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Ultrastructure of oesophageal melanocytosis

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Summary. Four examples of an endoscopically detected oesophageal melanotic lesion were examined by light microscopy, light microscope histochemistry and transmission electron microscopy, and were compared with 13 control samples of normal oesophageal epithelium. By light microscopy, pigmented melanocytes lacking atypia and mitoses were observed amongst the keratinocytes in the basal layer of the oesophageal mucosa. Junctional activity was absent. The mechanism of pigmentation was studied and found to consist of: an increase in the number of melanocytes in the basal layer of the mucosa, an increase in the quantity of melanin in these melanocytes, transfer of melanin from melanocytes to keratinocytes and to macrophages and fibroblasts in the tunica propria. Since all the lesions demonstrated increased numbers of both melanocytes and melanosomes, the term oesophageal *melanocytosis* rather than *melanosis* is suggested, to emphasise the essential character of the lesion as a cellular proliferation. The value of sampling these pigmented lesions during endoscopy is emphasised as a means of obtaining well-preserved material for the evaluation of a lesion which some authorities have viewed as a possible precursor for oesophageal malignant melanoma.

Key words: Oesophagus – Melanocyte – Melanoma – Ultrastructure – Endoscopy

Introduction

Primary pigmented melanocytic lesions of the oesophagus are rare. Malignant melanoma, whilst preferentially affecting skin and the eye, but also less frequently such sites as oral mucosa (Trodahl and Sprague 1970), upper respiratory tract (Mesara and Barton 1968) and the anorectal area (Wanebo et al. 1981), has been documented at this site. Oesophageal malignant melanoma was originally considered to be an exclusively metastatic lesion, a view partly founded on the low incidence and inconsistent distribution of melanocytes in the normal oesophageal epithelium: these cells seem to be present in only 2.5–8% (de la Pava et al. 1963; Shibata 1973; Tateishi et al. 1974) of human subjects. Since the first description by Piccone et al. (1970), primary malignant melanoma of the oesophagus has been firmly established conceptually, and there are 100 or more recorded examples (Chalkiadakis et al. 1985; Ohashi et al. 1990).

In addition to solitary oesophageal melanocytes, on the one hand, and malignant melanoma, on the other, melanocyte proliferations have been documented which present as pigmented lesions for which the term *oesophageal melanocytosis* has been suggested (Kreuser 1979). Oesophageal melanocytosis has been reported synchronously with (Kreuser 1979; Guzman et al. 1989) or preceding (Kanavaros et al. 1989) malignant melanoma and the possibility that it might be a premalignant lesion has been suggested. Melanocytosis, though not referred to as such, has also been observed in association with oesophageal carcinoma (Ohashi et al. 1990), introducing an additional possibility of a reaction to a stimulus from adjacent neoplasia as a cause for this proliferation. Given their uncertain significance, therefore, such lesions deserve attention whenever they are encountered, especially in view of their exceptionally low incidence.

The oesophageal epithelium is notoriously poorly preserved at autopsy because of the reflux of gastric juices. Endoscopy, however provides an opportunity of obtaining well-preserved material. Using this technique, Ohgami et al. (1984), Makuuchi and Mitomi (1986) and Ohmori et al. (1990) have documented clinical aspects and the incidence of oesophageal melanocytosis. The present work represents an extension of these studies; new findings are reported on the histopathology and particularly the fine structure of oesophageal mealnocytosis, and comparisons with the skin briefly pointed out.

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Table 1. The location, number, size and appearance of lesions examined

Case no.	Age/Sex (years)	Medical condition/ history	Lesions at endoscopy
1	70, F	Discomfort in epigastrium	6 black lesions 3–5 mm diameter lower $^1/_3$ of oesophagus Fig. 1a
2	62, M	Ulcerative duodenal and gastric lesions	1 pale brown and black lesion 7 mm long and band-like 35 cm from incisors
3	56, M	Diabetes mellitus, cigarette smoker (15–20/day)	1 macular lesion, almost black 30 mm diameter 30–33 cm from incisors occupied $^1/_3$ of marginal wall of oesophagus Fig. 1b
4	66, M	Cigarette smoker	Cluster of black lesions 7–8 mm diameter lower $^1/_3$ of oesophagus

Materials and methods

Four cases of oesophageal melanocytosis and 13 normal oesophageal epithelium samples acting as controls were studied. They were all taken from elderly Japanese individuals without oesophageal symptoms and presenting for routine gastrointestinal cancer screening by endoscopy. Two patients had evidence of gastrointestinal tract disease. Clinical data are presented in Table 1.

