The Occurrence and Morphogenesis of Melanocytes in the Connective Tissues of the PET/MCV Mouse Strain

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WITH ONE PLATE

INTRODUCTION

MAMMALS, as a rule, are described as having melanocytes of neural crest origin confined almost entirely to the skin. Of the organs other than skin which have been described as possessing melanocytes are portions of the gonado-genital apparatus of the Opossum (Burns, 1939), and, in the house mouse, tissues of the nictitans, the meninges of the brain, the parathyroids, the thymus and harderian glands (Markert & Silvers, 1956), and the parathyroids of C58 mice (Dunn, 1949).

The present investigation has been made in a strain of mice in which melanocytes are found in the connective tissues throughout much of the body. This strain originated several years ago in the Department of Genetics, Medical College of Virginia, from a cross between inbred C3H and black mice of unknown breed obtained from a local pet shop. Because of the latter circumstance, the line-bred progeny have been termed the PET/MCV strain. Upon the discovery by the authors (Reams & Nichols, 1959) of melanocytes in the serosae of new-born PET/MCV mice, a stock was developed for investigation.

The presence of melanocytes in the coelomic lining of the chick and other fowl has been known for years (Willier & Yuh, 1928; Eastlick, 1939; and Rawles, 1940, 1945). Recently, Reams (1956) has shown that peritoneal pigment cells are excellent subjects for the study of the ontogeny of cell form since they are easily obtained for experimentation, and since the changes which they undergo in their morphogenesis are readily apparent due to the onset of melanogenesis prior to the development of their definitive shape. The melanocytes within the connective tissues of the PET/MCV mouse similarly lend themselves to analysis by experimental techniques.

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It is the purpose of this paper to describe the distribution of melanocytes in the connective tissues of the PET/MCV mouse and to present experimental data on the morphogenesis of these pigment cells.

MATERIALS AND METHODS

In order to survey the progressive appearance and morphology of melanocytes throughout the body of the PET/MCV strain, individuals ranging in age from 14 days prenatal to adults were killed and fixed in Telyesniczky’s formol-aceto-alcohol. The mice were then hemisected longitudinally, dehydrated in alcohol, cleared in oil of wintergreen, and examined for the presence of melanocytes. Five or more mice from different litters of each group were examined.

A technique of intracoelomic grafting similar to that described by Rawles (1947) was employed to investigate the developmental capacity of melanocytes from the serosae. In addition, grafts were made to the skin of host chick embryos by the method of Willier, Rawles, & Hadorn (1937). In all cases the donor tissue (0.2 mm.$^3$ fragments of serosa bearing spherical melanocytes) was taken from PET/MCV mice varying in age from 18-day prenatal to 4-day postnatal and grafted into 60–70-hour White Leghorn chick embryo hosts. Following the grafting of mouse tissue, the hosts were returned to the incubator until killed 12–17 days after incubation, when they were examined for the presence and state of the donor melanocytes in the manner described above for mice.

DISPOSITION OF MELANOCYTES IN THE PET/MCV MOUSE

Examination of one-day postnatal mice of the PET/MCV strain reveals that melanocytes are found within the connective tissue of many organs. Their distribution is not, however, consistent from litter to litter, or even within the members of the same litter. Melanin-laden pigment cells are seen not only in those tissues previously noted, but also within the lungs, heart, gonads, nucleus pulposus, epiphyses of many of the bones, ribs, and muscles (especially the intercostals and the extremities) (Plate, figs. 1, 2, 3, 5, 7, 8); in the pericardium, serosae, the mesenteries (Plate, figs. 4, 6); in the adventitia of the esophagus, trachea, kidney, blood-vessels, and ducts; and in many other positions. Melanocytes are in fact consistently absent only from the connective tissue of the gut mucosa.

As in the fowl (Reams, 1956), the pigment cells within the connective tissues of the PET/MCV mouse first occur as melanoblasts which had probably migrated from the neural crest. With the onset of melanogenesis they are seen as spherical, unbranched melanocytes which later assume a branched or dendritic shape. That the spherical melanocytes arise from melanoblasts present in the area is indicated by the development of melanocytes in grafts of tissue, taken 2 days prior to their expected appearance, which are grown in the coelom of host chick embryos.

