British Journal of Dermatology (1982) **106,** 19–25.

A long-term hypopigmentary effect of thorium-X on freckled skin

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Accepted for publication 26 March 1981

SUMMARY

Sixteen years ago, an area on the forearm of a freckled subject was painted with a solution containing 500 esu of thorium-X per ml. Since then, despite much exposure to solar irradiation, there has been a complete absence of freckles from this area, which stands out in marked contrast to the surrounding skin. Biopsy of the skin from the area was examined by the Dopa technique and by electron microscopy. Melanocytes are present in apparently normal numbers, but in a condition of almost complete inactivity. This depression of melanogenesis is difficult to account for either on the basis of an environmental effect, or of intracellular inhibitory mechanisms.

The apparently simple phenomenon of freckling presents a number of fascinating and still unsolved problems in the general context of the genesis and determination of patterns of skin pigmentation, and the control of melanogenesis (Breathnach, 1957, 1958, 1959, 1960). Though probably genetically determined, being linked to the gene for red hair, freckling is not a congenital trait, and only becomes manifest two to three years after birth, apparently in association with exposure to solar ultraviolet irradiation. The distribution of freckles seems to be entirely random, and not associated with any other obviously punctate anatomical or physiological feature of skin. It is not known what determines the original site of appearance of individual freckles, or what controls their expansion into surrounding paler epidermis. It has been established that, paradoxically, freckles contain significantly fewer melanocytes per unit area than adjacent paler epidermis, but they are more active melanogenically, and electron microscopy has revealed that cells in the two situations produce melanosomes of different shape and internal structure (Breathnach & Wyllie, 1964). And that is really about all that is known concerning this human characteristic which is sometimes described as 'abnormal' by authors of dermatological text-books, and which those who manifest it find can be, depending upon circumstances in the eyes of others, both an asset and a cosmetic liability.

Figure 1 shows the forearm of a freckled subject who, 16 years previously, applied a solution of thorium-X to rectangular areas of his forearms in pursuit of an investigation into the then alleged relationship between melanocytes and Langerhans cells (Breathnach, Birbeck & Everall,

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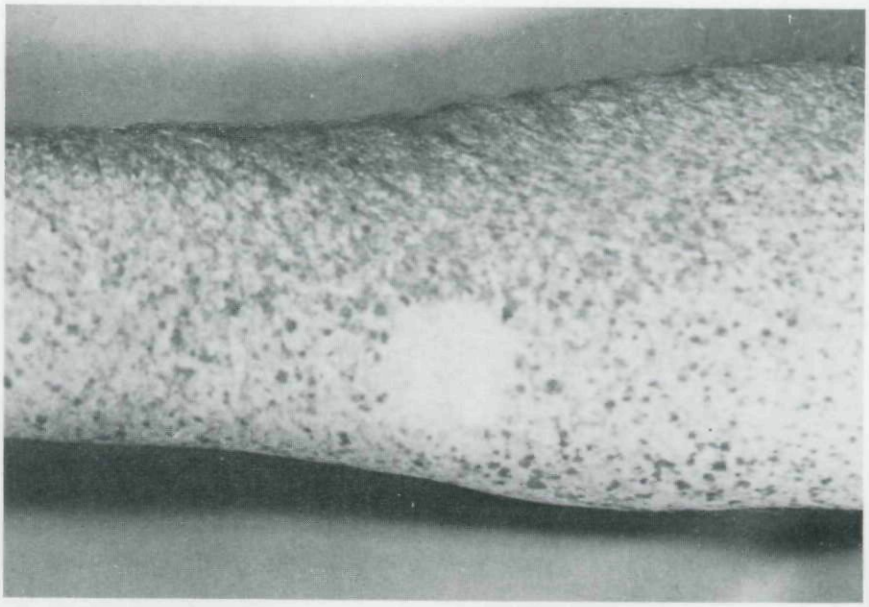

FIGURE 1. Forearm of freckled subject 16 years after application of thorium-X solution to quadrilateral area which since then exhibits no freckles. Photograph taken under UV light.

