Acta Dermatovener (Stockholm) 60: 495–501, 1980

METHACYCLINE HYPERPIGMENTATION: A FIVE-YEAR FOLLOW-UP

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Abstract. A five-year follow-up is presented of a unique material of patients who acquired a greyish black hyperpigmentation during long-term methacycline therapy for chronic bronchitis. The melanosis disappeared in cases in which tetracycline therapy was discontinued during the observation period but not in those in wich doxycycline had been substituted. In two autopsy cases pigment deposits were also found in cartilage and atherosclerotic lesions. Overall clinical and microscopic findings suggest a similarity to the pigmentation occurring in iatrogenic ochronosis.

Key words: Methacycline; Tetracyclines; Pigment; Ochronosis

In 1974 (3) we reported on pigment deposits in eyes and light-exposed skin during long-term methacycline therapy. The deposits had induced a greyish black dyschromia, "melanosis Malmö", in 7 out of 250 patients with chronic bronchitis given this type of therapy. The pigment, presumed to be a drug–melanin–calcium complex, was localized to the actinically damaged elastotic tissue. This dermal and extracellular localization of the pigment made its spontaneous disappearance within a reasonable time highly questionable. Therefore, we have now re-examined our patient material 5 years after discontinuing the methacycline treatment. One of the patients died during the follow-up period and an autopsy was performed.

RESULTS

Of the original 7 patients (ref. 3, Table I) 5 were available for a clinical and pathological study 5 years after stopping the methacycline treatment. Patient 1 was deceased (see autopsy below) and patient 7 could not be reached; however, she had only conjunctival deposits at the original examination. In cases 2–6 a punch biopsy was taken at a site corresponding to that in the original study.

Clinical examination

Patient 2. No other tetracycline given. Marked clearing of the dyschromia, only slight residues on sides of forehead.

Patient 3. No other tetracycline given. Marked clearing of the dyschromia, only slight residue on the base of the nose. Residual pigmentation of the conjunctivae suspected, not confirmed, however, by the ophthalmologist (K. Dyster-Aas).

Patient 4. Doxycycline substituted for methacycline and taken continuously, 100 mg a day throughout the 5 years. Hyperpigmentation of skin and conjunctivae similar in intensity to the original findings.

Patient 5. Doxycycline given for a few short periods, otherwise no tetracycline. Clinically, complete disappearance of dyschromia.

Patient 6. Doxycycline substituted for methacycline and taken continuously 100 mg a day throughout the 5 years. Hyperpigmentation of skin similar in intensity to that in the original study but that of conjunctivae disappeared.

Further material. Since the original report 4 more cases of melanosis Malmö have been observed in the material of about 250 bronchitis patients taking methacycline 600 mg/day continuously. A side effect incidence of at least 4% can thus be calculated.

Morphologic examinations

The *original biopsies* demonstrated the following features (Fig. 1–4 A): A dermal lesion with the histologic appearance of severe actinic elastosis. The elastotic fibres had a brownish tint in hematoxylin and eosin staining. They contained numerous small brown granules, easily seen in unstained sections. The granules stained black in the von Kóssa reaction and also to a varying degree in Masson-Fontana's reaction. They were green, like melanin in toluidine blue stain. They appeared to correspond to osmiophilic material situated within "empty" spaces in the elastotic fibres, as seen in the electron

