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EXPERIMENTAL HEMOSIDEROSIS: RELATIONSHIP BETWEEN SKIN PIGMENTATION AND HEMOSIDERIN

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Abstract. Iron (totalling 7.5 mg per mouse in three doses) was injected into hairless mice to determine the relationship between skin pigmentation and hemosiderin deposition. The skin color reached its maximum 24 to 48 hours after the last injection and then gradually faded over the subsequent 8 months. In the skin, hemosiderin granules were present extracellularly between collagen bundles as well as within dermal macrophages, Langerhans cells and indeterminate dendritic cells of the epidermis. A larger amount of iron was deposited in the facial than in the dorsal skin, resulting in darker pigmentation of the former. This study suggests that brownish discoloration of skin in hemochromatosis might be attributable in some degree to accumulation of hemosiderin and that pronounced hyperpigmentation of the face in hemochromatosis might be due to increased activation of melanocytes by a high content of hemosiderin.

Key words: Experimental hemosiderosis; Hemosiderin; Skin pigmentation; Hairless mice; Hemochromatosis

Hemochromatosis is a chronic disease characterized by the deposition of iron in body tissues, with eventual fibrosis and functional insufficiency of those organs severely affected. Depending on the manner in which excess iron deposits are produced, hemochromatosis may be characterized as idiopathic, dietary, or due to blood transfusion.

The pigmentation of hemochromatosis is one of its more frequently studied signs, having been noted in the first description of the disease. The physiopathology of the increased pigmentation is unknown and raises several questions, such as (i) What is the histological factor(s) that determines clinical pigmentation? Is it hemosiderin, melanin (6), or both (3)? (ii) Why is the color in the face darker than that in other parts of the body?

In order to obtain additional information on the above questions, iron was injected into hairless mice. These mice are useful for the study of skin color changes, since they have no hair and neither have they any pigment in most of the skin. In addition, we can expect melanin formation by indeterminate dendritic cells, or α-dendritic cells which can produce melanin following certain sorts of irritation (4, 7, 10, 11).

MATERIALS AND METHODS

Animals. Twenty hairless mice, ranging in weight from 23 to 28 g and from 3 to 4 weeks of age were selected from a colony of dark, hairless mice. The mice are offspring of a C57HR/Ch mutant which was established by crossbreeding C57HR/Ch with haired C57BL/10-H2^d (B10 D2) black mice to introduce more pigment capacity into the strain. The mice were kept in separate cages and received drinking water and food ad lib up to sacrifice.

Iron. The iron used was 0.5% iron dextran in water.

Methods. The iron dextran was given intraperitoneally in single doses of 2.5 mg every other day (totalling 7.5 mg of iron per mouse). Specimens were taken from the face and back of the animals before injection, or 24 hours, 48 hours, 7 days or 4 months after the late injection. Specimens were also taken from the liver, heart, pancreas and other organs of control animals or 7 days after the last injection.

For light microscopy, 7 μm sections were cut and stained with haematoxylin-eosin and Berlin-blue.

For electron microscopy, specimens were fixed at 4°C for 1 hour in 2% glutaraldehyde buffered with 0.2 M phosphate (pH 7.4). After thorough rinsing they were immersed in 1% osmium tetroxide buffered with 0.2 M phosphate (pH 7.4) for 1 hour and then dehydrated by graded ethyl alcohol and embedded in Epon 812 *ad modum* Luft (5). Ultrathin sections were cut with a Porter-Blum ultramicrotome MT-2. These sections were stained on a 100-mesh copper grid for 1 hour with a saturated aqueous solution of uranyl acetate followed by immersion for 10 minutes in Reynolds' lead citrate.

Some were examined with a Hitachi HS9 electron microscope; others were subjected to energy-dispersive microanalysis as follows. The analytical electron microscope was a Hitachi HU-600 electron microscope equipped with a Hitachi H 5010 scanning attachment and an energy-

disperse Kevex 7000C X-ray spectrometer system. Accelerating voltage of 75 KV was used throughout the study. The Kevex 7000C X-ray multichannel analyser was operated at 10 eV per channel, and probe size was approximately 150 Å in diameter. A sample current of 5×10^{-10}A measured on a copper grid was used for the electron beam focused on microareas of interest, and X-ray intensity of iron K-alpha peak was collected from a 7-channel window set placed over the energy range 6.34–6.46 KeV. Point micro-analysis was done in chosen areas. All data displayed on the cathode ray tube were recorded photographically with Polaroid film.

