Virchows Archiv A Pathol Anat (1990) 417:137–143

Virchows Archiv A
Pathological Anatomy and Histopathology

Melanocytes and melanosis of the oesophagus in Japanese subjects – analysis of factors effecting their increase

Kenichi Ohashi [1], Yo Kato [2], Jun Kanno [1], and Tsutomu Kasuga [1]

[1] 2nd Department of Pathology, Faculty of Medicine, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113, Japan
[2] Department of Pathology, Cancer Institute, 1–37-1, Kami-Ikebukuro, Toshima-ku, Tokyo 170, Japan

Received October 30, 1989 / Received after revision January 12, 1990 / Accepted January 15, 1990

Summary. Normal oesophagus specimens taken from 65 autopsy cases and surgical specimens from 127 oesophageal carcinoma cases were examined histopathologically to determine melanocyte incidence and distribution. Melanocytes were found in the epithelio-stromal junction in 7.7% of normal oesophagus specimens examined at autopsy, and in 29.9% of surgical cases with oesophageal carcinoma. Positive specimens in the latter groups, especially from pre-operatively irradiated individuals, showed a more remarkable increase of melanocytes than was evident in any of the normal oesophageal samples. There were no significant differences in incidence between males and females, or between age groups. In cases where the cancer invaded into deeper stroma, the melanocytes were mainly observed in the normal epithelium around the carcinomas. Epithelial and stromal elements of the melanotic mucosa commonly showed hyperplastic changes such as acanthosis or basal cell hyperplasia, and chronic oesophagitis. Melanocytes were observed most commonly in the lower part of the oesophagus, the site where malignant melanoma of the oesophagus, most often originates. These results strongly suggest that the melanocyte increase observed in areas of hyperplastic epithelium and chronic oesophagitis may play an important role as a precursor lesion for malignant melanoma in the oesophagus.

Key words: Melanocytes – Melanoma – Oesophagus – Oesophageal neoplasms – Melanosis

Introduction

One of the major characteristics of malignant melanoma in Japan is that it develops at sites other than the skin with a relatively high frequency. In Western countries, malignant melanoma of mucosal origin comprises about 2% of the total melanomas observed. In Japan, however, 21.7–33.5% of melanomas have been reported to originate from mucosae (Mori 1979; Kasuga 1987). However, the histogenesis of mucosal melanomas has not been studied in as great detail as skin melanomas.

Malignant melanoma of the oesophagus is a relatively rare tumour, about 100 cases having been described in the literature up to the present date (Baur 1906; Raven and Dawson 1964; Bullock et al. 1953; Piccone et al. 1970; Kreuser 1979; DiConstanzo and Urmacher 1987). In histogenetic studies, some authors have demonstrated melanocytes in normal oesophageal epithelium, but at a very low incidence (2.5–8%) and with the numbers of cells in each melanotic lesion being very small (LaPava et al. 1963; Shibata 1973; Tateishi et al. 1974). There remains some doubt as to whether uncommonly encountered melanocytes in the oesophagus are really the precursor cell for malignant melanomas.

The purpose of the present study was first to confirm the incidence and distribution of melanocytes using normal oesophagus speicmens and surgical specimens from oesophageal carcinoma. The second aim was to clarify how host factors such as age and sex, local factors such as location in the oesophagus, existence of carcinoma, degree of carcinoma invasion into adjacent tissues, and histological changes in the epithelium and stroma might be related to occurrence of melanocytes. The mechanism of melanocyte increase and histogenesis of malignant melanoma are discussed briefly and compared with those in the skin.

Materials and methods

The normal oesophagus from 65 non-selected cases obtained at autopsy, performed at Japan Red Cross Medical Center and Tokyo Medical and Dental University Hospitals, was taken. These cases comprised various age groups from infants to the aged, who died without malignant lesions or ulceration in the oesophagus. Specimens were all removed less than 10 h after death.

As surgical specimens of oesophageal carcinoma, 127 non-selected cases from operation at the Cancer Institute or Tokyo Medical and Dental University Hospital were used for study. Of these

Offprint requests to: K. Ohashi

127 cases, 53 were preoperatively irradiated in the range of 1800 rad-8800 rad/patient.