Routine endoscopic examinations of the upper gastrointestinal tract were performed using an Olympus fine diameter panendoscope GIF-P20 with direct-view facility. Immediately before endoscopic examination the oesophagus was washed with 50 ml water to remove mucous material adherent to the oesophageal wall.

Pigmented lesions and normal oesophageal epithelium were sampled as approximately 1- to 1.5-mm^3 pieces of tissue. They were fixed either in 20% formalin for light microscopy or 2.5% glutaraldehyde in 0.1 M pH 7.4 phosphate buffer for electron microscopy. The formalin-fixed specimens were processed according to a conventional histopathological technique for subsequent haematoxylin and eosin (H&E) staining. The study of melanin was pursued by examining unstained sections as well as sections after Masson-Fontana staining. For electron microscopy, glutaraldehyde-fixed material was rinsed in buffer, post-fixed with 1% osmium tetroxide, and embedded in Luft's Epon. Ultrathin sections were cut on a diamond knife, stained with uranyl acetate and lead citrate, and examined in a JEOL JEM-1200 EXII electron microscope.

Results

The location, number, size and appearance of lesions are given in Table 1. All the lesions were flat and their colour varied from pale brown to completely black (Fig. 1a, b). The cases were followed up for between 6 months and 6 years without any local or general change in the medical condition of the patients or the appearance of the lesions.

In all 4 cases of oesophageal melanocytosis most of the pigment was found in the lower layers of the oesophageal mucosa as seen on H&E (Fig. 2a) or unstained sections. Small amounts of pigment were also seen in the upper keratinocyte layers. Melanocytes showed no signs of nuclear or cellular atypia and mitoses were not encountered. Junctional activity was not observed. Those samples incorporating an area of tunica propria also contained abundant pigment in the cytoplasm of mononuclear cells interpreted as macrophages. This pigment was shown to be melanin by the Masson-Fontana technique (Fig. 2b). No pigment was found in any of the 13 normal control oesophageal epithelium samples. Inflammatory cells, including lymphocytes, plasma cells and neutrophils, were present in both lesional and normal specimens.

On electron microscopy four cell types were found to contain melanin in the pigmented lesions: melanocytes, keratinocytes, macrophages and fibroblasts. In control samples, neither melanosomes nor melanocytes were encountered. Melanocytes were interspersed amongst keratinocytes, principally those of the basal layer. They were free cells, lacking junctions with adjacent cells. Typically, elliptical nuclei contained fairly evenly dispersed chromatin and possessed several shallow indentations (Fig. 3a). The perikaryon consisted of a narrow rim of cytoplasm from which radiated several cytoplasmic processes, the so-called *melanocyte dendrites*, which intermingled with keratinocytes (Fig. 3a). Both the perinuclear cytoplasm and the melanocyte dendrites contained many melanosomes. These were about

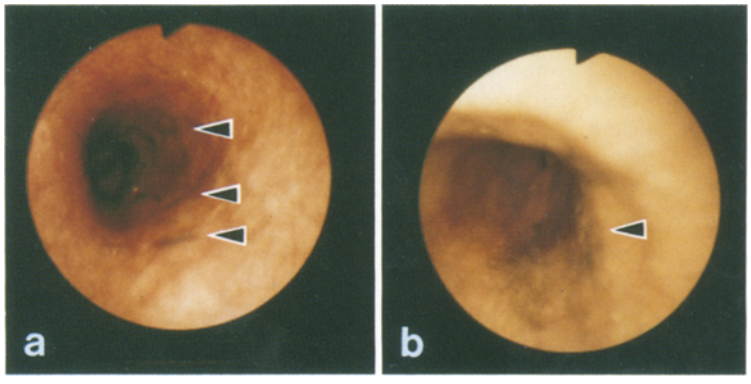

Fig. 1a, b. Endoscopic features of oesophageal melanocytosis. **a** Multiple dark brown lesions with elongate/oval profile (*arrowheads*) (case 1). **b** Macular pigmented lesion (*arrowhead*) (case 3)