In order to determine the amount of iron deposited in the skin, specimens of about 1.5×1 cm were taken from the face and back of each of 5 mice before injection, or 48 hours or 4 months after the last injection, separately. The iron content was measured according to the method of atomic absorption analysis as follows.

Skin samples (wet samples), after drawing off water with filter papers, were placed on a quartz boat. They were ashed for 3 days using Yamatokagaku plasma reactor model PR-151 type (power: 80W; 20 ml of oxygen per minute). The ash was dissolved in a mixed solution consisting of 0.2 ml conc. HNO_3, 0.2 ml conc. H_2SO_4 and 0.2 ml 6 N HCl, and diluted with 250 ppm $SCl_2 \cdot 6H_2O$ solution.

Iron content was measured using an atomic absorption spectrophotometer (Toshiba Beckman NF-1B), equipped with the following gas burner and light sources. Gas burner: An air–acetylene gas flame was used, consuming 10.0 liters of air and 2.2 liters of acetylene gas per minute. Light source: A hollow cathode lamp (Hamamatsu TV, Japan) was used with wavelength control set at 2 483 Å and slit width at 0.3 mm.

RESULTS

One of the 20 mice died within 24 hours of the first injection of iron, but the others survived until sacrifice.

Gross changes. The skin color of the mice before injection was pinkish white except for the ears and tail which were black to dark brown. The color started to change to light yellowish brown 24 hours after the second injection of iron and the color reached its maximum degree 24–48 hours after the last injection and then gradually faded over the subsequent 8 months. The face and back showed different degrees and shades of hyperpigmentation, noted from around the 7th day after the last injection; that is, the color on the face was darker than that on the back. The difference continued up to around 3 months after the last injection.

Light microscopic findings. Iron was noted as dark yellow granules in H&E stained sections and blue granules in Berlin-blue stained ones. These granules of iron were seen mainly in the upper dermis in all the specimens taken 24 hours, 48 hours, 7 days and 4 months after the last injection. No iron deposit was seen in the specimens before injection. It appeared that histologically more iron was deposited in the facial than in the dorsal skin in all of the specimens taken 48 hours and 7 days after the last injection (Fig. 1). Aggregates of iron were also seen in the other organs, including the liver and spleen, after injection of iron.

Table 1. *Amount of iron in skin*

Mouse	Face (mg/100 g)	Back (mg/100 g)
(1) *48 hours after injection*		
1	30.6	18.1
2	35.1	29.0
3	28.1	15.3
4	37.9	21.3
5	20.3	15.5
Mean±S.D.	30.4±6.8*	19.9±5.7
(2) *4 months after injection*		
6	8.0	7.2
7	6.5	6.3
8	7.1	7.0
9	4.5	5.3
10	8.9	7.0
Mean±S.D.	7.0±1.7 NS	6.6±1.6

*$p<0.01$.
NS = not significantly different.

Electron microscopic findings. Sharply outlined aggregates of electron-dense granular material, up to 1.5 μm in diameter, were seen in the dermal intercellular space, within macrophages, Langerhans cells, indeterminate epidermal dendritic cells and pilosebaceous gland cells (Fig. 2). It was also noted that some of these macrophages contained Langerhans granules. Some aggregates were free from limiting membranes, and the others, particularly in the macrophages and Langerhans cells, were bound by membranes. These aggretates were proved to contain iron, according to an electron X-ray microanalysis (Fig. 3) and are thought to be hemosiderin. There were no active melanocytes containing melanosomes.

Amount of iron in skin. As seen in Table 1, the amount of iron in the facial skin was significantly greater ($p<0.01$) than that in the dorsal skin in all specimens 48 hours after the last iron injection. However, no significant differences were seen between the two areas 4 months after the last injection. The amount of iron before injection was below 1.0 mg per 100 g in the facial and dorsal skin.