After fixation in phosphate-buffered formalin solution, each oesophagus was serially cut transversely at 7–8 mm. The cut tissues were then routinely processed through appropriate graded solutions to obtain paraffin blocks, and sectioned at 4 μm. For screening of oesophageal melanocytes, four blocks were cut from the total length of each normal oesophagus, and for surgical specimens with oesophageal carcinoma four blocks around the carcinoma were taken. All blocks which were positive for melanocytes were examined to clarify the distribution of melanocytes and their relationship to surrounding tissues. In 20 of the normal cases, four blocks of fresh tissue were also obtained, frozen immediately and cut serially at 8 μm in a cryostat for the dopa reaction and other stainings.

Haematoxylin and eosin (H&E) and Masson Fontana (MF)-argentaffin staining were performed to confirm the melanocytes histologically. The influence of up to 10 h delay in fixation on the stainability of melanocytes had been previously examined; fresh skin and oral mucosa specimens were taken of operation, and each specimen was cut in half. One half was fixed immediately, and the other half was incubated in 4° C saline for 10 h and fixed. Stainability and count numbers of melanocyte after 10 h delay were well-preserved in comparison with immediately fixed halves. Up to 10 h delay in fixation of autopsy specimens in the present study was therefore not considered to influence the number of stainable melanocytes.

Frozen sections were stained serially by H&E, MF and the dopa reaction. For the latter each section was dried, fixed in acetone for 5 min, rinsed and washed in phosphate-buffered saline at pH 7.4, and then incubated in 0.1% L-3,4-dihydroxyphenylalanine (L-dopa; Sigma, St. Louis, Mo., USA) buffered with pH 7.4 phosphate saline solution at 4° C for 4 h. Control specimens positive for MF staining and the dopa reaction were provided by skin obtained from the same patients.

Serial sections, the first and second being stained with MF and dopa solutions, respectively, were used to confirm positivity in each case for the same cell. Cells positive for MF staining were also examined electron microscopically to confirm the presence of melanosomes and premelanosomes. For electron microscopical examination, blocks positive for melanocytes were deparaffinized, refixed in 2.5% osmium tetraoxide and embedded in epoxy resin. Electron microscopical examination was performed to exclude the possibilities of confusion with melanin-laden macrophages, neuroendocrine cells and Langerhans cells, which can not be accurately differentiated from melanocytes histologically.

Results

Melanocytes with fine brown granules which were positive for the dopa reaction and MF staining were found in the basal or parabasal layers of oesophageal epithelium (Fig. 1). Melanin-laden cells with coarse brown granules in the submucosal layer were confirmed to be melanophages by detailed examination. Cells of oval, spindle or dendritic (so-called activated melanocyte) shape, with typical extension of dendrites along the basal membrane, were not observed in the submucosal layer or around the oesophageal glands. Melanocytes with dendrites were located on the basal membrane, but some dendrites also extended into the stroma below the basal membrane. None of the cases was associated with detectable pigmented areas macroscopically.

Electron microscopically, melanosomes and premelanosomes confirmed the melanocyte phenotype. Many dendrite processes were observed to extend into intercellular spaces between squamous epithelial cells, and melanosome transference to epithelial cells was apparent (Fig. 2).

The overall incidences of melanocytes and sex distribution are summarized in Table 1. The incidence of melanocytes in surgical cases with oesophageal carcinoma, especially of irradiated cases, was significantly higher than that in autopsied normal oesophagus samples.

Males appeared to have melanocyte increase more frequently than females in normal autopsy cases. In surgical specimens, the ratio of male to female incidence was 1:1.2 with no statistically significant difference.