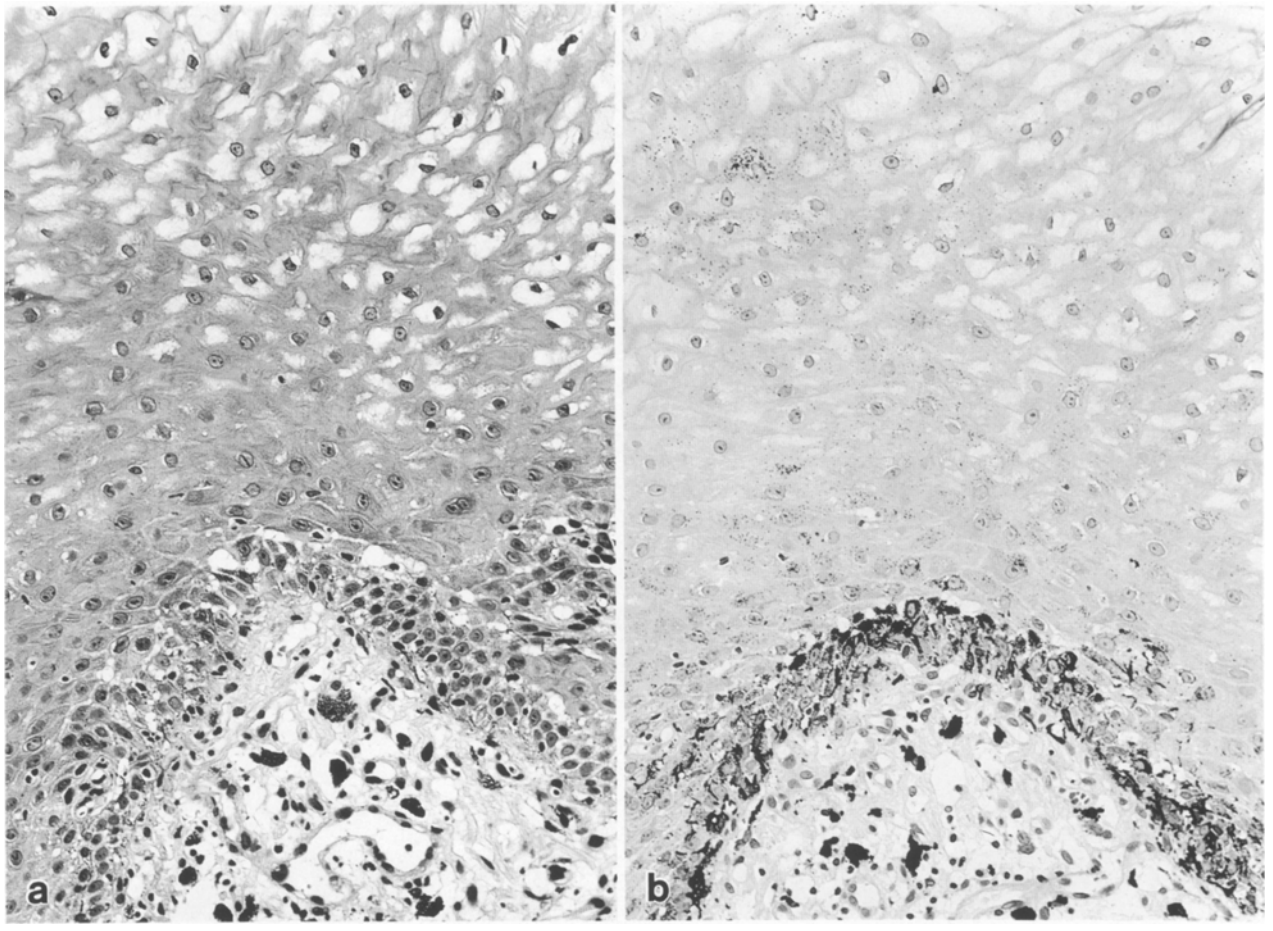

Fig. 2a, b. Light microscopy features of oesophageal melanocytosis. **a** H&E stain showing obvious melanin granules in cells of the basal mucosa and tunica propria. Case 1, ×200 **b** Masson-Fontana stain showing many minute black granules in the basal layer of the oesophageal mucosa. Aggregates of staining granules are also seen in the upper areas of the tunica propria and in the upper layers of the keratinocytes. Case 1, ×200

700 × 300 nm and mostly solitary, lying free in the cytoplasmic matrix (Fig. 3b). They showed varied degrees of pigmentation. Some non- or poorly pigmented organelles revealed the internal cross-striated lattice with a periodicity of about 10 nm, typical of melanosomes, while others were fully pigmented and contained an amorphous and intensely electron-dense interior (Fig. 3b).

