thy that the average NA concentrations within the principal superior cervical perikarya observed in our experiments and those based on the gelatin standard do not deviate substantially from those surmised by other authors[16].

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Giant pigment granules in dermal melanocytes of rat scrotal skin

M.E. Glimcher and G. Szabo[1]

Laboratory of Electron Microscopy, Harvard School of Dental Medicine, 188 Longwood Avenue, Boston (Massachusetts 02115, USA), 9 January 1978

Summary. Giant melanosomes, ellipsoidal (1.5 μm × 1.3 μm) or spherical (1.1 μm–1.4 μm in diameter) exist in the scrotal skin of the black pelted Long Evans rat. They are longer and wider than normal stage IV melanosomes (0.7 μm × 0.4 μm) found in these dermal melanocytes.

Giant pigment granules have been observed in the melanocytes of the skin of a number of mammalian species. Lutzner et al. reported the presence of giant pigment granules in the beige mouse[2] and in the Aleutian mink[3]. In humans, large accumulations of defective and large pigment granules have been found in patients with Chediak-Higashi Syndrome[4]; giant pigment granules (macromelanosomes) in the melanocytes and keratinocytes of the epidermis of patients with neurofibromatosis[5,6] and nevus spilus[7]. Here, we report for the 1st time the presence of giant melanosomes in the dermal melanocytes of a rodent. We feel it is necessary to report this single finding as no giant pigment granules have heretofore been found in dermal melanocytes nor in rodent skin.

Materials and methods. The effects of castration, testosterone replacement therapy, UV-light, and their combination, on scrotal skin pigmentation were studied using 51 black pelted, male Long-Evans rats, 50–160 days old (Blue Spruce Farms, Altamont, N.Y.). Periods of post-operative castration ranged from 11 to 109 days until sacrifice. Testosterone propionate (Sigma Chemical, St. Louis, Mo.) suspended in sesame oil was injected s.c. in the dorsum of the neck of 11–56-day post-operative castrates in dosages of 10^{-3}–10^{-7} g/100 g b.wt for up to 18 days. The UV-light source used was a Westinghouse FS-20 sunlamp with maximum output of 290–330 nm and peak at 310 nm. Daily dose ranged from 1.22×10^5 erg/cm^2 to 1.28×10^7 erg/cm^2 for exposures of 1–14 days.

The scrotal skin was excised and fixed in Ito-Karnovsky fixative[8,9], 5% glutaraldehyde, 4% formaldehyde, and 0.02% 2,4,6-trinitrophenol, for 2 h at 4 °C. The tissue was then washed in 0.1 M cacodylate buffer and post-fixed in 2% OsO_4 for 2 h at 4 °C. 1-μm-thick sections were stained with toluidine blue and photographed with a Zeiss Ultraphot II. Epon-embedded specimens were thin-sectioned using a Porter-Blum ultramicrotome, stained with uranyl acetate and lead citrate, and examined using an AEI Corinth C275 electron microscope.

Results and discussion. Giant melanosomes were observed in the course of an investigation in which the effect of castration and testosterone therapy on scrotal skin pigmentation was studied in black Long-Evans rats. Light and electron microscopic observation revealed the existence of giant pigment granules within the densely pigmented dermal melanocytes of 1 particular animal in the group which received daily testosterone replacement therapy (0.1 mg/100 g b.wt) for 10 days following 8 weeks of post-operative castration. These giant pigment granules are ellipsoidal (approx. 1.5 μm by 1.3 μm) or spherical (1.1 μm by 1.4 μm in diameter). Other melanosomes in dermal melanocytes of the same animal and of other animals were mainly in stage IV and approximately 0.7 μm by 0.4 μm.

The presence of normal melanosomes in stages II and III of formation in these dermal cells suggests that they are melanocytes and not macrophages. The histochemical tyrosinase reaction would clearly demonstrate this point.

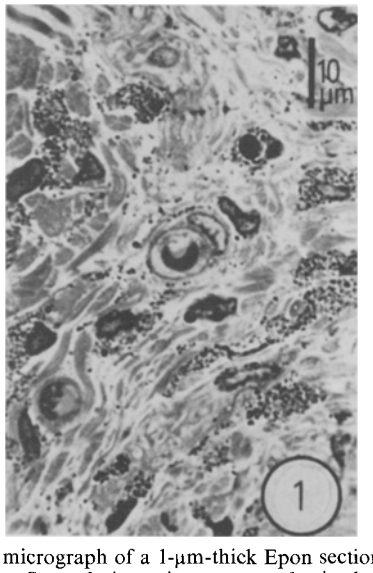

Fig. 1. Light micrograph of a 1-μm-thick Epon section stained with toluidine blue. Several giant pigment granules in the dermal cells. × 970.