Positive melanocyte cases were divided into three groups as follows. Grade 1 represents slight melanocyte

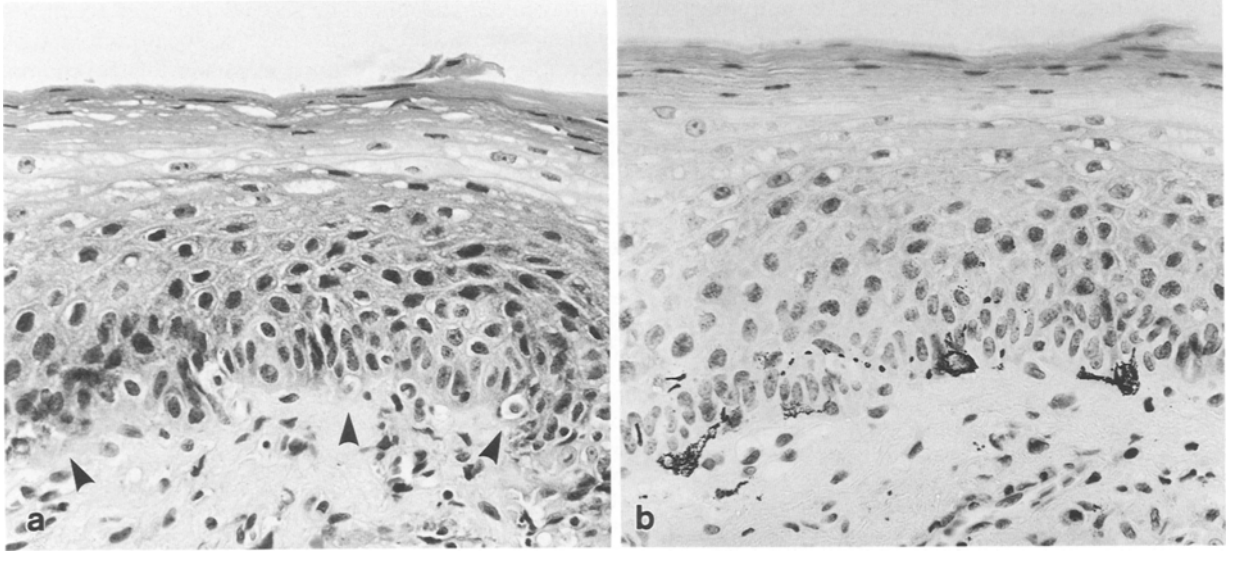

Fig. 1a, b. Semiserial sections through the same locus. **a** Melanocytes (arrows) demonstrate clear cytoplasm with fine brown granules. H&E, ×200. **b** Dendritic appearance of melanocytes in the basal layer. Masson-Fontana argentaffin, ×200

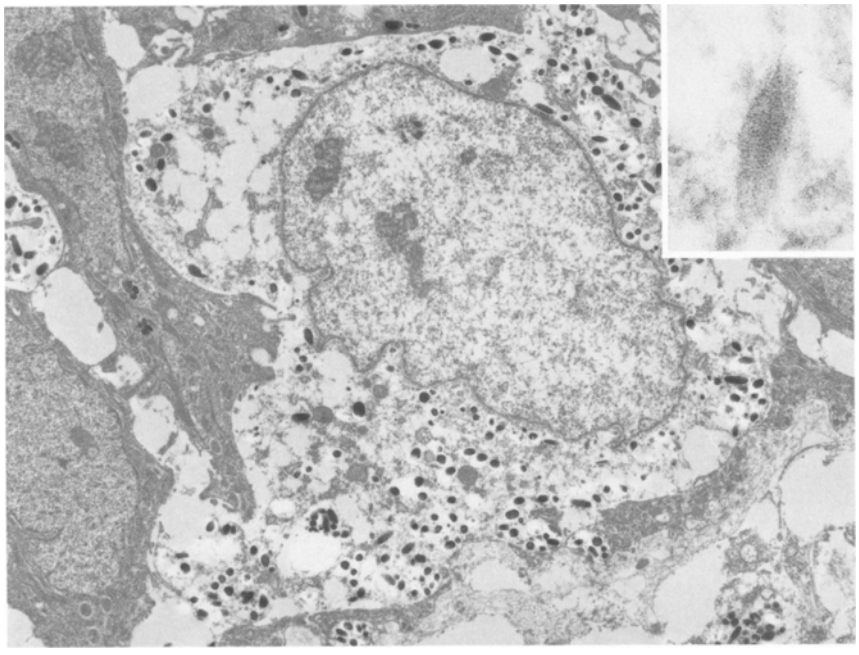

Fig. 2. Electron micrograph of an oesophageal melanocyte in the basal layer. Dendritic processes extending to intercellular spaces and also below the basal membrane. Melanosomes are apparently transfered to adjacent epithelial cells. ×4000. Premelanosome (*inset*) with an elliptical structure and a striated core observed in the melanocyte. ×35000

