Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 169–173, January 1991
Medical Sciences

Melanosis and associated tumors in transgenic mice

(simian virus 40 large tumor antigen/tyrosinase promoter/pigmentation/neoplasia/metaplasia)

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Contributed by Beatrice Mintz, October 8, 1990

ABSTRACT **Melanosis was found to various extents in a wide array of tissues of all 23 autopsied mice whose transgene consisted of the tyrosinase promoter fused to the simian virus 40 early-region oncogenic sequences. Pigmentation in a given animal was attributable to any or all of the following: an increase in numbers of some normally pigmented cells of neural crest origin (a result compatible with early stages of transformation); elicitation of melanin synthesis in some cells that normally have little melanin, or none at all (the latter possibly signaling metaplasia); unusual intercellular transfer of pigment granules from melanocytes into certain normally unpigmented epithelia and endothelia; and profusion of melanin-phagocytizing cells. Neoplasms, occasionally also containing melanin, arose in association with some of these melanotic tissues and included three choroid plexus tumors, three endocardial tumors, two peripheral nerve sheath tumors (schwannomas), two cochlear tumors, two pineal gland tumors, one salivary gland tumor, and one nasal mucosa tumor. These apparently originated independently of the ocular and cutaneous melanomas found in the same animals. The events involved in melanosis may thus contribute to neoplastic conversion.**

In the course of examining our Tg(Tyr-SV40E) transgenic mice [mice whose transgene consisted of the tyrosinase promoter fused to the simian virus 40 (SV40) early-region oncogenic sequences] of the C57BL/6 strain that were designed to develop malignant melanomas (1), we observed at autopsy that they had acquired pigmentation in a variety of internal tissues and had occasional tumors associated with such tissues. These results were unexpected for several reasons. Although expression of the transgene would be anticipated in naturally pigmented cells, due to the tyrosinase promoter, present knowledge did not account for expression in all the other tissues now seen to be pigmented. Moreover, the SV40 early-region genes, encoding transforming sequences and linked to the tyrosinase promoter, caused a *lightening* of the coat (Fig. 1), to different degrees, in the same animals. In addition, the ocular and cutaneous melanomas due to the SV40 large tumor antigen were characterized by hypo- rather than hyperpigmentation.

The presence of nonneoplastic melanin-containing cells in tissues or organs that usually produce a much smaller amount of melanin or none at all is termed melanosis or melanocytosis. Apart from the usual occurrence in skin and eyes, moderate numbers of pigmented cells have been noted in the nictitans, meninges of the brain, harderian glands, parathyroids, thymus (2), inner ear (3), and spleen (B.M. and M.B., unpublished observations). They have been seen more pervasively only in the PET mouse strain (now extinct) in connective tissues throughout the body (4).

We describe here the melanosis and associated tumors found in our transgenic mice and discuss their possible implications for development and neoplasia.

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FIG. 1. The light grey-colored male founder of Tg(Tyr-SV40E) transgenic line 5 is shown in comparison with a black control of the same inbred strain (C57BL/6).

MATERIALS AND METHODS

The same group of transgenic animals as in the accompanying paper (1) provided the material for this study; only the 23 mice that were completely autopsied are included (see Table 1). Tissue preparation for microscopy has been described (1); in addition, some samples of heavily pigmented tissues were demelanized with sodium permanganate and oxalic acid for further examination.

RESULTS

Melanosis. All 23 mice had some incidence of melanosis, involving 1–14 organs, organ systems, or tissue types per individual, often in multiple sites per organ. The number of affected organs varied within and among lines; in lines 5 and 6, which had the largest numbers of animals, melanosis was more widespread in line 5, which had more copies of the transgene (Table 1). To a lesser extent, age played some role.

