

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. 2C). Cinemagraphs taken for a considerable period of time after the treatment revealed no sign of cell damage.

Planimetry of the pigmented areas of the cell in Figs. 1A and 1B reveals that the fish cell is capable of reducing its pigmented region to about 50 percent of the original area. Similar measurements of Figs. 2A and 2B 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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References and Notes

1. Throughout this report we adhere to the standard terminology for pigment cells proposed by Myron Gordon in *Pigment Cell Growth* (Academic Press, New York, 1953), preface.
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5. We are indebted to T. C. Hsu of the M. D. Anderson Hospital Research and Tumor Institute, Houston, Tex., who provided laboratory facilities for the initial phase of this work, and also to C. H. Robertson of the M. D. Anderson Hospital for the original tumor material.
6. T. C. Hsu, *Texas Repts. Biol. and Med.* 12, 833 (1954).
7. The medium consisted of 500 ml of horse serum (Difco), 10 ml of chick-embryo juice, 0.2 g of Mycitradin sulfate (Upjohn), and

enough Gey's solution to make up a volume of 1 lit.

8. C. M. Pomerat, in *Methods of Medical Research* (Year Book Publishers, Chicago, 1951), vol. 4, p. 275. This design was modified by the use of a suction syringe outlet.
9. This equipment was purchased on a grant from the American Academy of Arts and Sciences. This work was also supported by grant C-3206 from the National Institutes of Health, U.S. Department of Health, Education, and Welfare.
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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

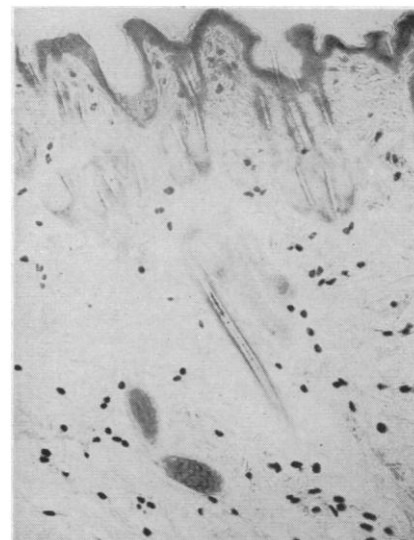


Fig. 1. Portion of the facial skin of a normal rat, stained with diluted Wright ($\times 114$). Mast cells in black.

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

Dura-Group	No.	No.	Total	Avg.
tion and	of	of	cells	per
in loca-	ani-	micro	count-	field
days tion	mals	fields	ed	($\pm$ S)
7 C, A	2	22	247	11.2 $\pm$ 1.1
7 D, A	5	123	1351	11.0 $\pm$ 0.3
7 C, F	2	41	1385	33.8 $\pm$ 2.4
7 D, F	5	77	2214	28.8 $\pm$ 1.4
14 C, A	3	34	340	10.0 $\pm$ 0.6
14 D, A	5	60	667	11.1 $\pm$ 0.6
14 C, F	3	50	2150	43.0 $\pm$ 2.3
14 D, F	4	43	1507	35.1 $\pm$ 3.0
28 C, A	3	35	338	9.7 $\pm$ 0.5
28 D, A	2	36	138	3.8 $\pm$ 0.5
28 C, F	3	34	1180	34.7 $\pm$ 1.6
28 D, F	2	30	590	19.7 $\pm$ 1.4

breakdown or environmental modification that are the result of vasodilation and edema (3, 8).

The next phase of the syndrome, during which blood magnesium returns to normal level (9), has shown blanching of the skin and regranulation of the mast cells. However, the dermal mast-cell population was only about one-half of that of the controls at 28 days (Table 1); this indicates a slow rate of regeneration consistent with that of mast-cell damage by osmotic environmental changes (3, 10).

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Gibberellin Effects on Temperature and Photoperiodic Requirements for Flowering of Some Plants

Several physiologic responses in plants that heretofore have not been subject to chemical regulation may be controlled by gibberellin. Dwarfism, both genetic (1) and physiologic (2), may be overcome, and growth rates characteristic of normal varieties may be established. The cold requirement for flowering of the biennial *Hyoscyamus niger* has been replaced by treatment with gibberellin (3), and encouraging results with other biennials and annuals have now been reported (4). In this report (5), the induction of flowering with gibberellin in a large number of genera and species that were grown under environmental conditions not conducive to flowering is described.

All plants were grown from seed and maintained in pot cultures of soil in the greenhouse during the summer, fall, and winter of 1956-57. Treatment was initiated when the plants were at a stage, defined by leaf number or by stem or root size, at which exposure to cold or to long days would have resulted in a

prompt flowering response. Gibberellin was applied, either as droplets of water solution on the apices of the plants or as an aqueous spray, to the foliage. Further details of the treating procedure, with a listing of the biennials and long-day annuals which have flowered with gibberellin under noninductive environments, are given in Table 1.