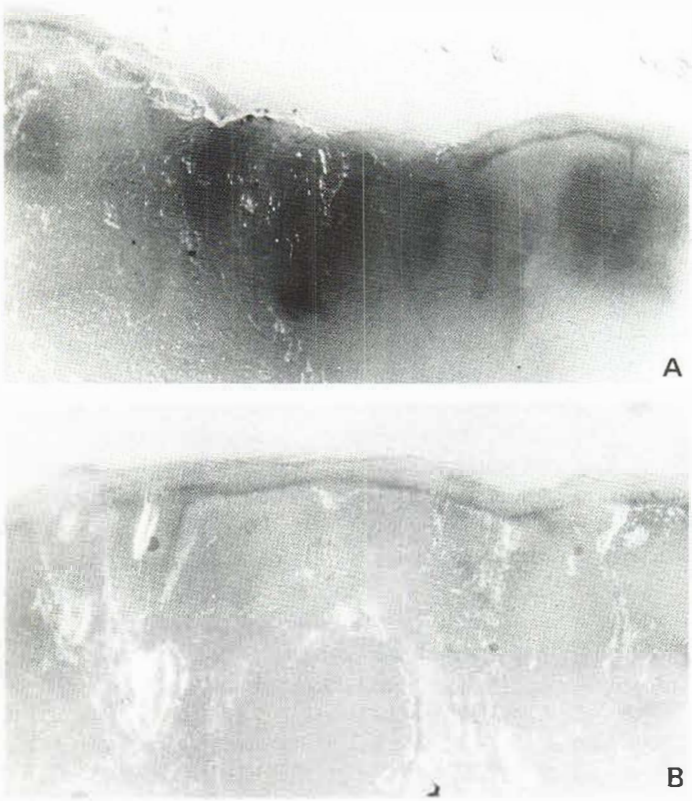

Fig. 1 A and B. Face of paraffin blocks of skin biopsies (A = original, B = follow-up). A demonstrates pigmentation of upper and middle dermis, corresponding to elastotic sites in Fig. 2 A. B demonstrates no such pigmentation. Unstained tissue. Both, ×60.

microscope. The granules occasionally coalesced to form large, angular bodies within elastotic material. Except for this pigment, which was considered the essential finding, there were numerous melanophages in the upper dermis, and possibly a slight epidermal hyperpigmentation. The histologic findings in the conjunctiva will not be dealt with here as they have not been followed up.

The *follow-up skin biopsies* after 5 years were treated with the same method as the original material (3).

Patient 4 was still pigmented and the findings were essentially unchanged histologically. The follow-up biopsy from patient 6 was technically unsuitable as it was superficial and was obtained from the lower leg and contained abundant amounts of iron pigment.

The biopsies from patients 2, 3 and 5 had between themselves similar light microscopical alterations when compared with the original tissue.

There was no change in the degree of elastosis but the brownish colour had disappeared. This was evident in hematoxylin and eosin stained sections but was most strikingly illustrated by comparing the unsectioned tissue in a microscope. Fig. 1 demonstrates the face of the paraffin blocks of the unstained biopsies. The photographs were taken with a combined transmitted and incident illumination. Fig. 2 represents sections of the blocks stained for elastin. It is obvious that the elastosis is similar but that the elastotic sites are pigmented in the original biopsy but not in the follow-up one.

The brown granules seen in the elastosis of the original biopsies had largely disappeared. In hematoxylin and eosin stained sections the fibres had a reticulated appearance like that in other cases of actinic elastosis. However, in the von Kóssa reaction, a moderate number of granules remained (Fig. 3 B). These were not visible in Masson-Fontana's reaction or in toluidine blue.

The melanophages were moderately reduced in number. The epidermal pigment was not noticeably different.