The following organs or tissue types exhibited melanosis with decreasing frequency in the indicated numbers of mice: nasal mucosa, 12; endocardium, 12; lungs, 12; meninges, 11; peripheral nervous system, 10; dermis (focally), 8; lymph nodes, 8; central nervous system, 7; genital organs and accessory glands, 7 (3 females, 4 males); skeletal muscle, 6; oral mucosa, 4; enamel organ of incisors, 4; pineal gland, 3; choroid plexus, 2; mammary glands, 2; larynx, 2; salivary glands, 2; bladder and urethra, 1. Examples are shown in Fig. 2 and in parts of Fig. 3 *a–c*.

In the nasal mucosa (Fig. 3*a*), melanin-containing cells were more frequently found in the olfactory than in the respiratory area and were located under the epithelium in contact with nerves and nerve endings; some were clearly part of the perineurium. In addition, there were scattered melanophages heavily laden with pigment. Pigment granules were sometimes seen in the nasal epithelium itself.

Abbreviation: SV40, simian virus 40.

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Table 1. Melanosis and associated tumors in Tg(Tyr-SV40E) transgenic mice

Line no.	≈ no. of transgene copies	Age at autopsy, wk	No. of mice with melanosis/ total	No. of organs with melanosis*	No. of mice with tumors†
3	15	15	1/1	1	1
4	8	13	1/1	6	1
5	6	2–18	6/6	1–14	3
6	4	3–19	10/10	1–7	3
7	4	15	1/1	4	
8	2	12–17	2/2	7–9	
9	1	22–23	2/2	5–6	

Only nonocular and noncutaneous tumors are included. All 23 mice had primary ocular melanoma and 4 had cutaneous melanoma, as described in ref. 1.

*Some organs (or organ systems or tissue types) often had multiple discrete foci of melanosis (e.g., in meninges, central or peripheral nervous system, dermis, lungs, skeletal muscle, or mammary glands).

†A total of 14 tumors, detected in autopsies at 12–18 wk, included 3 choroid plexus tumors, 3 endocardial tumors, 2 schwannomas, 2 cochlear tumors, 2 pinealomas, 1 salivary gland tumor, and 1 nasal mucosa tumor.

In the central nervous system, melanosis was most common in the olfactory bulb (Fig. 2*a*) and frontal lobe. The more intensely pigmented cells again appeared to be melanophages, here distributed around blood vessels. The general histopathological architecture resembled that of human infants with neurocutaneous melanosis syndrome (5), which includes melanosis of the leptomeninges, as in 11 of the transgenic mice. The perineurium of cranial and spinal nerves and of other large nerves and associated ganglia (Fig. 2*b*) frequently displayed melanosis. The choroid plexus occasionally included melanization in ostensibly normal cells (Fig. 3*b*).

Melanized cells were present in small clusters in the cortical areas of lymph nodes, chiefly in the head and neck. Most of the cells were rounded and markedly enlarged by melanin deposits in the cytoplasm (Fig. 2*c*). After bleaching, many had the large clear vacuolated cytoplasm and central nucleus of macrophage-like cells. Similar cells, sometimes observed in the red pulp and around the follicles in spleen, were S-100-positive. It is unlikely that all of these cells were melanophages, however, as premelanosomes and melanosomes in dendritic cells were demonstrated by electron microscopy (data not shown).

Melanosis was found in the endocardium of the atrioventricular valves (Fig. 3*c*). The cells retained their general structure and had variable numbers of melanin granules.

In lung, another frequent site of melanosis, very small foci of less than 20 heavily pigmented cells resembling melanophages were seen in the alveolar wall.

Skeletal muscle of the back and limbs of some animals contained conspicuous patches or streaks of pigmented cells (Fig. 2*d*). These were either stromal cells or neural cells in the endomysium or perimysium, rather than myocytes.

Some epithelial cells containing melanin, as well as melanophages, occurred in urogenital organs such as bladder, urethra, endometrium, prostate, preputial glands, etc.

Melanophage-like cells were also seen in the stroma of the salivary glands, in the connective tissue of the enamel organ in the incisors (Fig. 2*e*), and in the mammary glands (Fig. 2*f*). Dermal melanocytes as well as melanophages often formed small clusters or nodules, resembling early nevi, in the nipple areas, in the skin of the snout, and more rarely in the lamina propria of the larynx, pharynx, and oral mucosa.