increase with 1–20 melanocytes per slide section; grade 2 indicates moderate increase with 21–100 melanocytes; and grade 3 a marked increase with more than 101 melanocytes (Fig. 3). The results are presented in Table 2. In autopsied normal oesophagus, all 5 positive cases were limited to grade 1. In surgical specimens with oesophageal carcinoma, grade 2 plus grade 3 (moderate and marked increases) accounted for about half of all positive cases. This shift was most remarkable in preoperatively irradiated cases.

In autopsied normal oesophagus, melanocytes were found in adult cases over 51 years but not in cases less than 50 years old. In surgical specimens with oesopha-

Table 1. Overall incidence of melanocytes in specimens

	No. of cases with melanocytes/ oesophagus specimens (%)		
	Male	Female	Total
Autopsy specimens without abnormality	4/38 (10.5)	1/27 (3.7)	5/65 (7.7)*
Surgical specimens with oesophageal carcinoma	28/97 (28.9)	10/30 (33.3)	38/127 (29.9)*
Non-irradiated	15/58 (25.9)	5/16 (31.3)	20/74 (27.0)
Irradiated	13/39 (33.3)	5/14 (35.7)	18/53 (34.0)

* $P < 0.01$

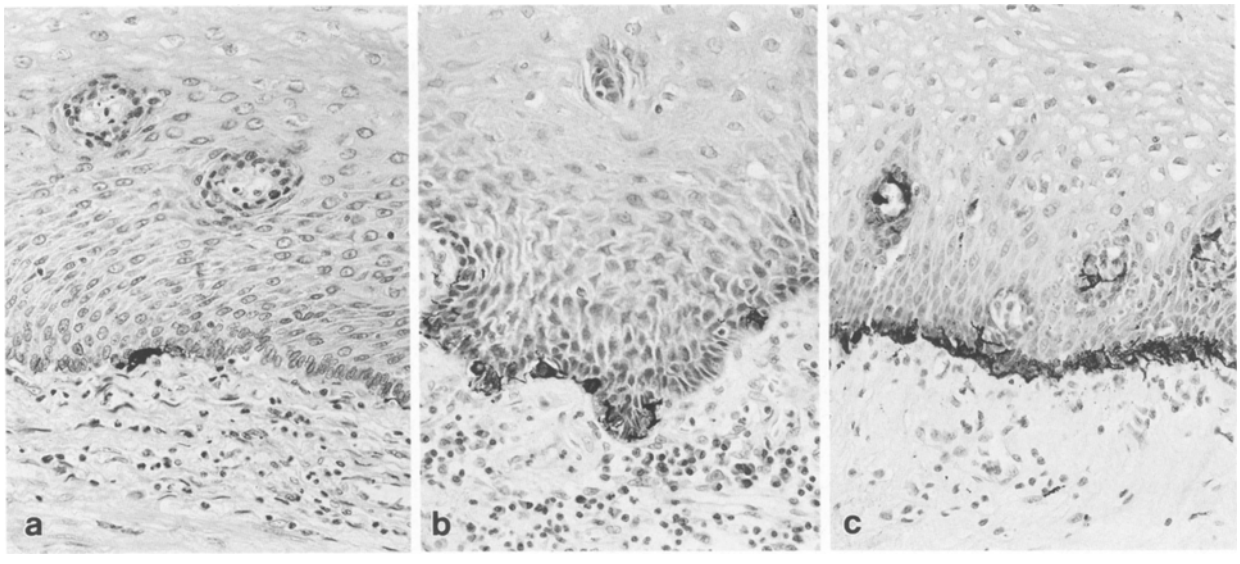

Fig. 3a–c. Three degrees of melanocyte occurrence in the oesophagus. **a** Slight, **b** moderate, **c** severe increase in numbers. Masson-Fontana argentaffin, ×100