Keratinocytes in the basal part of the mucosa (Fig. 4a) surrounding the melanocytes and their dendrites also contained melanin pigment. Here, it lay within compound melanosomes (secondary lysosomes) which contained small numbers of rounded or elliptical melanosomes, intensely electron-dense with melanin, enveloped by a common limiting membrane (Fig. 4b). A less dense granular material occupied the spaces between the pigmented melanosomes and the membrane in these compound organelles. Many of the pigmented melanosomes had the appearance of being solitary and lying free in the cytoplasm at low magnification. However, in many of these examples, a peripheral layer of finely granular and less dense material (identical to that in unambiguous compound melanosomes) was present between the melanin pigment and the membrane (Fig. 4b). Free solitary melanosomes, like those in melanocytes, were therefore considered to be absent from keratinocytes. Non-pigmented melanosomes were also difficult to find. Many of the melanosome dendrites, retaining their own surface membrane, were intimately associated with the keratinocytes and appeared to penetrate their cytoplasm.

In the tunica propria, melanin was unambiguously present within compound melanosomes, equivalent to secondary lysosomes, in both macrophages and fibroblasts. Compound melanosomes were up to 2 μm across, and somewhat rounded or irregular in shape (Fig. 5a) and of identical ultrastructure in macrophages and fibroblasts. They contained several rounded or elliptical and intensely pigmented melanosomal remnants embedded in a finely granular and less dense material which also contained an occasional lipid droplet (Fig. 5b). In some of these compound melanosomes, a fine space of uniform width could be seen beneath the limiting membrane. Fibroblasts were less pigmented, but cell processes containing abundant rough endoplasmic reticulum and believed therefore to be of fibroblastic origin contained small numbers of compound melanosomes (Fig. 6).