One biopsy only was availabe for follow-up elec-

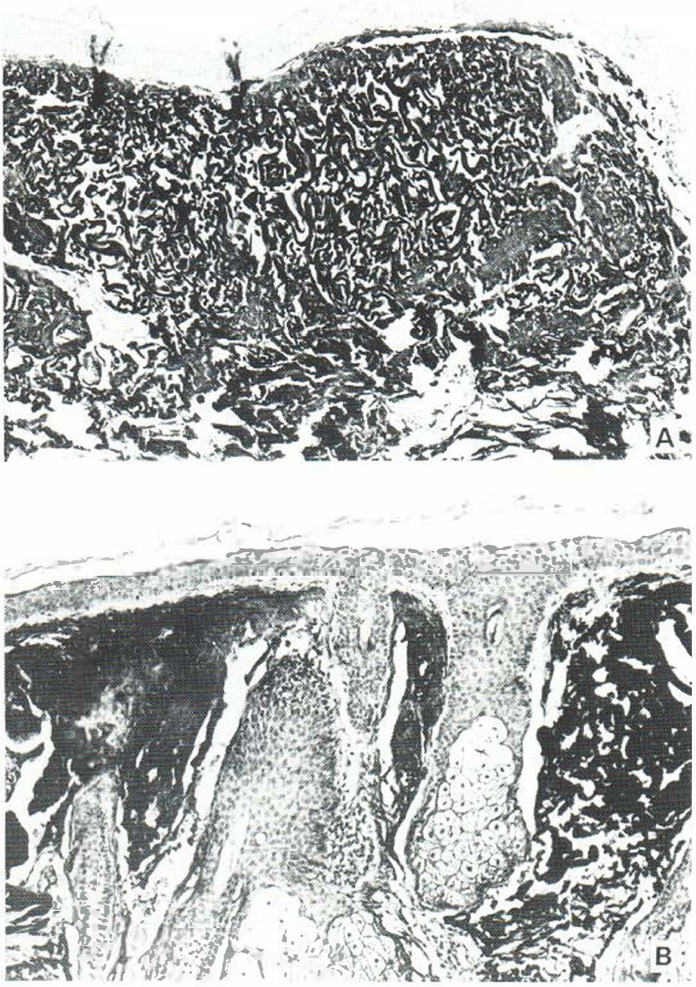

Fig. 2 A and B. Sections of paraffin blocks in Fig. 1 A and B. Elastosis (dark staining areas) is similar. Elastic tissue stain. Both, ×80.

tron microscopy (patient 3). This demonstrated elastotic lesions which appeared even more pronounced than those of the original one in this patient (Fig. 4 A and B). The osmiophilic material within the "empty" spaces of the elastotic material, however, had lost much of its electron density and generally appeared as a flocculent, ill-defined substance of roughly the same density as surrounding degenerative material. An occasional dense granule remained, however (Fig. 4 B), probably precluding a technical explanation for the change.

Autopsy observations. Patient 1 had died of pneumonia 2 years after the original examination. The pigmentation of the dorsa of the hands and of the conjunctiva was apparent to the autopsy pathologist. The patient also had brownish lesions of the lower legs and, unfortunately, only this site was examined histologically, showing hypostatic changes. A moderately severe atherosclerosis of the aorta was found. The atherosclerotic plaques had a dark grey-brown colour, which appeared remarkable to the pathologist. Also the thyroid was said to be peculiarly dark brown.

An additional patient given long-term methacycline treatment but not included in our original material, underwent autopsy at the institute. This 69-year-old man who succumbed to emphysema and pneumonia had taken methacycline continuously