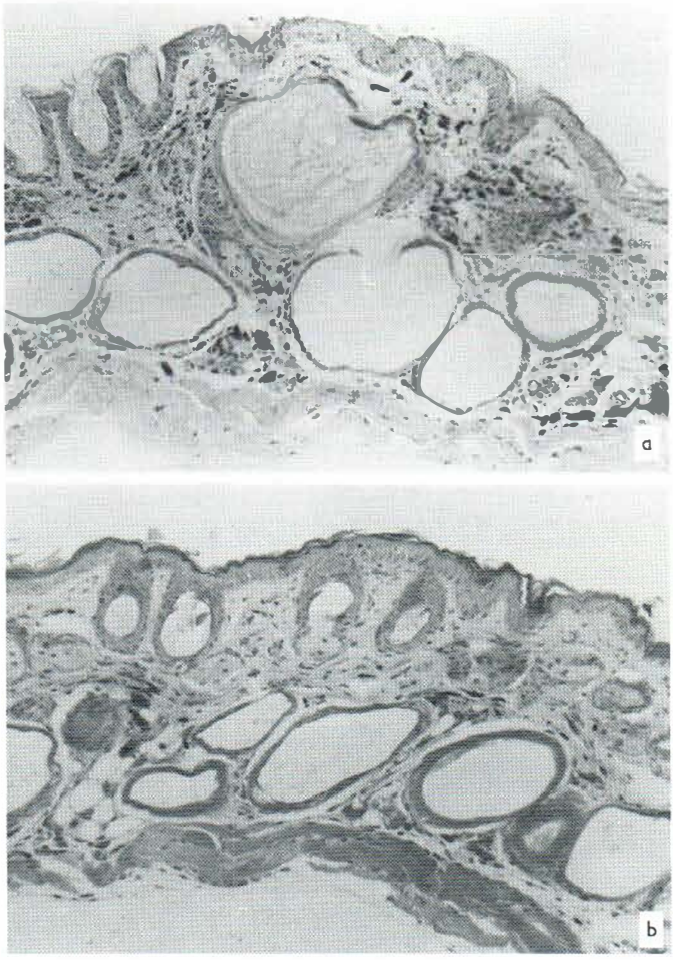

Fig. 1. Histology of skin 48 hours after injection (Berlin-blue staining). Hemosiderin granules are seen mainly in the upper dermis. It appears that more hemosiderin granules are deposited in the facial (*a*) than in the dorsal skin (*b*).

DISCUSSION

The present study revealed that a large amount of hemosiderin accounted for the yellowish-brown pigmentation of the mice after injection of iron and that the degree of the pigmentation depended on the amount of hemosiderin present. It was expected that indeterminate dendritic cells or α-dendritic cells in the epidermis would be activated and would produce melanin, since these dendritic cells have been proved capable of producing melanin (4, 7, 10, 11). However, they did not produce any melanin, although some of them contained dense aggregates of hemosiderin.

When comparing hemochromatosis with the experimental hemosiderosis seen in the present study, we notice three findings common to the two disorders: (*a*) an aggregation of hemosiderin in almost every tissue, structure, and organ of the body, including the skin, (*b*) the brownish skin color, and (*c*) the localization of the skin pigmentation, most prominently on the face. A difference between the two is that an increased content of melanin in the skin is seen in hemochromatosis, while no melanin accumulated in the skin of the experimental mice.

Discoloration of skin in cases of hemochromatosis has been attributed primarily to accumulations of melanin and, to a lesser degree, of hemosiderin (3). Recently Perdrup & Poulson (6) noted that vitiliginous areas in a patient with hemochromatosis were not hyperpigmented, in contrast to the surrounding skin. They concluded that the skin color of patients with hemochromatosis is due exclusively to an increased content of melanin. It is not possible to draw any firm conclusion about the discoloration of skin in hemochromatosis from the experimental results in

