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# Is it coincidence that iron and melanin coexist in hepatic and other melanomacrophages?

**Jeanne M. Henninger and W.A. Beresford**
Department of Anatomy, West Virginia University, Morgantown, USA

**Summary.** Use of a Prussian-blue histochemical method shows iron in some but not all hepatic melanomacrophges of turtle, alligator, caiman and anole. The hypothesis prompted is that melanomacrophages in general synthesize melanin to render less noxious free radicals arising from catalysis by the iron.

**Key words:** Free radicals, Iron, Liver, Melanomacrophage, Melanin

## Introduction

«Melanomacrophage» is applied to darkly pigmented macrophages in the kidney, spleen and liver of fishes, although there are few in the teleost liver (Agius, 1979; Herráez and Zapata, 1986). Closely related phagocytic cells exist in the liver and spleen of other ectotherms, but are variously and distractingly also termed Kupffer cells (Sichel et al., 1987), pigmented Kupffer cells (Ells, 1954), pigment cells (Hack and Helmy, 1964), extracutaneous pigment cells from histocytic origin (Sichel, 1988), or even melanophores (Harr and Hightower, 1976) or melanocytes (Ferrer et al., 1987). In amphibia, hepatic melanin can capture free radicals (Geremia et al., 1984); and in frog and turtle hepatic melanin is synthesized locally (Scalia et al., 1988; Sichel, 1988) rather than taken up from extrahepatic sources. Occasionally macrophages may ingest melanosomes of other cells, thereby meriting the name «melanophage» (Kordylewski, 1983), but the synthesis of melanin by melanomacrophages is an altogether different activity with its own significance.

Investigators histochemically have found iron in some but not all melanomacrophages in piscine kidney, spleen and liver (Agius, 1979; Herráez and Zapata, 1986), amphibian liver and spleen (Oppel, 1889; Berg, 1937; Hack and Helmy, 1964), and reptilian liver (Hack and Helmy, 1964; Ferrer et al., 1987). Here, we test for the presence of iron in hepatic melanomacrophages of four American reptilian species, and explicitly connect the probable role of melanin as a multimechanism scavenger of free radicals (Korytowski et al., 1986; Sichel, 1988) with unbound iron as a catalyst of free-radical production (Halliwell, 1987). Such radicals injure the mammalian liver (Arthur, 1988). The hypothesis offered is that the synthesis of melanin by the melanomacrophage is in some way a counterpart or response to the potentially hazardous iron.

## Materials and methods

Pieces of liver from eight adult box turtles (*Terrapene carolina*), eleven mature alligators* (*Alligator mississippiensis*), four juvenile caimans* (*Caiman crocodilus*), and one mature green anole (*Anolis carolinensis*) were fixed in Bouin's solution (* first in 10% formol-saline), embedded in paraffin, and sectioned at 8 µm. Sections were stained with Masson's trichrome, or Gomori's (1936) ferrocyanide method for iron with a neutral-red counterstain. Other sections were taken to water and bleached for 2 hours in 3% $H_2O_2$ with one drop of ammonium hydroxide added per 10 ml, before the Gomori staining. (All animals were taken and killed humanely and legally).

## Results

Hepatic melanomacrophages, known by their dark pigment, were numerous in all four species, with intraspecific variation in number among the turtles and alligators. Most of the pigment was bleached by ammoniated hydrogen peroxide and is taken to be melanin. With Masson's trichrome, the livers of box turtles had an appearance justly represented by

*Offprint requests to:* W.A. Beresford, Anatomy-HSN, West Virginia University, Morgantown, WV 26506, USA

**Fig. 1.** Turtle liver. Gomori, × 240

the recently published Fig. 1c of Sichel et al. (1987) and 1b of Ferrer et al. (1987) for Greek tortoise. Sichel et al. Fig. 1e for lizard is similar to the look of alligator, caiman and anole liver, except that melanomacrophages were more numerous in anole than in lizard, but displayed less clumping than in turtle and the crocodilians. The crocodilian cells were heavily pigmented and often in globular clumps.

In turtle (Fig. 1) and alligator, staining for iron showed a light granularity towards the bile-canalicular pole of hepatocytes, but the reaction at this site in caiman and anole was weak. In all four species, a far stronger iron reaction occurred in some of the melanomacrophages. The iron could be particulate or distributed as a dense partial shell around the pigment. Other melanomacrophages were so densely black that a reaction could pass unnoticed. Yet others were paler, but still displayed no iron. In the partly bleached sections some melanomacrophages lacked iron. On the other hand, few non-hepatocytic cells contained iron but lacked pigment.

## Discussion

The unity in the idea that many poikilothermic macrophages make and harbor melanin has been obscured by the variety of names for the melanomacrophages. The disparate naming has kept from its due recognition the often-reported coexistence of iron and melanin.

For iron-bearing melanomacrophages, the idea that melanin is there to capture free radicals generated by the iron is readily, if superficially plausible. In particular, tests for the hypothesis need: 1. to account for the inexact correlation between iron content and pigmentation in reptilian and other melanomacrophages; 2. to compare hepatic contents of iron and melanin; 3. to show that the iron in melanomacrophages catalyzes the formation of free radicals; 4. to relate the initiation or level of melanin synthesis to the rate of free-radical formation; and 5. to exploit amphibian albino mutants (Hack and Helmy, 1964), and the alterations in iron metabolism and melanomacrophage activity caused by experimental anaemia or starvation (Berg, 1937; Ells, 1954; Agius, 1979).

There are other complications for the hypothesis, for instance, ignorance of how reactive is the iron in melanomacrophages and hepatocytes: one aspect of the difficulty that the timescales of storage (as shown statically by histochemical methods), and of chemical activity related to free radicals, differ by several orders of magnitude. Also, fishes are very diverse in the organ distribution, clumping, pigmentation and iron content of their melanomacrophages (Agius, 1979; Herráez and Zapata, 1986).

Although demonstrated here by simple means, the coexistence of the two chemical entities presumably reflects a solution by these cells to a problem related to defence, haemopoiesis, or both. The breadth of the species and organs in which the iron-melanin coexistence occurs signifies that the solution is a generalised one, and hence deserves closer analysis on a briefer and more dynamic scale than was attempted here.

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