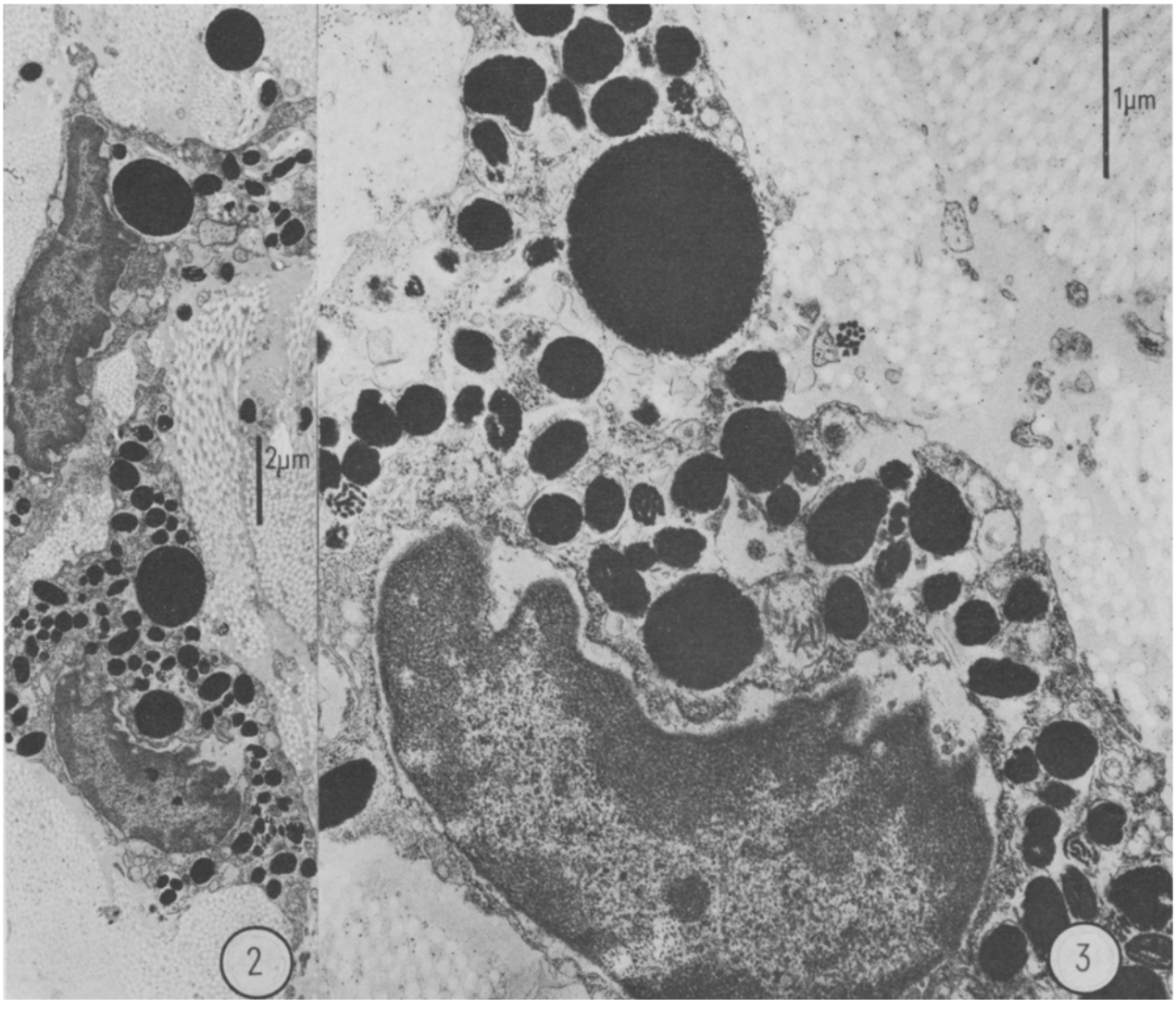

Fig. 2. Electron micrograph demonstrating 2 dermal melanocytes containing several single giant pigment granules. × 6,225. Fig. 3. Electron micrograph showing a portion of a dermal melanocyte containing several stage III and IV melanosomes and giant pigment granules. × 24,000.

The epidermal melanocytes and melanocytes of the superficial dermis, but not the lower dermis in the scrotal skin of the Long Evans rat, are dependent upon testosterone for the maintenance of their normal morphology[10]. After castration, with and without testosterone replacement therapy, the presence of dermal melanocytes is more pronounced especially in close proximity to the basal lamina.

These giant pigment granules are somewhat similar to type III giant melanosomes reported by Jimbow et al.[5] in human neurofibromatosis, although the human melanosomes are about 3 times larger than those found in the rat. The giant pigment granules reported here are not found in complexes, but are single, although several may be observed in an individual melanocyte in the dermis. No internal structure in the giant pigment granules is evident. No giant pigment granules have been observed in the epidermal melanocytes of the scrotal skin of the same animal or in other rats.

It is important to note that the dermal melanocytes of the scrotal skin of black pelted Long Evans rats are capable of forming melanosomes of normal size as well as abnormally large melanosomes. Generally, the presence of giant pigment granules is an expression of disease in humans as well as other mammals.

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