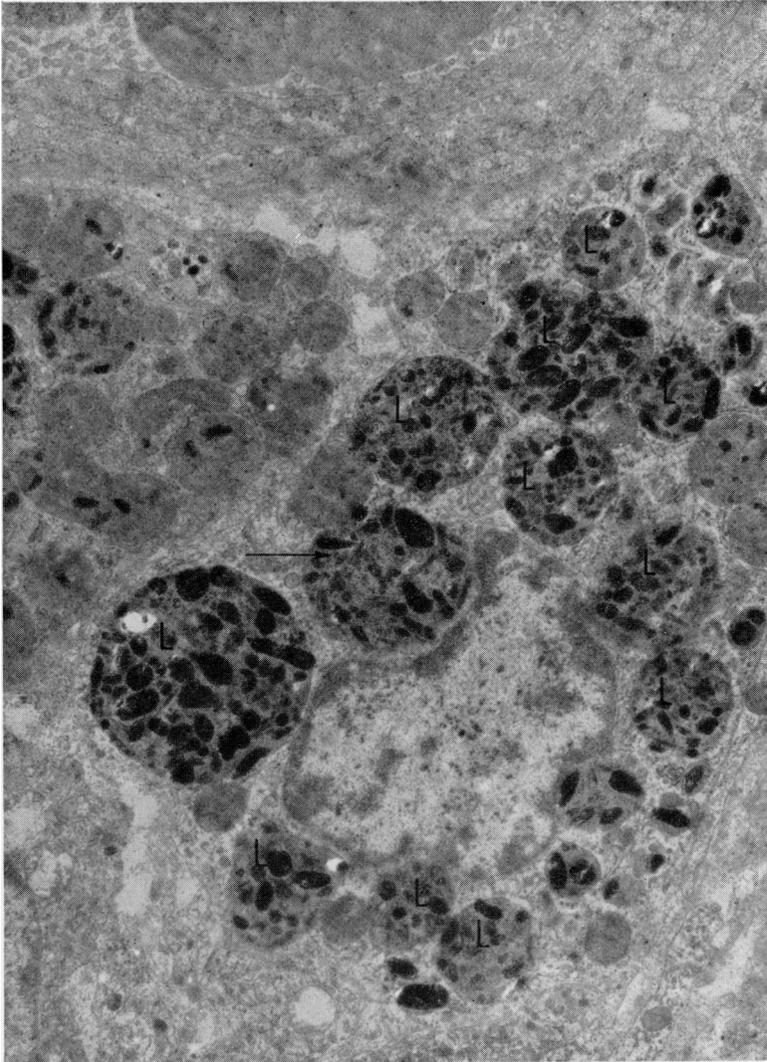


FIG. 13. Melanophages within the lung of the pig with melanoma. Associated with the tumors are large numbers of melanophages, their lysosomes (L) packed with large numbers of melanosomes. In some (see arrow) are seen partially digested melanosomes.  $\times 12,000$ .

interesting: We have seen large congenital nevi regressing by a factor of 30–50% in the first 4 months of life. We have also seen tumors appear in these animals clinically comparable to human junctional nevi and have seen them slowly enlarge. Thus far all these tumors have behaved in a benign manner. This current kindred with a high incidence of malignant tumors, however, is still too young to develop either spontaneous depigmentation or malignant degeneration.

The dramatic involution of these tumors results in near-total loss of melanocytes. This suggests potent regulatory mechanisms that could be of value in the treatment of human pigmentary abnormalities.

We feel that this animal model offers almost unlimited opportunities for studying the growth and regulation of melanocytic and nevocytic tumors in man.

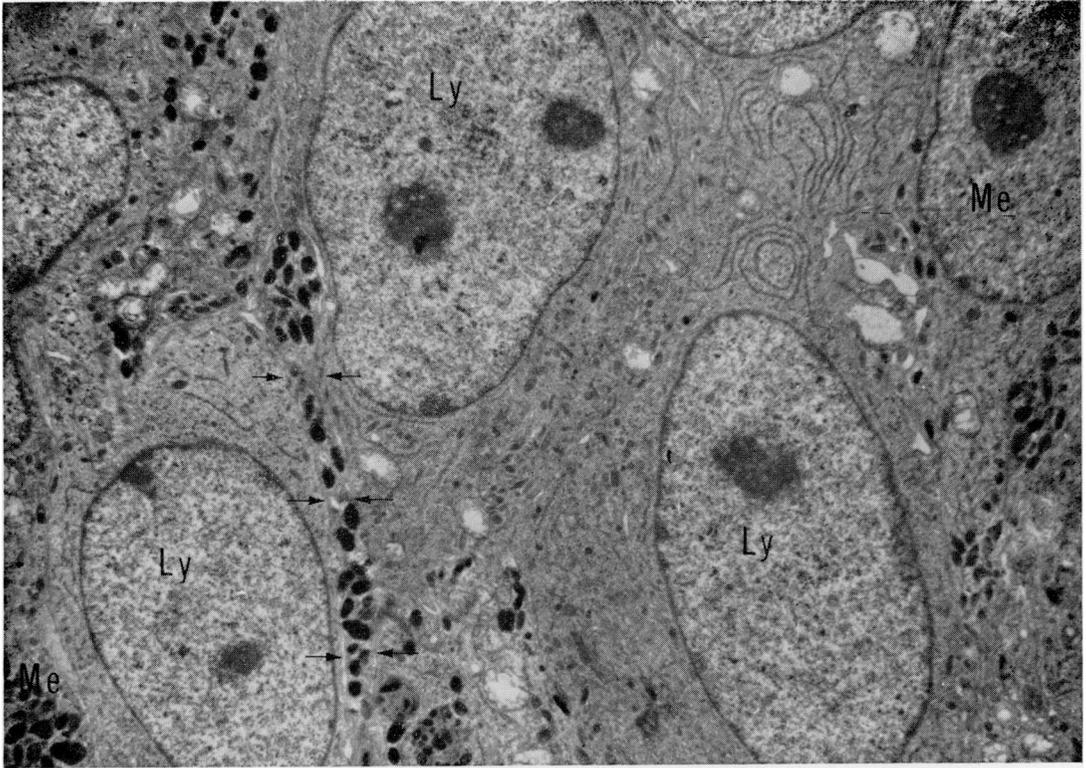


FIG. 14. Tumor metastatic to thoracic lymph node. Several lymphocytes (ly) are seen and in one corner the edge of a large melanocyte (me). Dendrites of another melanocyte (see arrows) interlace between lymphocytes. A melanophagocyte (mp) with lysosomes containing melanin (1) is seen in the upper center. (er-endoplasmic reticulum, n-nucleus, nu-nucleolus).  $\times 9400$ .

## SUMMARY

A large number of melanocytic nevi characterize the Sinclair swine at their present stage of breeding. These tumors often dramatically regress, associated with generalized depigmentation, and occasionally evolve into diverse and metastasizing lesions.

The melanocytes of these lesions and the clinical behavior of the tumors resemble the entire range of human melanocyte disorders from benign to malignant. Ultrastructural features of these tumors closely resemble human counterparts, varying only in amount of melanin. Malignant tumors show a close parallel to human superficial spreading melanoma and Klug's type-A melanoma.

Research into the etiology, progression, metastasis, and involution or therapy of melanocytic growth is now possible because of the close analogies demonstrated between this animal model and its human counterparts.

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