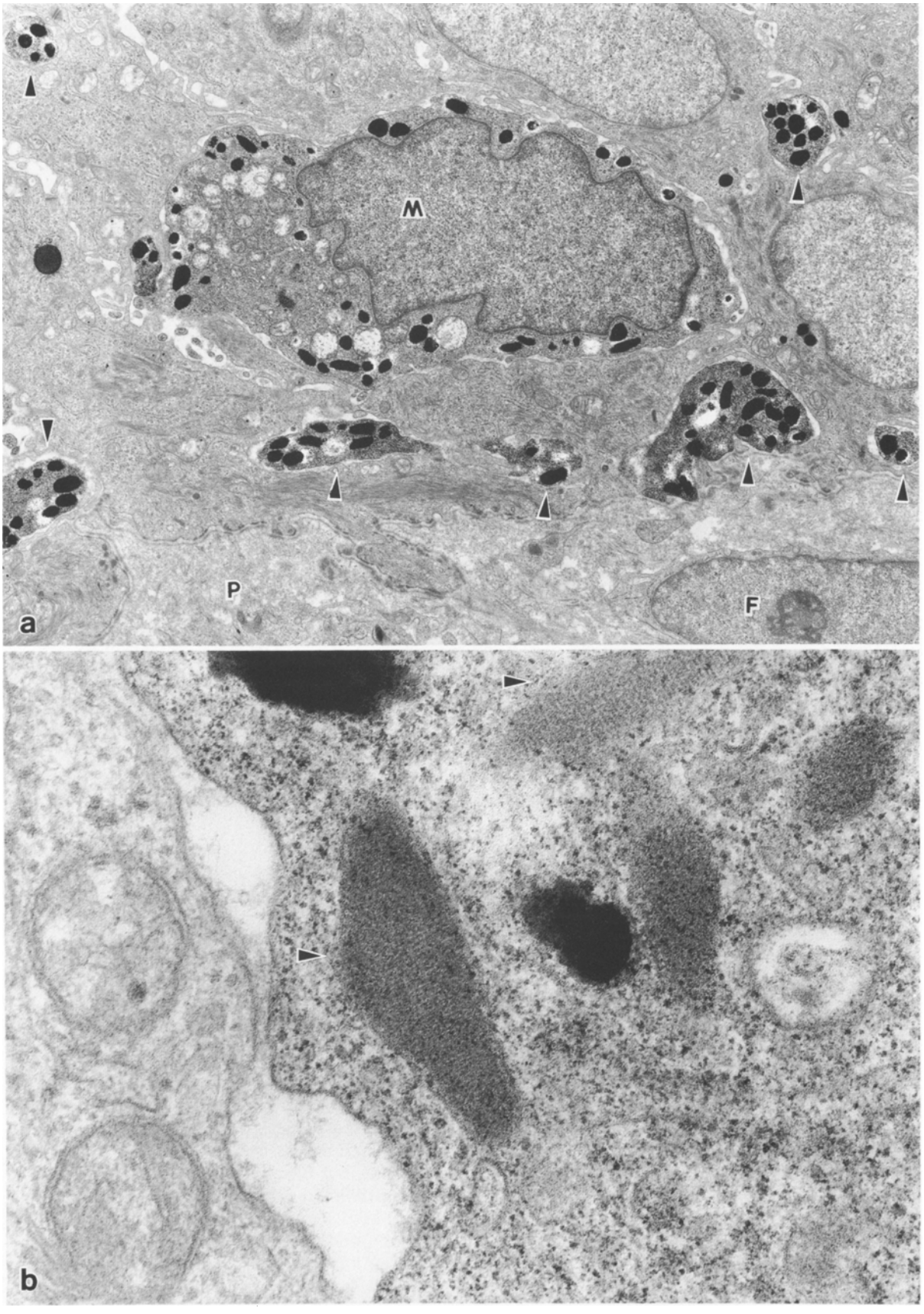

Fig. 3a, b. Transmission electron microscopy (TEM) of melanocytes. **a** Melanocyte (*M*) between the keratinocytes in the basal layer of mucosa, and melanocyte dendrites (*arrowheads*) containing many heavily pigmented melanosomes. *P*, Tunica propria; *F*, fibroblast. Case 1, ×7700. **b** High power view of melanosomes (*arrowheads*) in a melanocyte dendrite showing periodic internal structure. Case 1, ×77000