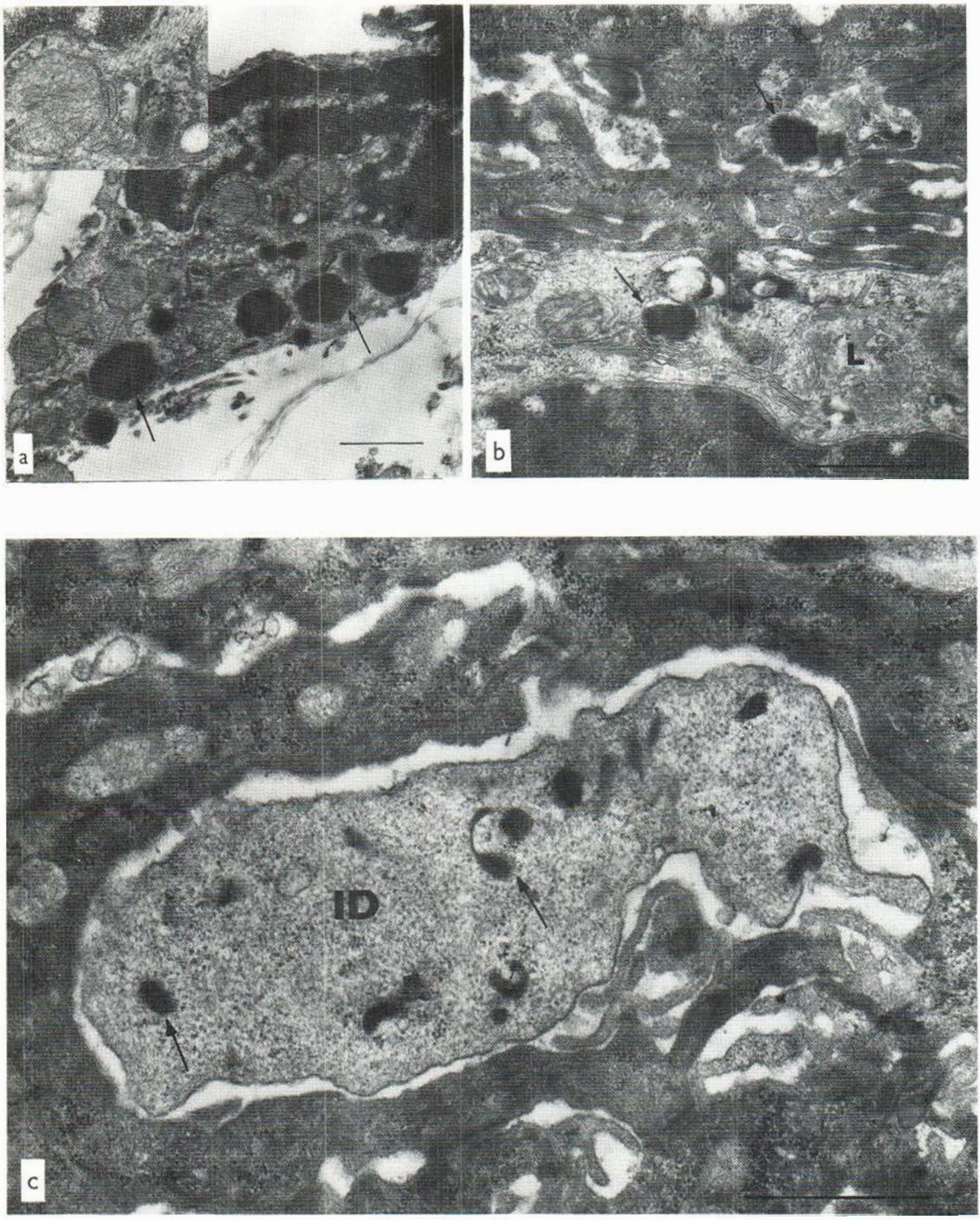

Fig. 2. Ultrastructure of skin 48 hours after injection. Hemosiderin aggregates (arrows) are seen within and adjacent to (*a*) a dermal macrophage containing Langerhans granules, (*b*) within a Langerhans cell (L), and (*c*) an indeterminate dendritic cell (ID). Some of the aggregates are free from limiting membranes, but others, particularly in the macrophages and Langerhans cell, are bound by membranes. Each bar indicates 1 μm. Inset in (*a*) indicates higher magnification of one of the Langerhans granules.