Table 2. Degree of melanocyte increase in surgical and autopsy specimens

Grade	Surgical specimens with oesophageal carcinoma No. of cases (%)			Autopsy specimens without abnormality No. of cases (%)
	Non-irradiated	Irradiated	Total	
Grade 1	9 (12.1)	8 (15.1)	17 (13.4)	5 (7.7)
Grade 2	10 (7.4)	6 (11.3)	16 (12.6)	0 (0)
Grade 3	1 (1.4)	4 (7.5)	5 (3.9)	0 (0)
Toal	20 (27.0)	18 (34.0)	38 (29.9)	5 (7.7)

Grade 1: 1–20 melanocytes/slide
Grade 2: 21–100 melanocytes/slide
Grade 3: >101 melanocytes/slide

Table 3. Incidence of melanocytes in the oesophagus, relative to examined site (127 surgical specimens with carcinoma)

Examined site	No. of cases with melanocytes/ oesophgus specimens (%)		
	Non-irradiated	Irradiated	total
Upper	1/5 (20)	2/10 (20)	3/15 (20)
Middle	11/42 (26.8)	9/30 (29.0)	20/72 (27.8)
Lower	8/27 (29.6)	5/13 (38.5)	13/40 (32.5)
Total	20/74 (27.0)	18/53 (34.0)	38/127 (29.9)

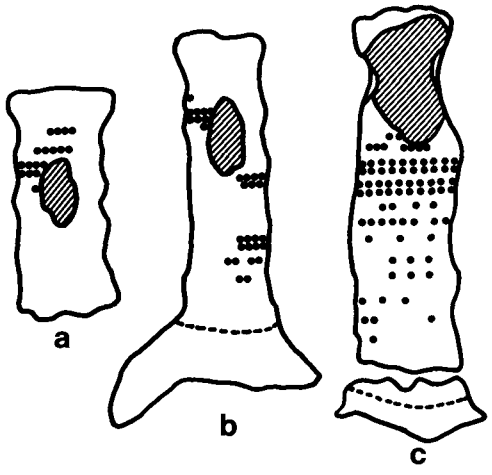

Fig. 4a–c. Diagrams of oesophageal melanocyte distribution, compared with carcinoma location. One dot represents approximately 10 melanocytes/slide section and *shaded area* shows extent of carcinoma development. **a** Melanocytes are localized in a mottled pattern a little cranially to the carcinoma. **b** Melanocytes are distributed in localized patterns around the carcinoma and also in an area caudally distant from the malignancy. **c** Melanocytes are diffusely distributed thoughout the entire oesophagus caudal to the lesion

geal carcinoma, neither significant differences between age groups over 31 years old nor proportional increase with aging were observed.

To clarify the relationship between the oesophageal site of melanocytes and their incidence at autopsy, a

Table 4. Histological findings in melanotic mucosa (21 cases with moderate or severe melanocyte increase)

	No. of cases (%)	
Basal cell hyperplasia	+	11/21 (52.4)
	++	10/21 (47.6)
	+++	0/21 (0)
	Total	21/21 (100)
Acanthosis	+	6/21 (28.6)
	++	7/21 (33.3)
	+++	2/21 (9.5)
	Total	15/21 (71.4)
Chronic oesophagitis	+	8/21 (38.1)
	++	4/21 (19.0)
	+++	0/21 (0)
	Total	12/21 (57.1)
Submucosal fibrosis	+	5/21 (23.8)
	++	1/21 (4.8)
	+++	0/21 (0)
	Total	6/21 (28.6)
Atrophy	+	0/21 (0)
	++	2/21 (9.5)
	+++	0/21 (0)
	Total	2/21 (9.5)
Submucosal invasion of carcinoma	+	2/21 (9.5)
	++	0/21 (0)
	+++	0/21 (0)
	Total	2/21 (9.5)
Epithelial dysplasia	+	1/21 (4.8)
	++	0/21 (0)
	+++	0/21 (0)
	Total	1/21 (4.8)
Ulcerative oesophagitis	+	0/21 (0)
	++	1/21 (4.8)
	+++	0/21 (0)
	Total	1/21 (4.8)

+ Slight change; ++ moderate change; +++ severe change

total of four blocks, one each from the upper part, the upper middle part, the lower middle part and the lower part of the oesophagus were chosen. In the total of 5 cases positive for melanocytes, 2 cases showed melanocytes only in the lower middle part, 2 cases showed only in the lower part, and 1 case showed both in the lower middle and lower parts. No melanocytes were found in the upper or upper middle parts of the oesophagus.