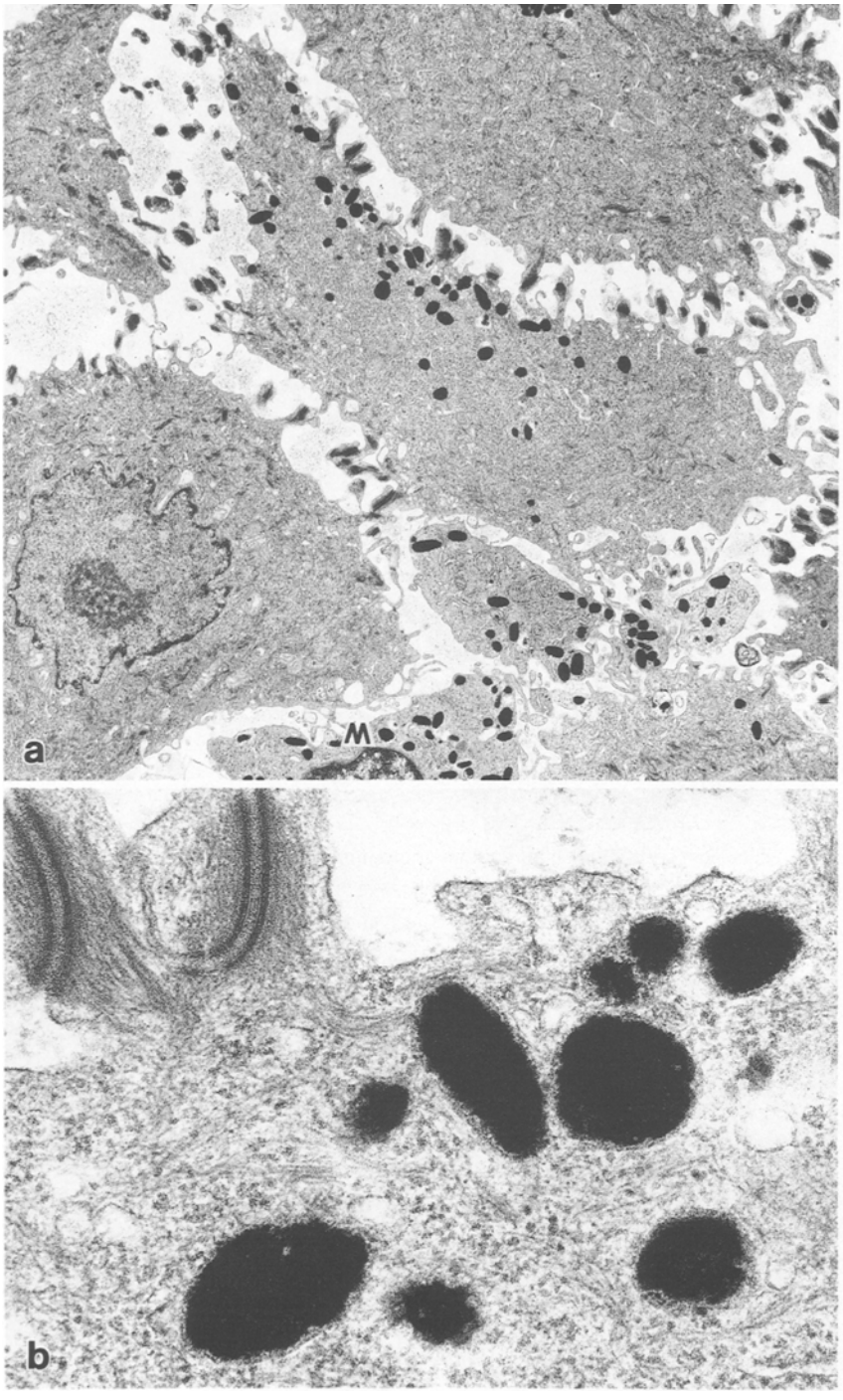

Fig. 4a, b. TEM of melanosomes in keratinocytes. **a** Low power view illustrating the large numbers of pigmented melanosomes in the cytoplasm of keratinocytes. Case 2, ×6000. **b** Densely pigmented melanosomes in a keratinocyte. Case 2, ×60000

Discussion

While primary malignant melanoma of the oesophagus is rare (Chalkiadakis et al. 1985), other primary pigmented lesions at that site occurring in isolation and not associated with other pathology, and lacking malignancy on the basis of the absence of mitotic activity, cellular atypia and junctional activity, are even more uncommon. Apart from the single case of Dumas et al. (1990), the most detailed clinical work on such lesions has been carried out in Japan (Ohgami et al. 1984; Makuuchi and Mitomi 1986; Ohmori et al. 1990). According to these authors, 24 individuals were found with discrete melanised lesions in approximately 33000 patients showing no clinical evidence of oesophageal pathology but undergoing endoscope screening for non-oesophageal and oesophageal, upper gastrointestinal tract disease. This represents an incidence of about 0.07% – slightly lower than the earlier studies indicating 0.1–0.15% but based on smaller numbers of cases (Ohgami et al. 1984; Makuuchi and Mitomi 1986; Ohmori et al. 1990). On a clinical basis and in the absence of histopathological