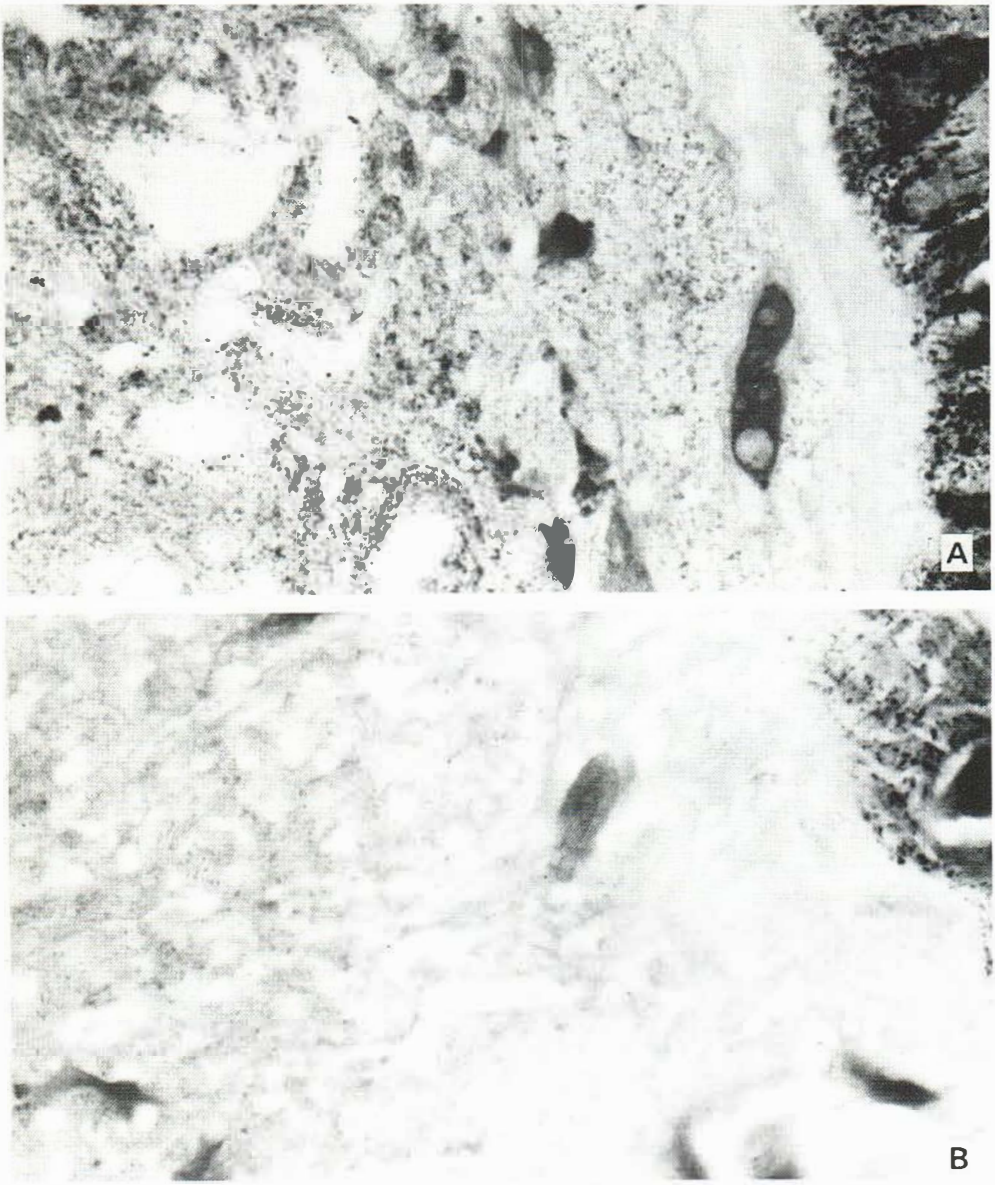

Fig. 3 A and B. High magnification of sections from blocks in Fig. 1 A and B. Elastotic sites in von Kóssa's reaction. A (original) demonstrates heavy granularity. B (follow-up) demonstrates very slight granularity. Basal epidermis to the right. von Kóssa's reaction. Both. ×1300.

for 6 years up to 3 years before his death. Treatment had then been discontinued because of severe pigmentation of the face and of the conjunctiva.

At autopsy the skin pigmentation was no longer visible but slight conjunctival discoloration was noted. In this case another pathologist remarked on a dark brownish colour of the aortic atherosclerotic plaques and of the thyroid gland. Furthermore, the rib cartilage was greyish brown.

DISCUSSION

Methacycline hyperpigmentation has been described from this department only (3), hence the