From a study of individuals of different age groups it was found that each area
### Table 1

**Appearance of melanocytes in major regions**

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*B: Branched melanocytes.  ●: Unbranched melanocytes.
has its own particular time for the appearance of the melanocytes and for the development of branches by the melanocytes. Table 1 summarizes the time of appearance and the condition of the melanocytes for certain of the areas. To illustrate, from day 15 to day 17 prenatal, the presence of pigment cells in the thoracic wall can be detected only by the dopa technique or by grafting tests. Melanocytes appear scattered in the connective tissue of the intercostal muscles at day 17 of embryonic life. At this time the melanocytes are spherical in form and are laden with melanin granules. Counts of the melanocytes at various ages show that the pigment cells continue to slowly proliferate until day 4 of postnatal life, when they change their shape by developing branches and concomitantly lose their ability to multiply. After this there is little or no increase in the number of melanocytes present among the fibres of the intercostal muscles.

The apparent decrease in the number of melanocytes from this stage on into adulthood is due to the increase in size of the region with relatively no change in the number of its melanocytes, as well as to necrosis, since many of the melanocytes are seen in varying stages of degeneration.

Spherical melanocytes are likewise seen in the tissue of the lung on the 17th prenatal day. However, they remain spherical here and maintain their population balance with the quantity of lung tissue even in the adult.

In general, melanocytes are found not only in the skin but also within much of the connective tissues of mice of the PET/MCV strain. Further, the time at which melanin production begins, and/or the production of branches is evoked, is characteristic of the organ in which the pigment cells reside.

TESTS OF THE DEVELOPMENTAL CAPACITY OF SPHERICAL MELANOCYTES

In view of the similarity between the descriptive phases of the PET/MCV mouse investigation and those for the chick (Reams, 1956), the question arises as to whether the melanocytes of this mouse will exhibit a similar developmental capacity and morphogenetic behaviour. Techniques of intracoelomic grafting and grafting to the skin of the leg-bud of host chick embryos offer a means to this end. Since grafts of serosa which contained spherical melanocytes, regardless of the age of the donor (18 days prenatal to 4 days postnatal) or the area (pleural or peritoneal), presented similar results, the data will be grouped for convenience.

A total of twenty-seven intracoelomic grafts of serosa have been recovered and examined. The graft melanocytes and their progeny were generally found scattered within a limited area of the coelomic lining of the abdominal wall and in six cases were buried within the substance of the rump. The great majority of the melanocytes observed in eleven hosts recovered prior to day 15 of incubation were of the spherical type. There had been a fivefold increase in the melanocytes since the time of grafting. Sixteen hosts recovered after the 15th day contained
donor melanocytes which were branched and whose number was essentially no different from those recovered just prior to day 15.

To test the proliferative capacity of the spherical melanocytes of the serosae, intracoelomic grafts were also made of dermis taken from 18-day PET/MCV mouse embryos. The absence of epithelial spheroids and/or hairs in the recovered grafts was used as the criterion for the purity of the original dermal grafts. Although dendritic melanocytes were in most of the grafts, melanoblasts were present also—as evidenced by the great number and wide distribution of melanocytes found in the eleven hosts at recovery (cf. Rawles, 1940, 1947). In respect to the shape of the melanocytes, the data are in agreement with those previously described.

Only 5 out of 24 grafts of serosa with its spherical melanocytes made to the leg-bud could be located upon recovery of the host embryos at 12 days. Since some grafts did take, it might be presumed that many of the grafts had been rejected by the host and lost. However, of those hosts in which mouse pigment cells could be detected, the melanocytes were comparatively few in number and were branched.

Thus it would appear that the spherical melanocytes of the PET/MCV mouse serosae are capable of proliferation but not as rapidly as melanoblasts. In addition, there is a critical time within the host at which the melanocytes branch, regardless of the age of the donor from which the melanocytes were removed.