The biennials that were induced to flower with gibberellin were grown at temperatures slightly higher than the critical temperature for flower formation. That these temperatures (10° to 13°C) were noninductive was indicated by the absence of flowering in controls. The original finding (3) for *Hyoscyamus niger* was no exception. At temperatures of 18°C or above, extensive stem elongation was induced in cabbage, kale, beets, rutabagas, turnips, and celery, but flowering was not consistent. In contrast, gibberellin has caused stem elongation and flowering in carrots over a wide range of noninductive temperatures (13° to 25°C) and under short (9 to 11 hours), as well as long (14 to 16 hours), photoperiods.

With long-day annuals, flowering was induced under a noninductive environment of a short (9 to 11 hours) photoperiod and low (10° to 13°C) temperature. Under the same conditions, but in the absence of gibberellin, lettuce, endive, radish, spinach, dill, and mustard remained vegetative and acaulous.

Development of most gibberellin-treated biennials and annuals during flowering differed from that of the nontreated plants. On nontreated plants, bolting and flower initiation occurred simultaneously, while treated plants developed stems from 20 to 100 cm higher before flower buds could be identified.

Regardless of whether the effects of gibberellin on flower formation are direct or indirect, it has now been established that treatment with gibberellin, a naturally occurring plant product, has resulted in complete flowering responses. Often a single application is sufficient to induce flowering in a wide variety of economic crops grown under nonflowering conditions of temperature (the cold-requiring biennials) and photoperiod (long-day-requiring annuals).

Table 1. Cold-requiring biennials and long-day annuals in which flowering has been induced by gibberellin. (Plants grown under noninductive conditions.)

Biennials		Long-day annuals	
Plant	Treatment	Plant	Treatment
<i>Brassica oleracea</i> var. <i>capitata</i> (cabbage). Golden acre and Ferry's round dutch	100 µg weekly for 8 wk. First treatment at stem diameter of 1 cm and 7-9 leaves (As above for cabbage)	<i>Anethum graveolens</i> (dill)	1 foliage spray of 100 ppm at 5-6 leaf stage
<i>Brassica oleracea</i> var. <i>acephala</i> (kale). Siberian and Dwarf blue curled	100 µg weekly for 6 wk at 7-9 leaf stage and 1 cm stem diameter	<i>Brassica pekinensis</i> (Chinese cabbage). Michihli	2 foliage sprays (3-wk interval) of 1000 ppm at 6-7 leaf stage
<i>Brassica oleracea</i> var. <i>acephala</i> (col-lards). Georgia and Louisiana sweet	3 foliage sprays of 1000 ppm at 2-wk intervals at the 6-9 leaf stage (As above for rutabaga)	<i>Brassica juncea</i> (mustard). Southern giant curled and tendergreen	100 µg weekly for 3 wk at 8-10 leaf stage
<i>Brassica napobrassica</i> (rutabaga). Purple top	20-100 µg/plant when roots were 1 cm or larger in diameter	<i>Cichorium endivia</i> (endive). Full heart Batavian and green curled	100 µg weekly for 8 wk at 8-10 leaf stage
<i>Brassica Rapa</i> (turnip). Purple top	100 µg/plant for 6 weeks at the 6-8 leaf stage or 1000 ppm foliage spray (As above for rutabaga)	<i>Lactuca sativa</i> (lettuce). Great Lakes Bibb	3 applications (4-wk interval) of 20 µg at 8-10 leaf stage 2 applications (4-wk interval) of 20 µg at 8-10 leaf stage
<i>Daucus Carota</i> var. <i>sativa</i> (carrot). Chantenay and Imperator	100 µg/plant for 6 weeks at the 6-8 leaf stage or 1000 ppm foliage spray (As above for foxglove)	Grand Rapids and Tendergreen	20 µg at 8-10 leaf stage
<i>Digitalis purpurea</i> (foxglove)	20-100 µg/plant at the 6-8 leaf stage or a foliage spray of 100 ppm (As above for stock)	<i>Raphanus sativus</i> (radish). Crimson giant and Icicle	100 µg or foliage spray of 100 to 1000 ppm at 3-5 leaf stage
<i>Bellis perennis</i> (English daisy)		<i>Spinacia oleracea</i> (spinach). Prickly dark seeded	Foliage spray of 1000 ppm at 2-3 leaf stage, repeated after 4 wk
<i>Matthiola incana</i> (stock)		<i>Petunia hybrida</i> (petunia)	Foliage spray of 10-100 ppm at 4-8 leaf stage
<i>Viola tricolor</i> (pansy)			