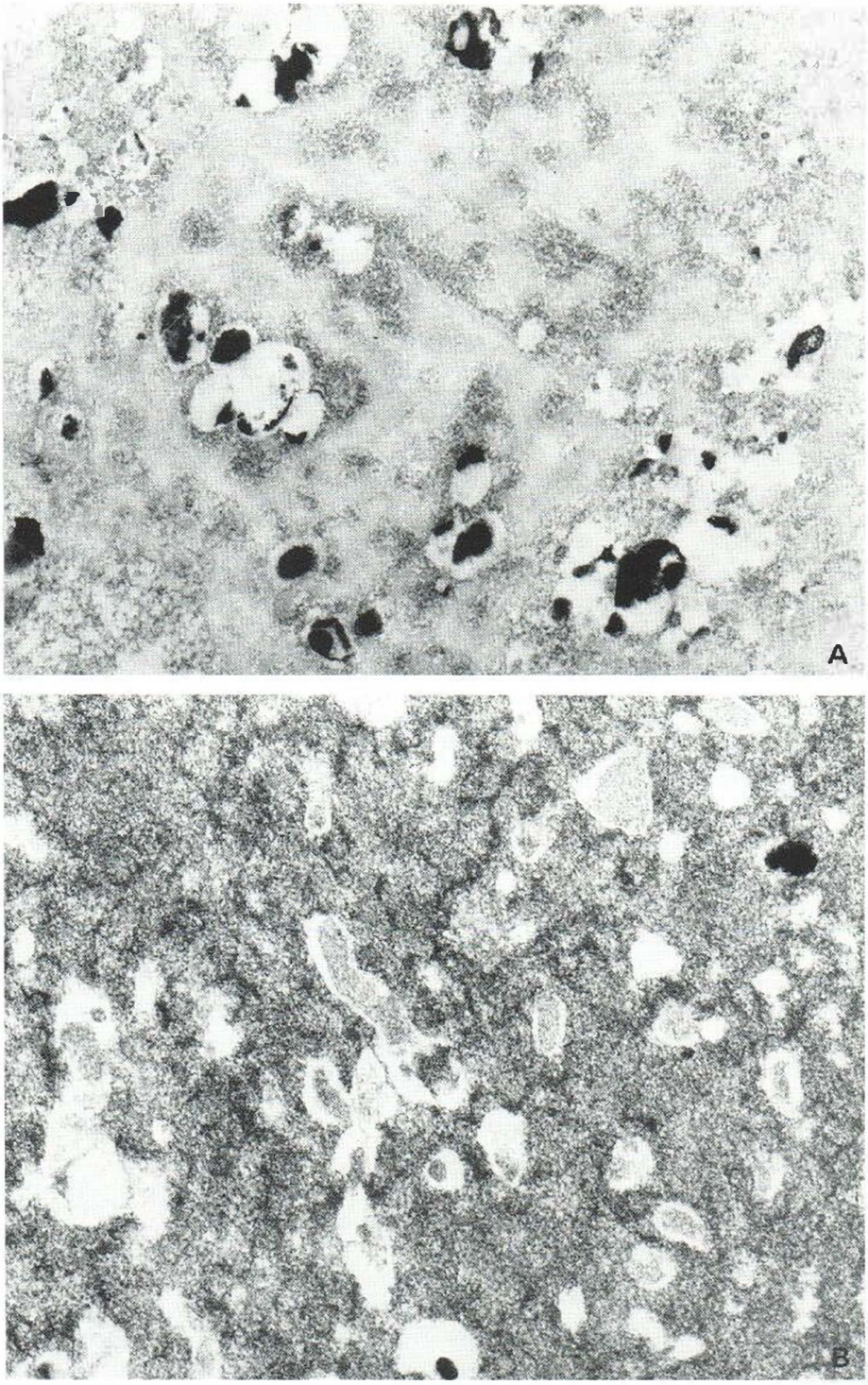

Fig. 4 A and B. Electron micrographs of elastotic fibres in dermis. A (original) demonstrates electron-dense granules in fibre. B (follow-up) demonstrates marked reduction of electron density at same sites. Glutaraldehyde and osmium tetroxide fixation, uranyl acetate and lead citrate. Both, ×34 000.

eponym melanosis Malmö. Tetracyclines are often used in the treatment of chronic bronchitis but the schedule practised in our material, methacycline 600 mg daily and continuously for several years, is probably unique.

We previously suspected that the side effect was not associated with methacycline in particular but might arise following the use of other tetracyclines too. The suspicion was confirmed by the follow-up findings of remaining pigment deposits, clinically and microscopically, in the cases in which doxycycline had been substituted.

In the patients in whom methacycline had been discontinued without substitution the melanosis had almost completely disappeared, portending a favourable prognosis. It was our impression, however, that about 5 years were required for the disappearance. This lengthy recovery time may be due to the extracellular location of the pigment.

