12 samples collected under a variety of conditions. Rafter (*7*), on the other hand, found that four CO_2 samples collected in New Zealand in 1954 and 1955 had higher concentrations of C^{14} than had contemporary wood (+4.7 percent for one sample). Our collections at Washington, D.C., during the summer of 1955 gave values for the C^{14} content of atmospheric CO_2 appreciably higher than those previously reported. It seems difficult to account for these high values on the basis of isotopic fractionation, and therefore the increase in the C^{14} content of atmospheric CO_2 from 1952 to 1956 is probably the result of the addition of radiocarbon from thermonuclear sources. The delayed appearance of the C^{14} increase at ground level may indicate a stratospheric reservoir of this isotope.

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3 March 1957

Melanin Mobilization in Pigment Cells of the Mouse

Many lower vertebrates possess the ability to change color rapidly by effecting redistribution of pigment in the chromophores (*1*) of the skin. The pigment cells have been shown to be quite sensitive to a number of chemical and physical agents (*2*). Epinephrine causes a rather rapid migration of the cytoplasmic melanin granules to the center of the melanophore. Fig. 1*A* shows a melanophore on a fragment of fish fin in Holtfreter's solution. After the saline is replaced with 0.5 m*M* epinephrine in Holtfreter's solution, the pigment mass assumes the configuration of Fig. 1*B* within 30 seconds. If the epinephrine is removed, the pigment is redistributed throughout the cell.

It has been generally assumed that the ability to mobilize melanin is restricted to the pigment cells of only those animals that are capable of effecting rapid

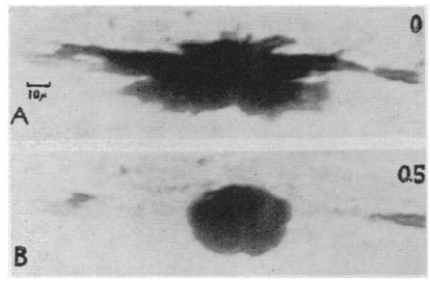

Fig. 1. (*A*) A melanophore from the caudal fin of the fish *Xiphophorus helleri* in Holtfreter's solution. (*B*) The same cell shown in *A* exposed to 0.5 m*M* epinephrine.

changes of skin color. Although no functional melanophores are found in mammals, the mammalian melanocyte does occur, and it is quite similar to the melanocytes of lower vertebrates, especially to those found in pigmented tumors (*3*). Since in earlier studies we had observed pigment mobilization in the tumor melanocytes of *Xiphophorin* fish melanomas (this has also been reported by Greenberg *et al.*, *4*), we suspected that such a phenomenon might also occur in the pigment cells of mammalian melanomas.

The mammalian melanocytes that we first studied were those found in tissue cultures of the Cloudman mouse melanoma (*5*). Melanocytes were abundant and rather easily distinguished from the larger macrophages and pigmentless cells. The nuclei of melanocytes were small and contained only one or two nucleoli, whereas other cell types had large, multinucleolated nuclei. Furthermore, melanocyte behavior, as observed in time-lapse motion pictures, was quite characteristic. Cell shape was ignored, since it tended to be variable.

Tissue cultures of the mouse melanoma were made according to the standard roller tube method (*6*). After about 1 week of culturing in horse serum medium (*7*), the cover-slip cultures were incorporated into a perfusion chamber (*8*). The perfusion medium used throughout the test was that in which the cultures had been grown. Since cytological changes in rounded cells are difficult to observe, the culture outgrowths were searched for flattened melanocytes of the type shown in Fig. 2*A*. A record of the subsequent testing was made with cinephotomicrographic time-lapse equipment (*9*). Color film was used so that the brown melanin granules could be distinguished from other cytoplasmic granulation, especially spherical mitochondria.

Time-lapse motion pictures made preliminary to treatment established that normal activities consisted typically of rapid membrane undulation, occasional pynocytosis (cell drinking), and considerable erratic motion of the melanin granules and mitochondria in the body of the cell. Such a cell in untreated medium is shown in Fig. 2*A*.

When the chamber medium was replaced with culture medium containing 0.5 m*M* epinephrine, normal membrane action was immediately suspended, the motion of the cytoplasmic granulation ceased, and all cell movement appeared to be frozen. Melanin granules clumped together, and the pigmented mass in the cytoplasm slowly began to contract. Some of the spherical mitochondria remained in the clear cytoplasmic regions (Fig. 2*B*), but their motion was halted by the treatment.

After the pigmented mass reached what appeared to be a maximum contraction (Fig. 2*B*), the chamber medium was replaced with untreated culture medium. Immediately the cell membrane

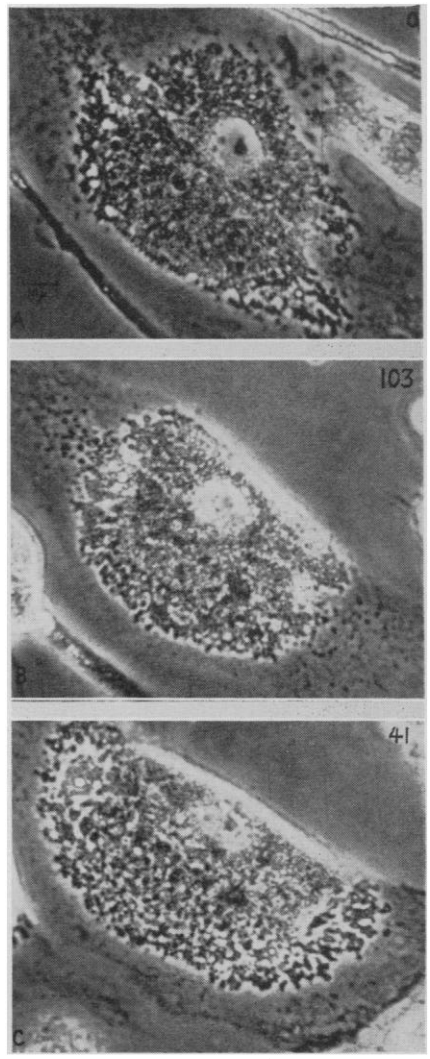

Fig. 2. (*A*) A melanocyte from the Cloudman mouse melanoma in tissue culture. (*B*) The same cell exposed to 0.5 m*M* epinephrine. (*C*) The cell after the removal of the epinephrine. The number in the upper right of each photograph indicates the time interval in minutes from the preceding picture.