In surgical cases, a total of four blocks from around the carcinoma were chosen in each case. Comparing with localization of the carcinoma, the surgical cases were divided into three groups by site, the upper, middle and the lower oesophagus.

In both autopsied normal oesophagus and in surgical cases with oesophageal carcinoma, the lower part was the most common site for melanocytes, the middle part

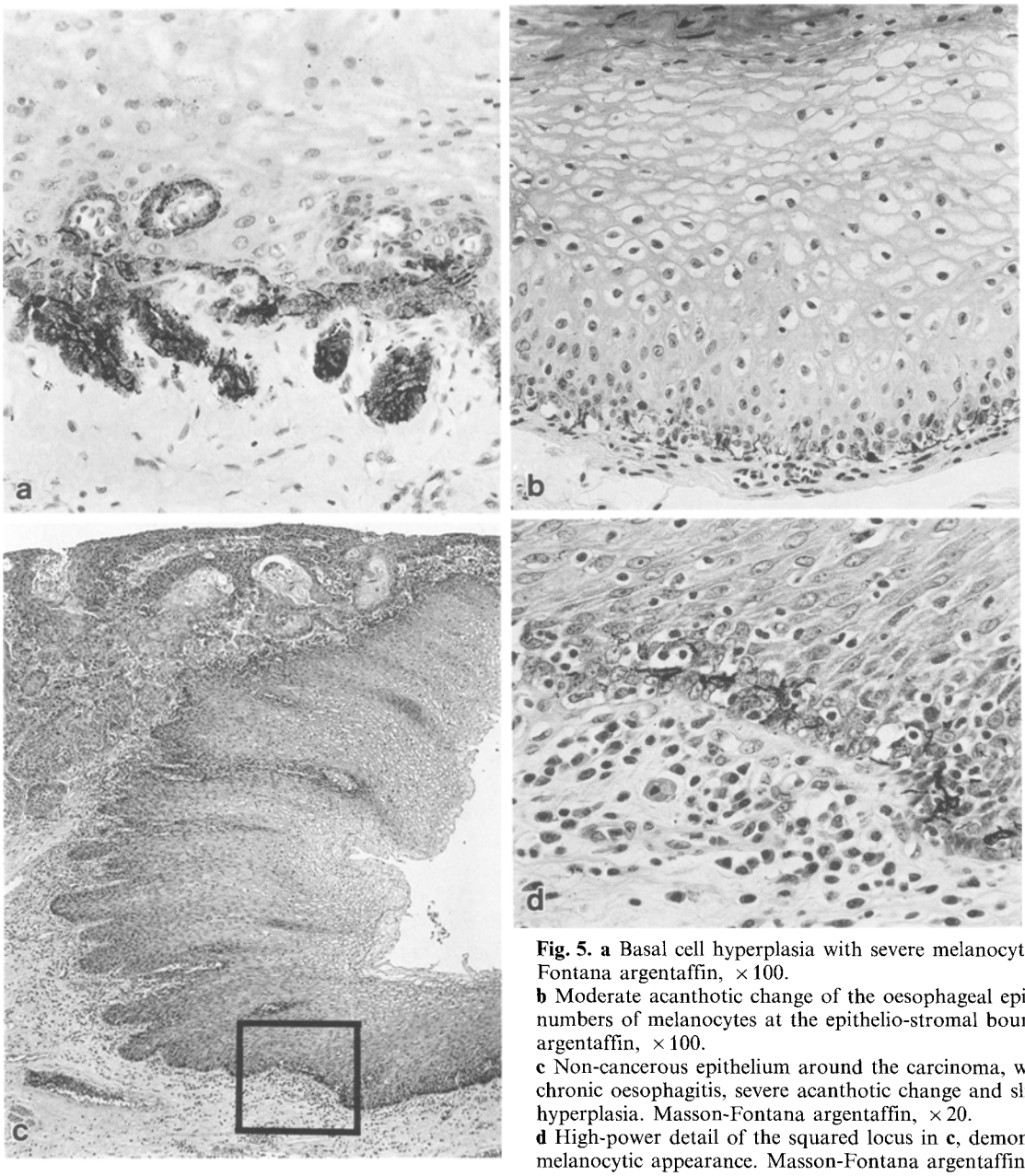

Fig. 5. **a** Basal cell hyperplasia with severe melanocytic appearance. Masson-Fontana argentaffin, ×100.
b Moderate acanthotic change of the oesophageal epithelium with increased numbers of melanocytes at the epithelio-stromal boundary. Masson-Fontana argentaffin, ×100.
c Non-cancerous epithelium around the carcinoma, which shows moderate chronic oesophagitis, severe acanthotic change and slight basal cell hyperplasia. Masson-Fontana argentaffin, ×20.
d High-power detail of the squared locus in **c**, demonstrating moderate melanocytic appearance. Masson-Fontana argentaffin, ×200

being the second most common site. In surgical specimens, irradiation was associated with a small increase of incidence in the lower part, although no significant difference was evident (Table 3).

Screening for oesophageal melanocytes revealed 21 surgical specimen cases showing grade 2 and 3 melanocyte increase. In these cases all blocks with melanocytes were examined with respect to their location, three examples being illustrated in Fig. 4.

Summarizing the results concerning relationship between distribution of melanocytes and carcinoma, 17 cases (81%) showed localized patterns as with cases (a) and (b) in Fig. 4, and 4 cases (19%) demonstrated a diffuse pattern as with case (c). In 19 cases (90.5%), melanocytes were distributed mainly in the areas adjacent to the carcinoma, and in only 2 cases (9.5%) were they predominantly distant from the carcinoma. As for cranial-caudal site predominance, in 12 cases (57.1%) the degree of distribution density was more remarkable on the cranial than the caudal side, relative to the tumour.

The relationship between depth of cancerous invasion and incidence of melanocytes was examined. In cases where invasion of carcinoma was limited to mucosal and submucosal layers, the incidence of melanocytes was 10.5%, that is almost equal to the incidence in autopsied normal oesophagus. However, in cases where invasion extended to the muscular layer and adventitia, the incidences of melanocyte were 30% and 34.1%, respectively.

