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PIGMENT FORMATION IN MAST CELLS IN TISSUE CULTURE*

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The concept that mast cells are capable of melanin synthesis was proposed at the turn of the century (1–4), and was based on the observation of melanin granules in some mast cells. Critics of this theory (5, 6) explained the presence of melanin in mast cells on the basis of phagocytosis rather than synthesis.

Recently, a series of observations in our laboratory (7–10) provided evidence that mast cells are capable of melanin synthesis and are histogenetically related to melanocytes. These observations included: a) the presence of preformed melanin in some mast cells; b) the presence of dopa oxidase activity in mast cells; and c) the presence of histochemically demonstrable tyrosinase activity in mast cells stimulated by ultraviolet light. Studies currently in progress in our laboratory also suggest that tyrosinase activity can be demonstrated in mast cells by the histochemical autoradiographic method.

Embryological evidence (which has been reviewed by Hörstadius (11)) that the neural crest gives rise to connective tissue (12, 13) and that the connective tissue of the dermis is at least partly of neural crest origin (14, 15) provides a developmental basis for the theory that cutaneous melanocytes and mast cells derive from a common stem cell.

In this study mast cells were observed in tissue culture in an attempt to get more information concerning their melanogenic potential.

MATERIALS AND METHODS

Mast cells were obtained from peritoneal washings of adult Sprague-Dawley (albino) and ACI/Mai (black agouti) rats, and were cultured using a modification of the method described by Burton (16). Ten ml of Gey's solution were injected into the peritoneal cavity; after 30 minutes as much fluid as possible was withdrawn from the peritoneal cavity (usually 6 to 8 ml), using a plasma needle. This fluid was centrifuged at 1000 rpm for 4 minutes, then the sediment was re-suspended in 2 ml of culture medium containing 0.25 per cent viokase and centrifuged again at 1000 rpm for 4 minutes. The supernatant fluid was then withdrawn and the sediment was finally suspended in 0.5 ml of the culture medium (MEM Eagle, Spinner modified, containing 10% calf serum). A drop of this suspension, containing 6000 to 20,000 mast cells per mm^3, was applied to a coverslip in a Petri dish and incubated for 30 minutes at 37° C, in an atmosphere containing 5 per cent CO_2. At this time a coverslip containing a drop of the suspension was removed from the incubator to be used for comparison with the culture after growth. Enough culture medium was then added to cover the bottom of the other Petri dishes and the culture was allowed to proceed under the conditions described above.

In some of the specimens 21.6 i.u. of corticotropin† was added to each ml of the culture medium initially, while in others culture medium containing this concentration of corticotropin was introduced after 4 days of growth.

The cultures were observed by bright field and phase contrast microscopy, both unstained and stained (after methanol fixation) with toluidine blue, Perl's stain, Masson-Fontana ammoniated silver nitrate stain, and Lilly's ferrous iron uptake technic (17).

RESULTS

The specimens observed before culture contained many round mast cells with characteristic metachromatic granules. No mast cells with pigment or dendritic processes were seen. Occasional red blood cells and mesothelial cells were present. After 3 days of growth the cultures not containing corticotropin showed both round and stretched mast cells, some of which contained brown to black pigment. As the culture progressed the proportion of mast cells containing pigment increased somewhat. The pigment in some instances occupied part of the cytoplasm of the cell, but in other instances was distributed throughout the cytoplasm (Figs. 1, 2, 3). With toluidine blue the non-