Tumors. Eight of the transgenic mice (Table 1) had a total of 14 tumors in 7 organs, other than eye or skin melanomas. All were detected in autopsies at 12–18 wk of age and occurred only in lines with multiple (4 to 15) transgene copies. These animals represent 47% of the 17 mice with multiple (2 to 15) copies of the transgene that were autopsied in this age range. The neoplasms included 3 choroid plexus tumors, 3 endocardial tumors, 2 peripheral nerve sheath tumors (schwannomas), 2 cochlear tumors, 2 pineal gland tumors, 1 salivary gland tumor, and 1 nasal mucosa tumor. One mouse (in line 5) had 5 tumors (choroid plexus, schwannoma, cochlear, pineal, and salivary); another (also in line 5) had 3 tumors (choroid plexus, cochlear, and nasal).

The nasal mucosa tumor (Fig. 3*a*) was an amelanotic melanoma in close contact with the melanotic nasal mucosa already referred to.

The choroid plexus tumors were well or moderately differentiated papillary carcinomas accompanied in two of the three cases by melanosis of the neighboring normal choroid plexus (Fig. 3*b*). Pigmented cells of melanophage morphology were in the connective tissue and vascular papillae of the normal area and at the adjacent border of the tumor.

Endocardial tumors were amelanotic poorly differentiated melanomas attached to a heart valve in which some melanized cells were present (Fig. 3*c*).

Cochlear duct tumors in the inner ear were hypopigmented epithelioid melanomas (Fig. 3*d*). (The normal pigment cells of the cochlea are also present in these mice.)

Schwannomas were found near the lumbar spinal nerves. In one case, the tumor was an intraspinal benign schwannoma with melanosis in the perineurium of the nerves. The other was a deeply pigmented infiltrating malignant tumor with melanosomes visualized by electron microscopy (data not shown). The tumor involved the vertebral column and the lumbar muscles (Fig. 3*e*). After bleaching of sections, spindle cells were seen arranged in whorls and bundles (Fig. 3*f*). This tumor closely resembles the malignant melanocytic schwannoma described in humans (6).

The pineal tumors were small and were associated with melanin-containing cells in the parenchyma of the gland as well as in the neighboring meningeal cells.

The salivary gland tumor was an adenocarcinoma infiltrating the tongue and pharynx; melanosis was present in nonneoplastic cells of the salivary gland.

DISCUSSION

The most noteworthy feature of this array of 14 tumors (encompassing 7 kinds of noneye, nonskin tumors) in Tg(Tyr-SV40E) transgenic mice is the presence in most of them (except choroid plexus and salivary gland tumors) of some melanized cells, and the proximity of all of them to nontumorous cells that are melanized. The schwannomas were distinctly melanotic (as in some human schwannomas) and four other types—the cochlear, endocardial, pineal, and nasal tumors—had very little pigmentation but were characterized as melanomas on the basis of their histopathologic resemblance to the hypomelanotic or amelanotic ocular and skin melanomas in the same animals (1). "Melanoma" has been used classically to designate melanized tumors rather than tissue of origin; thus, human primary melanomas have been reported in various tissues, including not only skin and eyes but also nasal mucosa, oral mucosa, bronchial mucosa, urethra, etc. (7).

The tumors seen in these animals rarely occur spontaneously in mice. Choroid plexus (8, 9) and pineal (10) tumors have been produced in transgenic mice with constructs containing SV40 large tumor antigen sequences, as in the present cases, but not with the tyrosinase promoter. Possibly this promoter is not completely tissue-specific; its expression was in fact detected in transgenic brain RNA preparations (S.P. and B.M., unpublished data). Salivary gland tumors have appeared in transgen-