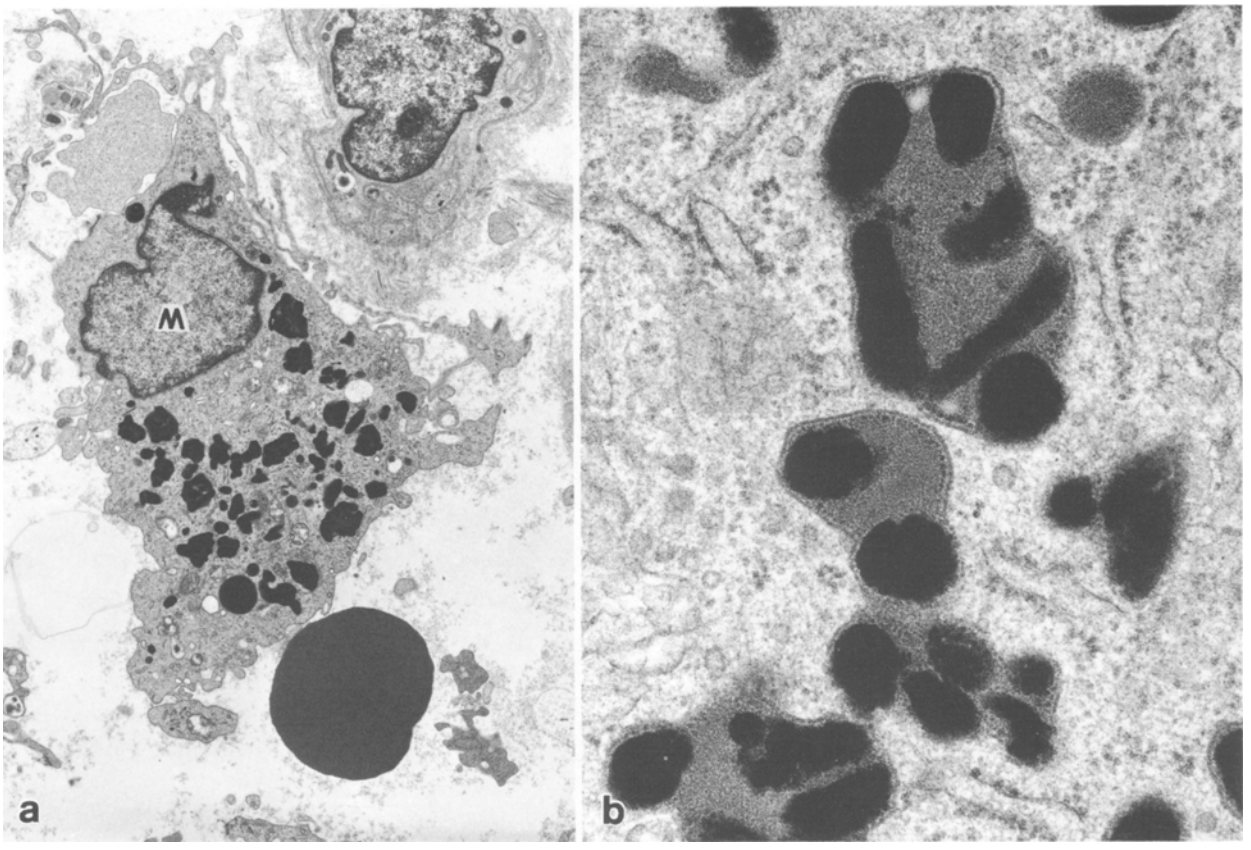

Fig. 5a, b. TEM of melanophages in tunica propria. **a** Melanophage (*M*) with numerous compound melanosomes in an oedematous tunica propria. Case 1, ×4600. **b** Detail of compound melanosomes (secondary lysosomes). Case 1, ×4600

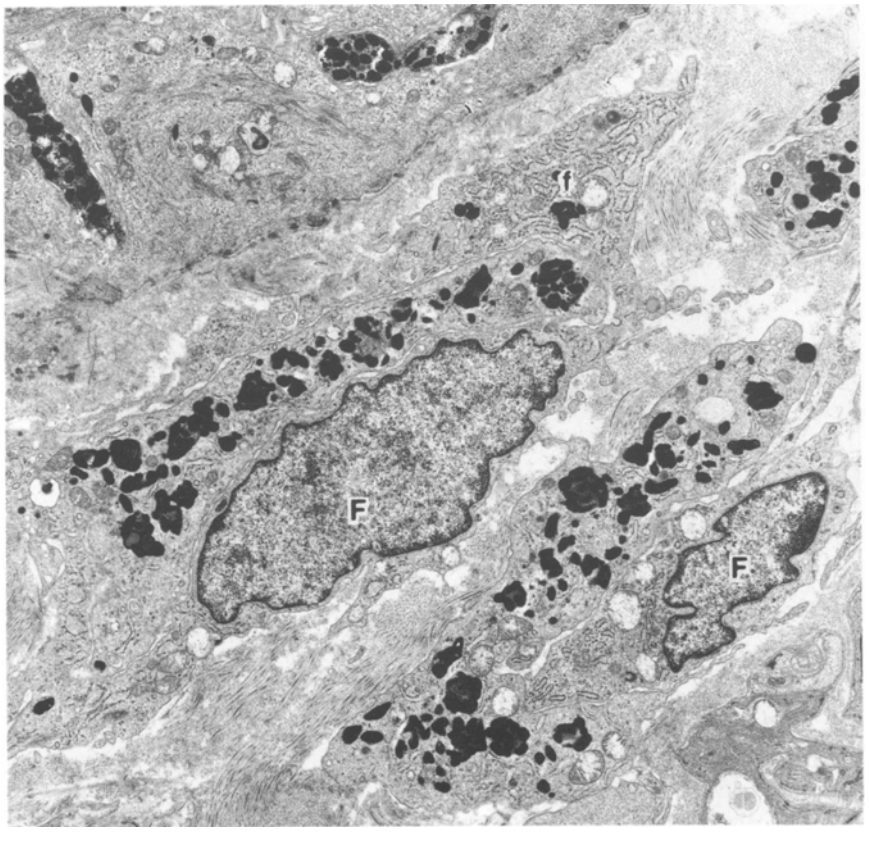

Fig. 6. TEM of melanophages and fibroblasts in tunical propria. Spindle-celled fibroblasts (*F*) and fibroblasts process (*f*) containing rough endoplasmic reticulum and secondary melanosomes. Case 1, ×4600