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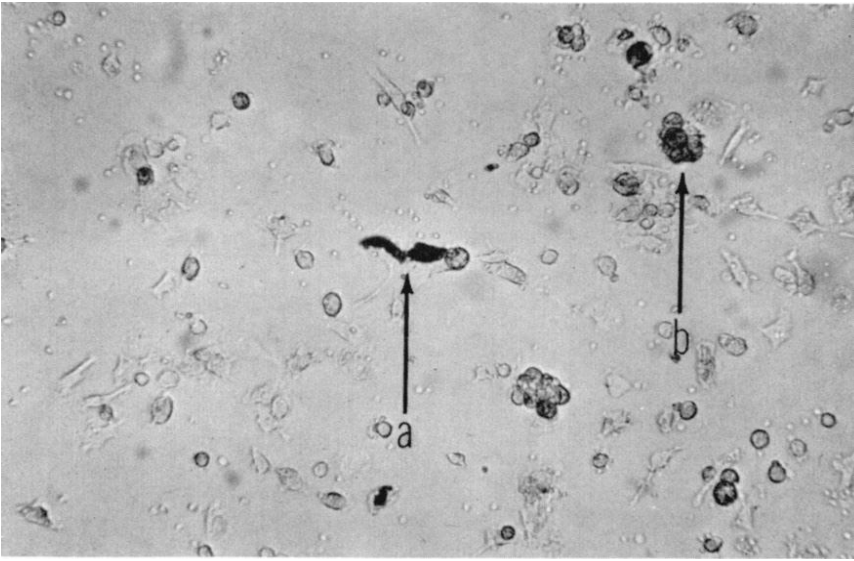

FIG. 1. 5-day-old culture of mast cells from peritoneal washings of a Sprague-Dawley rat (corticotropin not present). The field shows many round and stretched cells, some of which show varying degrees of pigmentation (arrows). Bright field illumination, unstained. × **360.**

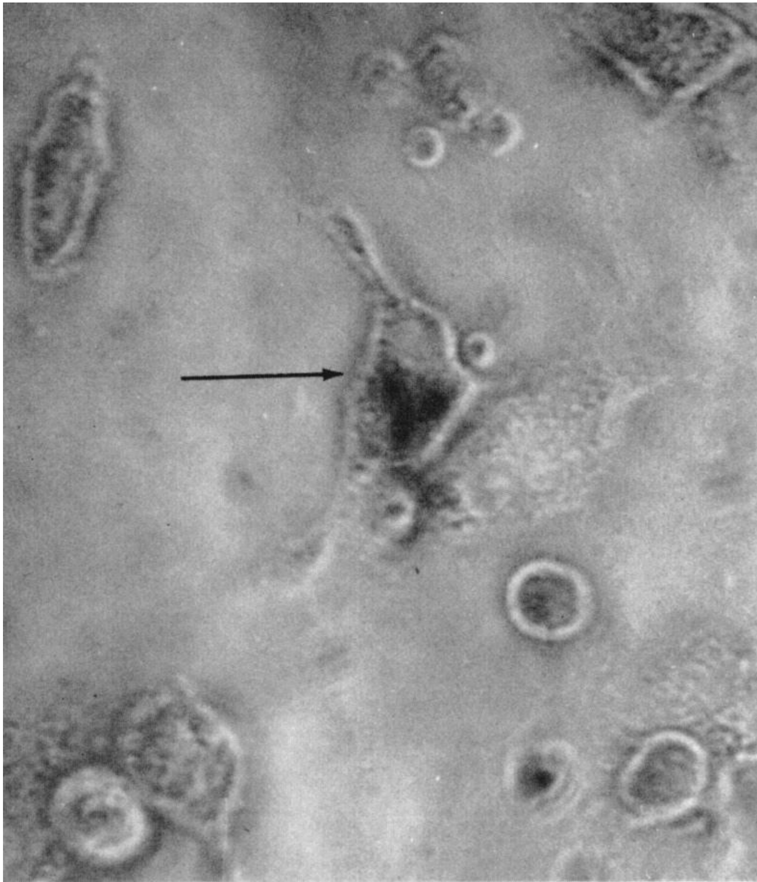

FIG. 2. 5-day-old culture of mast cells from peritoneal washings of a Sprague-Dawley rat (corticotropin not present). In the center of the field (arrow) a stretched mast cell shows a focus of pigmentation in its cytoplasm. Phase contrast illumination, unstained. × 2000.