In 21 surgical specimen cases which showed grade 2 or 3 melanosis, changes of epithelial and stromal elements were examined, the results being summarized in Table 4. The frequencies of basal cell hyperplasia (Fig. 5a), acanthosis (Fig. 5b) and chronic esophagitis (Fig. 5c) were relatively high. Atrophy of epithelium, ulcerative oesophagitis, and submucosal fibrosis were rarely observed. No clear increase in degree of melanocyte development was evident with increase in any of these changes. Although melanocytes were mainly distributed around carcinomas (Fig. 5c, d), they were rarely observed in intraepithelial invasion areas of cancer or in dysplastic epithelium covering submucosally invading cancer.

Discussion

In general, malignant melanoma of the skin has been considered to originate from melanocytes or nevus cells, both of which are assumed to be derived from the neural crest. Malignant melanoma has therefore been proposed as classifiable into two broad groups, these originating from melanocytes and these from nevus cells (Clark et al. 1979; Paul 1984). The propriety of this classification is not yet confirmable cytologically and histologically, but it is agreed that malignant melanoma develops from melanocytes located in the epidermal-dermal junction. Malignant melanomas of mucosal origin are similarly assumed to originate from melanocytes or nevus cells located in the mucosal-stromal junction.

Recently it was demonstrated that the melanocytic tumours of hamsters and mice induced experimentally by consecutive 9,8-dimethyl-1,2-benzanthracene(DMBA) and 12-o-tetradecanoylphorbol-13-acetate (TPA) treatments originate from perifollicular dermal melanocytes or perifollicular Schwann cells (Nakai and Rappaport 1963; Kanno et al. 1986, 1987). In human nasal mucosa, it has been reported that the melanocytes, which are mainly distributed in the submucosal stroma, also act as the parent population for nasal malignant melanoma development (Uehara et al. 1987). These reports suggest that some malignant melanomas, especially those arising in mucosae, may originate not only from junctional melanocytes or nevus cells but also from submucosal melanocytes, nevus cells or peripheral nervous cells such as Schwann cells (Kanno et al. 1987; Cramer 1984).