analysis these lesions may be, and have been referred to, as oesophageal melanosis by analogy with melanoses of other parts of the gastrointestinal tract, notably the duodenum and colon (Ghadially and Parry 1966; Bisordi and Kleinman 1976; Sharp et al. 1980). The term, however, seems to be an imprecise one since, as pointed out by Dumas et al. (1990), it encompasses melanotic hyper-pigmentation with or without melanocyte proliferation, quite apart from its usage for lesions which are pigmented with substances other than melanin. Histopathological studies, such as the one presented here, have the value of distinguishing those melanoses where there is also a cellular proliferation of melanocytes, and in such cases the term *oesophageal melanocytosis* is preferred; the term also carries the advantage of clarifying the nature of the pigment.

There have been few histopathological studies of oesophageal melanosis or melanocytosis (de la Pava et al. 1963; Kimball 1978; Kreuser 1979; Guzman et al. 1989; Kanavaros et al. 1989; Dumas et al. 1990; Ohashi et al. 1990). Of these, Kreuser (1979), Guzman et al. (1985), Kanavaros et al. (1989), Dumas et al. (1990) and Ohashi et al. (1990) have emphasised, as we have done in our study, the nature of the lesion as a cellular proliferation, thereby justifying the term *oesophageal melanocytosis*. The oesophageal pseudomelanosis of Kimball (1978) which is due to a non-melanin type of pigment is therefore unrelated biologically to our cases or to those described by Dumas et al. (1990). The studies by de la Pava et al. (1963), Kreuser (1979), Guzman et al. (1989) and Kanavaros et al. (1989) have recorded the condition in association with malignant melanoma, while Ohashi et al. (1990) documented it in oesophageal epithelium adjacent to carcinoma. Our cases, like that of Dumas et al. (1990), have been found in isolation from other histopathological conditions or lesions.

The synchronous or metachronous association of oesophageal melanocytosis with malignant melanoma (Kreuser 1979; Guzman et al. 1989; Kanavaros et al. 1989) has raised the question of its being a precursor lesion. In particular, Guzman et al. (1989) reported a malignant melanoma-associated melanosis in which there was melanocytic atypia extending through to melanoma in situ. The finding of melanocytosis in relation to oesophageal carcinoma (Ohashi et al. 1990) has not been interpreted as arguing against this idea. The most vigorous melanocytosis was found in the lower part of the oesophagus, a preferential site for malignant melanoma. The data suggest, however, that some melanocytoses, or some components of any given melanocytosis, may be due to stimuli arising from local lesions unrelated histogenetically to the melanocyte. Our data may have some relevance in this context, in that in both light and electron microscopy the proliferating melanocytes seem to lack atypia and appear to be essentially of an untransformed phenotype. Ultrastructurally they show no cytopathological features such as extreme nuclear irregularity or the aberrant melanosomes seen, for example, in the precancerous cutaneous melanosis studied by Anton-Lamprecht and colleagues (1971, 1972) or in dysplastic melanocytic naevi (Rhodes et al. 1988). Further techniques, including more sensitive probes of transformation, such as flow cytometry to elucidate ploidy levels, investigations into the expression of cytoadhesins (McGregor et al. 1989) or recognition of antigen by the antibody HMB45 (which is reported to be absent from normal adult melanocytes; Sun et al. 1990) may help to clarify the nature of these melanocytes[1]. In comparison with pigmented lesions and melanocyte proliferations of the skin, the oesophageal melanocytoses studied here probably correspond most closely to lentigo (MacKie 1984).

This ultrastructural study – the first of an oesophageal melanocytosis – provides a further parallel with normal skin melanocytes in the context of the mechanism of pigmentation of surrounding cells. The present study offers no new insights into the means by which pigment finds itself in the tunica propria. However, the transfer of pigment to adjacent keratinocytes in the oesophagus seems likely to occur by a mechanism similar to that in the skin (Fitzpatrick and Breathnach 1963) by which melanin-containing melanocyte dendrites or free melanosomes in the extracellular matrix are ingested by keratinocytes. This mechanism is supported by our observations, which show an exceptionally close intimacy between melanocyte dendrites and the keratinocyte cell bodies through which they ramify.

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