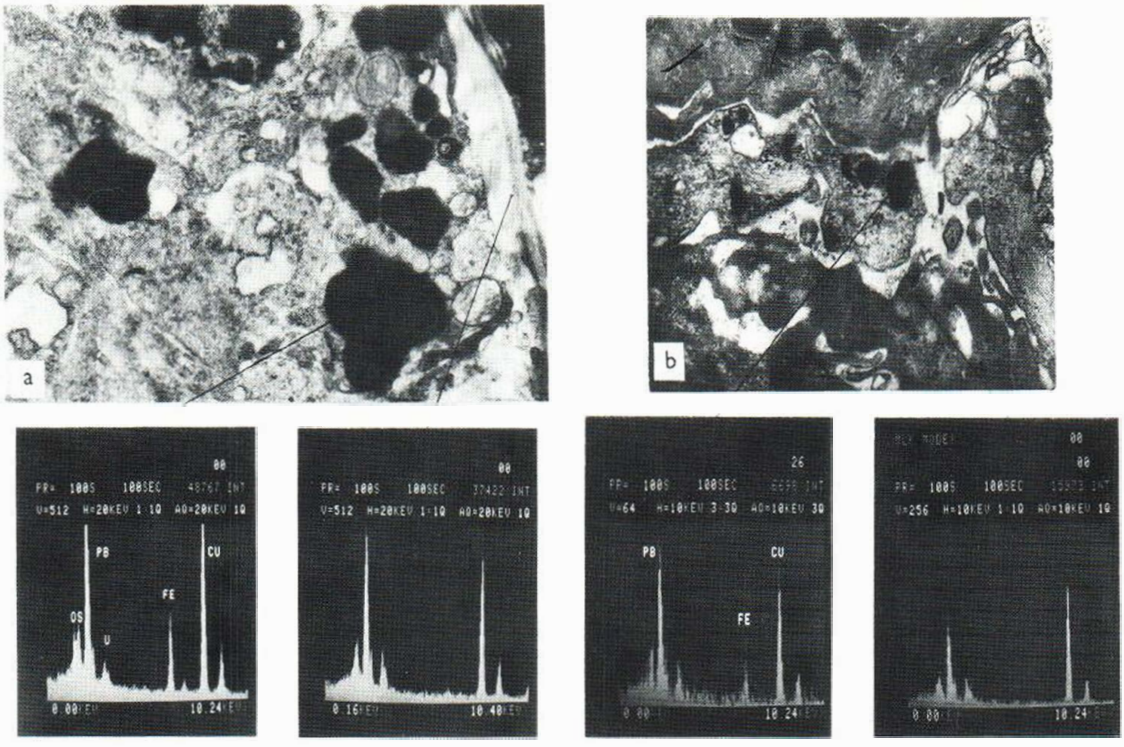

Fig. 3. Electron X-ray microanalysis (*a*) Electron-dense aggregates in macrophage and (*b*) an indeterminate dendritic cell are proved to contain iron (*EF*). Marks *CU*, *FE*, *OS*, *PB* and *U* at the peaks indicate copper, iron, osmium, lead and uranium respectively.

mice, since there are genetic differences between human and mouse skin. In general, human skin is thicker than mouse skin. In hemochromatosis, however, conspicuous thinning of the epidermis was reported (1). This suggests that there may be no great difference between the two disorders in the dermal portion of the skin where hemosiderin is deposited. Thus the present study suggests that brownish discoloration of the skin in hemochromatosis might be attributable in some degree to accumulate of hemosiderin in the dermis.

Concerning the increased melanin observed in the skin of most patients with hemochromatosis, there are several hypotheses. Hellier (3) thought that it might be due to adrenal dysfunction. Robert & Zurcher (8) have suggested that the presence of metal in the skin favors the formation of melanin, possibly by enhancing the oxidative processes. Moreover, Cawly et al. (1) suspected that it is the result of injury to melanocytes themselves or to surrounding tissue by hemosiderin. The fact that deposition of hemosiderin was seen in the indeterminate dendritic cells of the mice is consistent with the possibility of injury by hemosiderin to the melanocytes in hemochromatosis, resulting in hyperpigmentation of the skin.

Cawly et al. (1) have also suggested that pronounced hyperpigmentation of the face in patients with hemochromatosis is related to the relatively large number of melanocytes in this location. The present study revealed that a higher content of hemosiderin was present in the facial than in the dorsal skin of the experimental mice. This might be true also in hemochromatosis, and pronounced hyperpigmentation of the face might be due to increased activation of melanocytes by a high content of hemosiderin rather than to the large number of melanocytes in this location.

Several previous reports have shown that the Langerhans cell has the ability to phagocytose foreign material such as ferritin, exogenous protein, and latex beads (9, 12, 13). The present study has confirmed this. In addition, the fact that iron-phaging macrophages with Langerhans granules were

seen in the dermis suggests a close relationship between Langerhans cells and macrophages.

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