**DISCUSSION**

Mice of the PET/MCV strain differ little in external appearance from any other black mouse strain (for example, C57 Black). However, internally there is found a wide distribution of melanocytes throughout the connective tissues of the body. If melanocytes may be presumed to be of neural crest origin, then this vividly emphasizes the extent to which the cells of the neural crest may be distributed within the individual. The neural crest has been designated as forming, or contributing to the formation of, a great number of structures (Hörstadius, 1950), particularly in the amphibia. To prove experimentally many of the contributions of the neural crest in amphibia is often difficult and open to doubt. To do so in mammals borders on the realm of the impossible. However, since melanocytes are derived exclusively from the neural crest (for a review of the evidence, see Rawles, 1948) they may be considered here as markers for the distribution of crest cells in the PET/MCV mouse. Considered in this way, the structures within the PET/MCV mouse containing crest cells form an impressive list. The distribution of neural crest cells in other mouse strains might therefore be inferred to be greater than is generally appreciated.

Rawles (1947) demonstrated the progressive spread of neural crest cells in the mouse and showed that the melanocytes of the skin and its derivatives were of neural crest origin. That the pigment cells found in the deeper tissues of PET/
MCV mice are indeed neural crest melanocytes and not melanin-bearing macrophages is shown by several lines of evidence. First, a macrophage must have an extrinsic source of melanin granules to phagocytize before it can become melanin-bearing. In the first sites of the PET/MCV mouse in which pigment-bearing cells appear, the large number of cells to develop granules simultaneously is notable. In addition, just prior to the time at which the black melanin granules appear, dopa-positive cells are to be found in equivalent numbers. If these cells do not manufacture their own melanin granules, whence then are they obtained? Second, the progressive spread of melanoblasts, as demonstrated by grafting experiments and the dopa-method, and later as observed with the melanocytes, is in conformity with the pathways and progress in time one might anticipate. Finally, when grafted into the coelom of host chick embryos, these ‘internal’ pigment cells show a behaviour identical to that found for coelomic melanocytes of the chick.

In the chick, a melanoblast in its differentiation towards a definitive melanocyte passes through an intermediate phase of either a branched melanoblast or an unbranched melanocyte (Reams, 1956). The sequence of migratory melanoblast to spherical melanoblast to branched melanoblast to branched melanocyte is characteristic of the skin. In other areas the procedure generally is migratory melanoblast to spherical melanocyte to branched melanocyte. The presence of ‘dendritic cells’ (melanoblasts) in the skin and their subsequent production of melanin have been described by Billingham (1948), Reynolds (1954), and others. A sequence comparable to that described here for the pigment cells within the skin of the chick has been found for the differentiation of melanocytes in human skin (cf. Zimmermann & Becker, 1959). In the present investigation it has been shown that the sequence of morphogenesis for the pigment cells in the PET/MCV mouse is in accord with that found in the chick for the skin and internal tissues.

Since Rawles (1940) demonstrated that the chick coelom was a favourable environment for the growth of mouse tissue, it has been employed for diverse investigations on pigment cell behaviour in the mouse. In all instances host chick embryos containing grafts of mouse tissue were not killed until late in development, and the melanocytes found were of the branched type. The discovery of ‘internal’ melanocytes in the PET/MCV mouse and the observation that differentiation is similar to that of melanocytes in the chick suggested an investigation of the phases of differentiation of mouse pigment cells within early stages of chick embryo hosts. Hence, grafts of serosa and of dermis were made to chick embryo hosts. These hosts were recovered at varying ages and the condition of the donor pigment cells from the grafts determined. The mouse melanocytes, regardless of whether they were the spherical melanocytes of the serosa or whether they came from the melanoblasts of the dermis, were found to be of the unbranched (spherical) type in all chick hosts prior to day 15 of incubation. After this time, the mouse melanocytes produced branches and became dendritic
in shape. The melanocytes within grafts of mouse skin were found to be branched from the time of their first appearance, without regard to the age of the host. In addition, spherical melanocytes grafted to the skin of chick hosts also branched prior to day 15. Thus it appears that the epidermis has a strong capacity to evoke the branching of pigment cells. In its absence the pigment cells seem to be unable to branch until comparatively late in the development of the host.