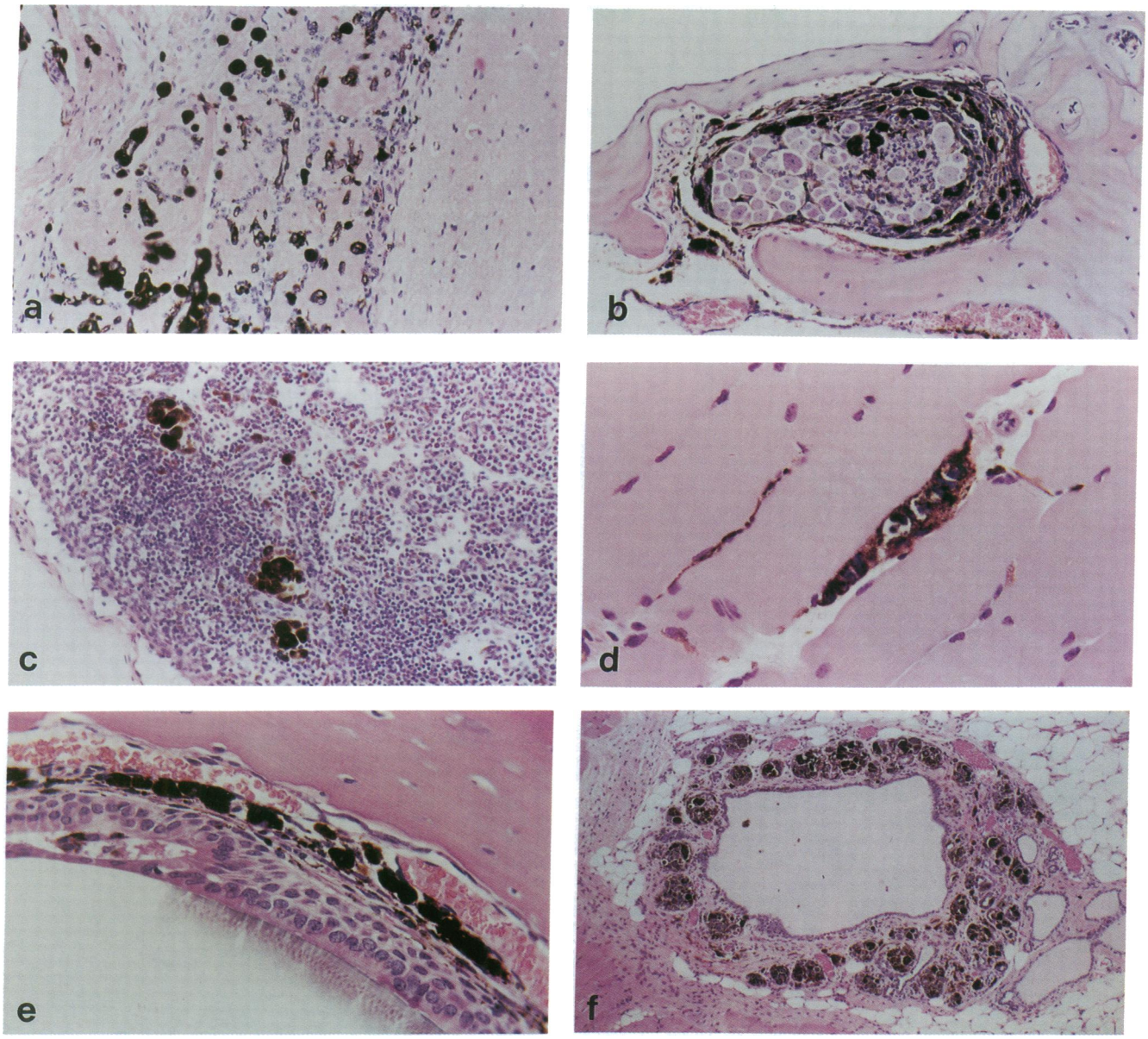

FIG. 2. Examples of melanosis in various tissues of the transgenic mice. (*a*) Pigment-laden cells in this olfactory bulb of the brain. (*b*) A vestibular ganglion with the most pigmentation in the spindle cells around the periphery. (*c*) Melanin-containing cells in the cortical and paracortical areas of a cervical lymph node. (*d*) Melanosis in striated muscle. (*e*) Enamel organ of an incisor with melanin-bearing cells in the connective tissue of the dental sac. (*f*) Melanosis in the stromal cells in contact with a main mammary duct. (*a–c*, ×80; *d*, ×160; *e*, ×320; *f*, ×50.)

ics with the *int-1* oncogene (11). Pigmentation or melanomas were not reported in any of these mice.