The comparison of the original and follow-up biopsies obviously sustained our early impression that the pigment was associated with the elastotic sites. A striking change was the disappearance of the dark granules from the elastotic fibres. On the other hand, reduced numbers of granules were still observed in silver staining according to von Kóssa. Such silver-positive granules are found in all cases of actinic elastosis and were also seen in our initial control specimens. They correspond reasonably well to the "empty" spaces seen electron microscopically, possibly containing lipid. The difference between our biopsies thus appears to be, not the existence of the granules, but that they are heavily pigmented during methacycline therapy. They also appear to stain more intensely in von Kóssa and also with Masson-Fontana and toluidine blue stains. The electron microscopic difference could possibly be incidental, as only one control biopsy was examined. However, it does not appear improbable that the more dense appearence in the original specimen was due to the presence of an associated substance, representing the pathological pigment.

The autopsy findings indicate that the pigment is not confined to light-exposed sites but may combine with internal structures. The atherosclerotic plaques obviously contain both lipids and calcium and thus do not give any clue to which, if any, of those substances is pigment binding. It is difficult to evaluate the colour of the thyroid as it may be quite dark under normal conditions but it seems curious that two experienced autopsy pathologists should regard the findings as remarkable in the two cases described. Other tetracyclines have been reported to discolour the thyroid (1). The discoloration of cartilaginous tissue is remarkable. There is a considerable similarity between methacycline pigmentation and ochronosis, which also includes a pigmentation of cartilage and atherosclerotic lesions (2). Ochronosis has been reported to enhance elastotic lesions and induce pigmentation of degenerating connective tissue structures in elastosis (4, 5).

Ochronosis or an ochronosis-like pigmentation may be caused by exogenous chemicals such as certain antimalarials and phenolic compounds used in dressings (2). This pigment is deposited in the same regions as methacycline pigment, externally as well as internally. Patients with such exogenous ochronosis do not have an increased amount of homogentisic acid in the urine, as do those with hereditary ochronosis (alkaptonuria). Such ochronotic pigment is said to reduce ferricyanide but not silver nitrate. The granules within the elastotic material in our cases do reduce both ferricyanide and silver nitrate (Schmorl's and Masson-Fontana's reactions). A peculiar fact, however, is that the large extracellular pigment clumps seen occasionally in the skin of our patients, and in considerable amounts in the conjunctiva, reduce silver nitrate and ferricyanide only to a slight degree. These pigment clumps correspond exactly to descriptions in the literature of ochronotic pigment, both light- and electron microscopically. On the other hand it is obvious from our biopsies that the large clumps are formed by a coalescence of the smaller granules. Apparently, the ability to reduce ferricyanide and silver nitrate is largely due to the size of the pigment aggregates. To test this hypothesis we stained sections of the aorta from a patient with fatal alkaptonuria. The theory was found to be correct. The very small pigment granules were black in Masson-Fontana, but not so the large clumps.

In this way, the staining characteristics of the pigment are better explained than by our original hypothesis that the silver staining properties were due to the presence of negative ions in conjunction with calcium—especially as the Alizarin red reaction for calcium was negative—or that they were due to melanin. The pigment-binding substance appears to be something else in elastotic material.

Theoretically, the pigment could be produced either by enzyme inactivation giving rise to a true ochronosis of the type seen in the hereditary condi-

tion, or by a drug metabolite with properties similar to the ochronotic pigment being deposited. The lack of urinary homogentisic acid excretion in other drug-induced ochronoses speaks in favour of the latter hypothesis. This could also explain the morphologic dissimilarities. Sweat glands were not involved in our cases. The patients had not noted dark urine. This is natural if there is no excretion in sweat or urine. Possibly the chemical nature of ochronosis-like pigment is somewhat different in cases with different background.

Methacycline pigmentation is most probably an ochronosis-like condition. This is interesting as the patients who were treated with another tetracycline during the observation period were still heavily pigmented. Tetracycline therapy may thus provide a non-toxic model by which to study the development of ochronosis in man.

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Received March 3, 1980

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