That the coelomic lining of the chick holds the production of branches by a pigment cell in check, and that branching is effected only by certain tissues of the embryo, has been shown by Reams (1956). Furthermore, the branching appears to be brought about by a chemical agent which is first effective locally at the site of its production and, later, humorally through a build-up in its concentration in the vascular system (Reams, 1957; Reams, Nichols, & Hager, 1959). That the morphogenesis of the melanocytes of the PET/MCV mouse is brought about in a similar manner is demonstrated by the following evidence.

In the breeds of pigmented fowl examined, melanocytes, when present in the coelomic lining, remain spherical until about day 13 of incubation, at which time they branch. When grafted into the coelom of White Leghorn chick host embryos, these spherical melanocytes produce branches at day 15. It is interesting to note that the time of branching of melanocytes of chick into chick or mouse into chick is the same. In all cases, where grafts were not contaminated with epidermis, the melanocytes branched at a time specific to the host and not in accord with the chronological age of the graft cells. To cite an example, melanocytes in grafts of pleura from an 18-day PET/MCV fetus branched at day 15 in a chick host—after a total of 12 days within the host. The melanocytes are all branched in the pleura of normal PET/MCV mice at day 4 postnatal—an equivalent length of time of about 7 days. Since the method of intracoelomic grafting does not notably slow up the rate of normal growth and differentiation of a graft, it is concluded that there is a real differential of 5 days between the normal and the experimental. Thus the data lead to the assumption that the branching of PET/MCV mouse as well as chick pigment cells is indeed brought about by a chemical entity which is active initially at the site of its production and, later, as its concentration builds up, elsewhere in the body. And, in addition, its action, as observed for chick and mouse, is not species specific.

**SUMMARY**

1. Mice of the PET/MCV strain are characterized by having melanocytes scattered in the connective tissues of most of the body. The 'internal' pigment cells generally first appear in fetuses as spherical melanocytes which later produce branches and lay down melanin. By contrast, pigment cells within the skin branch prior to the onset of melanogenesis.

2. Spherical melanocytes of the serosae of the PET/MCV mouse proliferate within the coelomic lining of host chick embryos. However, the degree of pro-
liferation and spread is considerably less than that of melanoblasts under similar conditions.

3. Under the conditions of experimental grafting, both spherical 'internal' melanocytes and melanoblasts from the dermis showed a similar pattern of differentiation. The time at which donor melanocytes produce branches within the host chick embryos is in accord with their location and the specific time expected of the host and not of the donor.

4. The data suggest that the branching of a pigment cell is evoked by a morphogenetic substance which is produced by certain tissues of the body, particularly by the epidermis. The effect, at least with regard to the mouse and chick, is not species specific, since grafted mouse pigment cells react in keeping with the chick environment.

REFERENCES

BURNS, R. K. (1939). The differentiation of sex in the opossum (Didelphys virginiana) and its modification by the male hormone testosterone propionate. J. Morph. 65, 79–120.

EXPLANATION OF PLATE

Fig. 1. Hind-foot of 1-day PET/MCV mouse. Note melanocytes in epiphyses. × 30.
Fig. 2. Marrow portion of centrum of tail vertebra with melanocytes. × 100.
Fig. 3. Melanocytes within muscles of thigh of new-born. ×40.
Fig. 4. Abdominal wall of 18-day PET/MCV fetus showing melanocytes in the peritoneum. ×40.
Fig. 5. Lung of 18-day fetus with unbranched melanocytes. ×100.
Fig. 6. Pigmented pericardium from 3-day PET/MCV mouse. ×100.
Fig. 7. Thoracic wall of 3-day PET/MCV mouse. Melanocytes in intercostales and in costal cartilage. ×100.
Fig. 8. Thoracic wall of 4-day mouse showing branching of melanocytes in intercostals. ×100.
All specimens unstained and cleared in wintergreen oil.

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S. E. Nichols and W. M. Reams