Malignant melanomas involving the oesophagus were originally considered to be metastases from other organs, because melanocyte or nevus mother cells could not be demonstrated (Becker 1927; Johnson 1910; Stout and Lattes 1957). Since 1963, when LaPava et al. first demonstrated the existence of melanocytes in oesophageal epithelium, primary malignant melanoma of the oesophagus has been acknowledged. LaPava et al. examined 100 cases of normal oesophagus at autopsy and found intraepithelial melanocytes in 4 cases. In 2 cases equivocal melanocytes were located in the upper third of the oesophagus, and in the other 2 cases melanocytes were located in the middle third. Later Shibata (1973) and Tateishi et al. (1974) performed similar examinations using Japanese autopsy subjects, and observed melanocytes in 2.5% and 8% of the cases, respectively, similar to the 7.7% of the present study. Meanwhile, cases of primary malignant melanoma of the oesophagus accompanied by marked melanosis around the tumours had been reported (Piccone et al. 1970), and this oesophageal tumour type was understood to originate from intraepithelial melanocytes following melanocyte increase. The melanocyte increase or melanosis was emphasized as an important precursor lesion (Raven and Dawson 1964; Kreuser 1979).

In the present series of studies, striking differences in melanocyte increase between normal and carcinoma subjects were shown. It has also been demonstrated that it is the existence of carcinoma, and not the sex, aging or irradiation factors, which plays an important role in the melanocyte increase. It is unclear whether this can be explained by the existence of carcinomas themselves or whether some other factors induced by tumours result in the remarkable increase in melanocyte populations.

Several mechanisms have been proposed to explain the increase in epidermal melanocytes: the proliferation of pre-existing melanocytes,; activation of resting melanocytes following activation of melanogenesis; migration of dermal melanocytes; and differentiation of peripheral neural cells (such as Schwann cells) to melanocytes. Some investigators have shown that melanocytes can be increased in number experimentally using ultraviolet irradiation and chemicals (Miyazaki et al. 1974; Rosdahl and Szabo 1978). In recent years it has been shown that several growth factors produced by fibroblasts and keratinocytes can activate melanocytes in vitro (Haraban et al. 1987; Gordon et al. 1989).

In the oesophagus, melanocytes were distributed like islands and/or macules in normal-appearing epithelium, mainly adjacent to associated carcinomas. Hyperplastic changes such as basal cell hyperplasia and acanthosis and chronic oesophagitis were closely related to the increase of melanocytes. These findings strongly suggest the possibility of a direct link between tumour promotion or proliferation and melanocyte appearance.

In seborrhoeic keratosis, an epidermal hyperplastic disease of the skin, it has been reported that the numbers of melanocytes increase (Tanaka 1988). It may be possible that some common mechanisms, such as local growth factors, influence the oesophageal melanocytes. The mitotic capacity of oesophageal melanocytes and differentiation of subepithelial totipotential cells were not fully examined in this study; we need to develop an analytical method for investigating these possibilities.

It was of interest that the lower part of the oesophagus was the most common site for oesophageal melanocytes. As a possible explanation the lower part of the oesophagus is subject to chronic stimuli (such as reflux of gastric juice) resulting in hyperplastic epithelial changes or chronic oesophagitis. This may lead to increased melanocytes. In the majority of case reports concerning primary malignant melanoma of the oesophagus, the site of the lesion was the lower region, followed by the middle part (Kreuser 1979). The fact that melano-

cytes commonly proliferate in the lower oesophageal regions is consistent with the sites where malignant melanoma most often develops.

In conclusion, the present results demonstrate that the melanocytes which exist at the epithelio-stromal junction may be activated by certain factors in association with hyperplastic changes in the epithelium and with chronic oesophagitis. This cell population may be related to development of oesophageal melanosis and malignant melanomas.

Acknowledgments. The authors wish to thank Dr. T. Takemura for her supply of materials, and M. Takeuchi, M. Kobayashi and H. Tsuboi for their technical advice.

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