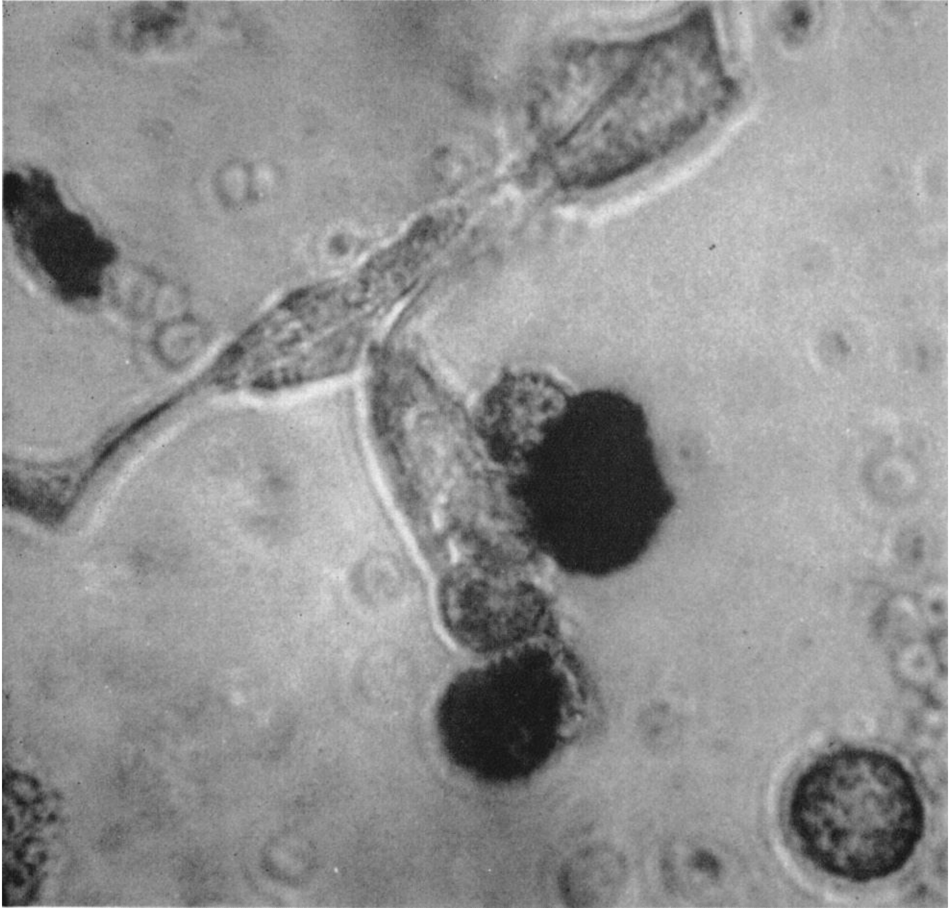

FIG. 3. 5-day-old culture of mast cells from the peritoneal washings of a Sprague-Dawley rat (corticotropin not present). Several round mast cells contain dense aggregations of pigment. Phase contrast illumination, unstained. × 2520.

pigmented granules of both the round and stretched mast cells were metachromatic, whereas the pigment stained greenish-brown. Some cells showing dual (metachromatic and greenish-brown) granulation were observed. Most of the pigment did not react with Perl's stain, although with this stain rare clumps of positive material were noted both intracellularly and extracellularly. This probably represented hemosiderin which was derived from red blood cells present in the initial specimen. Most of the pigment was argentaffin and with Lilly's ferrous iron uptake technic appeared dark green.

Dendritic mast cells were occasionally seen after 4 days of growth, but after 10 days of growth dendritic mast cells constituted the majority of the cells of the colony (Fig. 4).

Cultures in which corticotropin was added initially did not grow in monolayer, but rather as clumps of small round cells. Pigment formation was present in a greater proportion of cells than in the cultures not containing corticotropin; after 3 days of growth, pigment was present in many of the cells of the colony. In those cultures to which corticotropin was added after 4 days of growth the colony rapidly took on the appearance of the cultures in which corticotropin was added initially, being composed of clumps of small round cells which frequently contained pigment.