The evidence supports the conclusion that cells accounting for melanosis, or for pigmentation in or near the tumors, are independent of the ocular melanomas. Three mice with melanosis (two in line 5 and one in line 6) had early ocular melanomas that had neither invaded nor metastasized. And two mice (in line 6) with a pineal and a choroid plexus tumor, respectively, had advanced ocular melanomas with no indication of metastasis (1).

The melanized cells appear to have diverse developmental origins. Organs or tissues such as the cochlea (3) and leptomeninges (2) normally contain pigmented melanocytes which are believed to originate from the neural crest; these have now increased in number, compatible with early stages of transformation. Other pigmented cells may be neural crest-derived melanocytes whose migration during development could be more widespread than is usually thought but whose presence would be undetected if they are ordinarily unpigmented. Normal Schwann cells and some other neural and perineural cells, also from the neural crest, have long been known to be capable of melanogenesis, and some human schwannomas have been described as melanotic (6). Certain epithelia and endothelia may have an intrinsic developmental plasticity and dormant capacity for melanogenesis, particularly in neuroectodermal derivatives. However, melanosis in tissues such as mucous membranes may signify metaplastic conversion. Some epithelia (e.g., in the reproductive tract) seem to be receiving melanin granules injected by melanocytes, as more normally occurs in skin keratinocytes. Phagocytic cells or melanophages, as seen in the lymph nodes and elsewhere, may simply be derived from the macrophage pool. Some cells of similar appearance may instead be large melanocytes engorged with so-called "melanin macroglobules," as observed in pigmentary disorders such as the café-au-lait macules of patients with neurofibromatosis. The granules seem to be formed by fusion of phagosomes which contain numbers of melanosomes, with