resumed the normal rhythmic undulations, and melanin granules at the edge of the pigmented mass broke free into the cytoplasm and resumed erratic motion. The dimensions of the pigmented area gradually increased (Fig. 2*C*). Cinemagraphs taken for a considerable period of time after the treatment revealed no sign of cell damage.

Planimetering of the pigmented areas of the cell in Figs. 1*A* and 1*B* reveals that the fish cell is capable of reducing its pigmented region to about 50 percent of the original area. Similar measurements of Figs. 2*A* and 2*B* show a reduction to about 80 percent for the mouse cell.

Normal melanocytes found in cultures of the skin from the dorsal regions of 15-day-old mouse embryos were tested in the manner described, and the results appeared to be similar. No other mammalian species was tested.

Whether or not the cell herein described actually corresponds to a melanocyte or melanophore is largely a problem of definition. The presence of hair or fur coverings on mammals prevents such cells from fulfilling a functional role. We therefore recommend the retention of the term *melanocyte* for these cells and that they be distinguished from the melanophore of lower vertebrates on the basis of normal function, rather than on the ability to mobilize pigment.

This demonstration of a pigment mobilization system in mammalian melanocytes may provide a fresh approach to melanoma chemotherapy. The advantages are manifold: much information is available in this field (*2, 10*), the effects of appropriate chemicals are highly selective and are produced at low concentrations, and the effects are produced within minutes of the drug application.

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25 March 1957

Behavior of the Dermal Mast Cells in Magnesium-Deficient Rats

A diet that contains not more than 1 to 2 ppm magnesium produces peripheral vasodilation in young rats, which appears after 4 days (*1*), is most intense after a week or 10 days, and then subsides gradually. Since the mast cells have recently been associated with the production of histamine (*2, 3*), it seemed possible that they might be involved in the afore-mentioned syndrome.

Three groups of five rats of 40 g were fed a casein diet that contained less than 1 ppm of magnesium (*4*), for periods of 7, 14, and 28 days. An identical number of controls for each group were fed the same diet into which had been blended 0.6 g of magnesium sulfate per 100 g of diet. Portions of skin from the face and from the abdomen were fixed in formaldehyde-ethanol for maximal retention of mucopolysaccharides (*5*), cut at 10 μ in celloidin, and treated with diluted Wright stain to permit easy identification of the mast cells (Fig. 1), whose granules are azurophilic.

The cells were counted at a magnification of 264, which produced a field of 0.17 mm². The averages of cumulative counts (Table 1) have revealed a remarkable constancy between controls of the three groups as well as a constant difference of approximately 3 times between facial and abdominal skin. The magnesium-deficient animals produced comparable counts at 7 and 14 days. At 28 days, however, both abdominal and facial samples revealed a 40 to 50 percent difference in the mast-cell population as compared with controls.

In addition to the differences in mast-cell numbers, there were individual variations in shape and degree of granulation among individual cells. In all the animals, the mast cells were found to be most abundant and highly granular in the vicinity of the hair-bulbs (Fig. 1). Closer to the surface of the skin, the cells were less abundant; immediately under the epidermis, they appeared to have fewer granules (Fig. 1). In most of the animals that were fed the deficient diet for 7 days, this phenomenon was grossly exaggerated; the cells were poorly granulated over a wide area, and the cells located near the epidermal junction were practically empty of granules. The considerable variations in the shape of the cells seem to indicate accelerated ameboid activity. At 14 and 28 days, there appears to be progressively less degranulation and pleomorphism.

With an artificial histamine stimulator, Fawcett obtained degranulation accompanied by release of histamine (*3, 6*). This was followed by rapid regeneration of granules and cells. In the experiments described in this report (*7*), hyperemia was proportional to dermal mast-cell count (Table 1). It is thus possible that the sudden deprivation of magnesium might act as a histamine stimulator.

On the other hand, the disappearance of granules and pleomorphism in the mast cells can be interpreted as passive

Fig. 1. Portion of the facial skin of a normal rat, stained with diluted Wright (× 114). Mast cells in black.

Table 1. Mast-cell counts. C, control group; D, magnesium-deficient group; F, facial skin; A, abdominal skin; ± S, standard error.

Duration in days	Group and location	No. of animals	No. of microfields	Total cells counted	Avg. per field (± S)
7	C, A	2	22	247	11.2 ± 1.1
7	D, A	5	123	1351	11.0 ± 0.3
7	C, F	2	41	1385	33.8 ± 2.4
7	D, F	5	77	2214	28.8 ± 1.4
14	C, A	3	34	340	10.0 ± 0.6
14	D, A	5	60	667	11.1 ± 0.6
14	C, F	3	50	2150	43.0 ± 2.3
14	D, F	4	43	1507	35.1 ± 3.0
28	C, A	3	35	338	9.7 ± 0.5
28	D, A	2	36	138	3.8 ± 0.5
28	C, F	3	34	1180	34.7 ± 1.6
28	D, F	2	30	590	19.7 ± 1.4