The pigment present in the cultures contain-

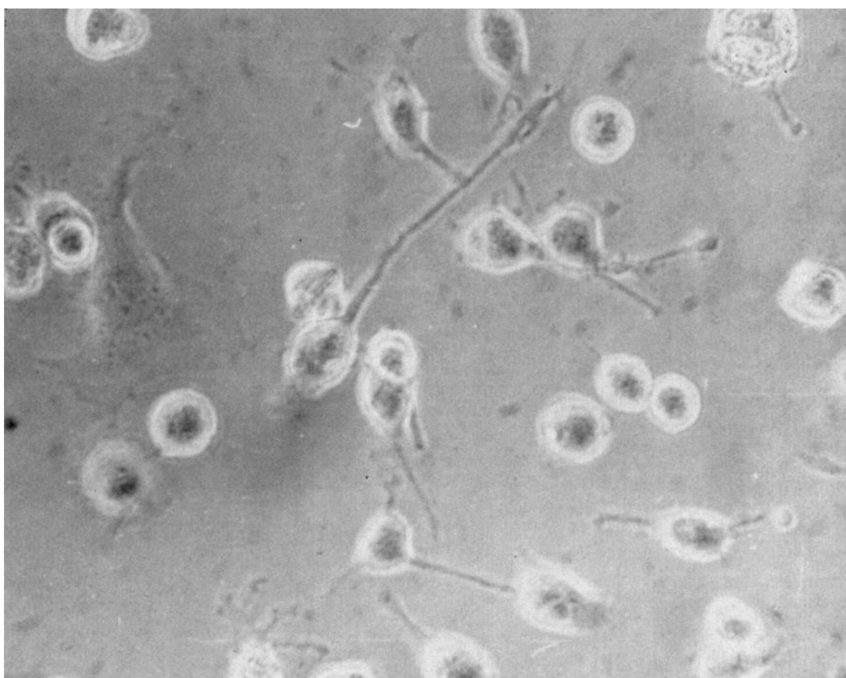

FIG. 4. 12-day-old culture of mast cells from peritoneal washings of a Sprague-Dawley rat (corticotropin not present). The field contains many highly dendritic mast cells. Phase contrast illumination, unstained. ×1800.

ing corticotropin reacted tinctorially in the same way as the pigment in the cultures not containing corticotropin.

There appeared to be no significant difference in the degree of pigmentation in the cultures derived from mast cells of albino rats as compared with those of black agouti rats.

DISCUSSION

The results of this study indicate that mast cells readily form pigment in tissue culture under the conditions described, and that most of this pigment shows the histochemical staining reactions for melanin. Our observations also suggest that corticotropin enhances the degree of pigment formation, but that corticotropin is not essential for pigment formation.

An explanation for the fact that mast cells form melanin in tissue culture, even without the stimulus of corticotropin, cannot be easily furnished. The problem is analogous to that posed by the melanization of human albino melanocytes in tissue culture observed by Mishima (18) in corticotropin-free culture medium containing L-tyrosine. He proposed that the tyrosinase of the albino melanocytes may be present in an inactive form or may be inhibited by some environmental biochemical factors *in vivo*. It appears possible that the same differential factors which prevent melanization of albino melanocytes *in vivo*, but allow their melanization *in vitro*, apply to mast cells.

The enhancement of the pigmentation of mast cells in tissue culture by corticotropin is consistent with the effect of this hormone on cultured albino melanocytes. Hu and Chavin (19), for example, demonstrated that corticotropin increases the degree of pigment formation in cultured albino melanocytes of goldfish. The mechanism of the melanization effect of corticotropin is unknown. Mishima (18) has postulated that corticotropin may promote the association of tyrosinase with the melanosome structure.

Although the factors responsible for the melanization of mast cells in tissue culture remain to be clarified, the fact that melanization does occur supports our previous work

suggesting that mast cells contain tyrosinase and are capable of synthesizing melanin *in vivo*.

SUMMARY

Pigmentation was observed in tissue cultures of mast cells obtained from peritoneal washings of rats. The degree of pigmentation appeared to be enhanced by the presence of corticotropin. Differential stains indicated that most of this pigment was melanin. These observations provide evidence supporting our previous work suggesting that mast cells contain tyrosinase and are capable of synthesizing melanin *in vivo*.

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