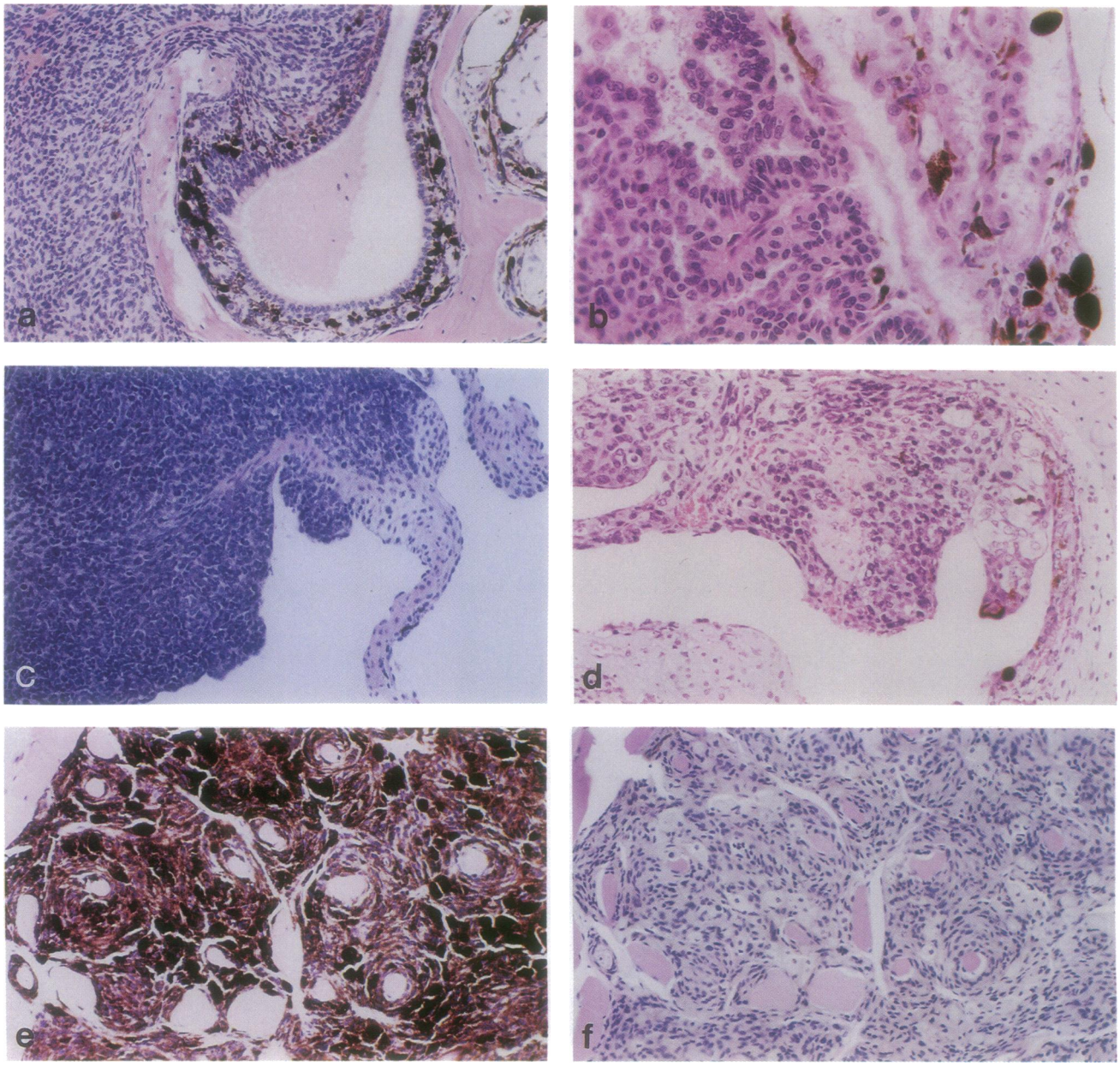

FIG. 3. Examples of tumors (other than ocular or cutaneous melanomas) in the transgenic mice. (*a*) A large amelanotic melanoma in contact with the nasal mucosa. Some cells under the epithelium are heavily laden with pigment, and the mucosa also contains a few melanin granules. (*b*) A well-differentiated choroid plexus carcinoma (left) adjacent to melanosis in an otherwise normal region of the choroid plexus (right). (*c*) Amelanotic, poorly differentiated melanoma attached to the mitral valve of the heart. Note melanin in a few endocardial cells. (*d*) Malignant epithelioid melanoma, with little pigment, arising in a cochlear duct of the inner ear. (*e*) Intensely pigmented malignant melanocytic schwannoma infiltrating a muscle near the vertebral column. (*f*) The same tumor section as in *b* after demelanization with sodium permanganate and oxalic acid. (*a*, *c*, *e*, and *f*, ×80; *b*, ×320; *d*, ×160.)

lysosomes, and can be transferred to other cells such as keratinocytes or macrophages (12).

Thus, the melanosis may originate in different ways and may contribute to neoplasia. It has been proposed (13) that the transition to malignancy occurs at the threshold of further differentiation of stem-like cells, when genetic "decisions" between ongoing proliferation and *de novo* differentiation are made, rather than at an earlier stage normally dedicated solely to proliferation. That decisions involving changes in gene expression may become aberrant in melanogenic cells is illustrated by the marked increased in melanin production after mouse melanoma cells are hybridized with teratocarcinoma cells (14).

This work was supported by U.S. Public Health Service Grants HD-01646 and CA-42560 to B.M. and CA-06927 and RR-05539 to the Fox Chase Cancer Center as well as an appropriation to the Center from the Commonwealth of Pennsylvania.

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