1963). Following the application of thorium-X (an alcoholic solution containing 500 esu, equivalent to 71 μCi of thorium-X), freckles faded and disappeared from the areas (Fig. 2). Since then, despite much periodic exposure to sunlight, they have remained absent for 16 years and there is also a general hypopigmentation of the areas compared with surrounding paler, i.e. non-freckled, epidermis. This report is concerned with the present condition of any melanocytes that might be present, and of general features of epidermis and dermis. The

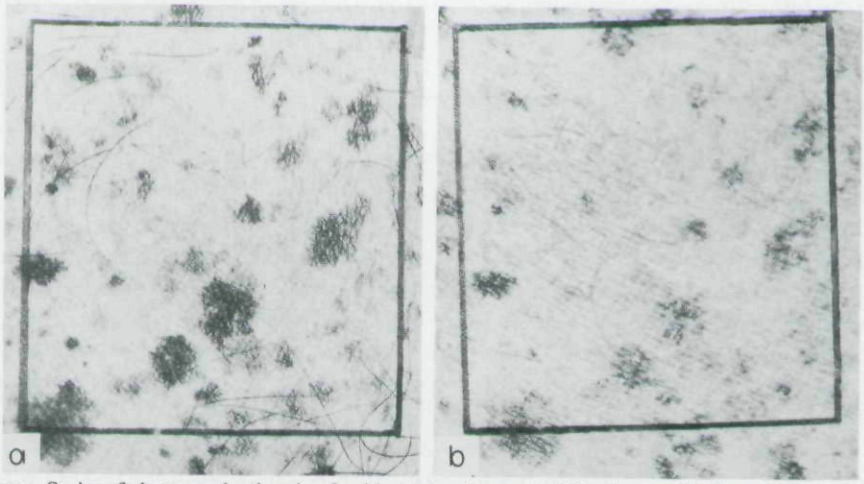

FIGURE 2. Series of photographs showing freckled skin at intervals following application of thorium-X. (a) Before application with the area concerned outlined. (b) Two months following application; many freckles are no longer apparent, and those present are less pigmented.

findings could be of interest in connection with some aspects of the control of melanin pigmentation of the skin.

MATERIALS AND METHODS

Biopsy of skin was made from the depigmented area in Fig. 1, and divided into two pieces. One piece was divided into small blocks which were fixed with 2% buffered glutaraldehyde, post-osmicated, dehydrated in graded ethanols and embedded in Araldite; thin sections of these blocks were stained on the grid with uranyl acetate and lead citrate, and examined by electron microscopy. The other piece of skin was floated overnight on a 0·1% solution of commercial trypsin at 2°C, following which the epidermis was stripped from the dermis as a sheet and, following fixation for 1 h in formol calcium chloride, was incubated for 6 h in a buffered 0·1% solution of Dopa. After further fixation and dehydration, the epidermal sheet was mounted dermal side up on a slide. Araldite-embedded blocks of tissue taken 16 years previously from an area of the same subect at 7 and 14 days following application of thorium-X, and examined at that time, were further sectioned in order more extensively to re-examine the effects of the application on melanocytes and epidermis and dermis generally. Control split-skin Dopa-incubated epidermal sheets of the subject's freckled skin were also available.

OBSERVATIONS

Light microscopy

Examination of the Dopa-incubated split-skin epidermal sheet from the treated area (Fig. 3) revealed the presence of melanocytes, the majority of which exhibited such a weak reaction that accurate estimates of numbers per unit area were not possible, though this appeared to be approximately the same as in paler areas of control epidermis. An occasional larger, more intensely Dopa-reactive melanocyte of the type characteristic of freckles was also present.

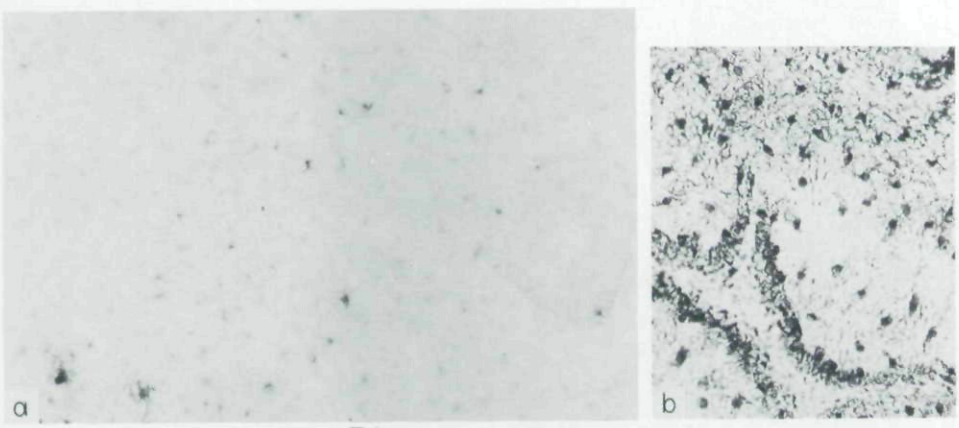

FIGURE 3. (a) Dopa-incubated epidermal sheet prepared from depigmented area of forearm 16 years after application of thorium-X. Melanocytes are evidently present in apparently normal numbers, but the intensity of Dopa-reaction in the great majority is extremely weak. (b) Melanocytes of normal epidermis of freckled subject in the region of a freckle showing large active melanocytes with melanin in keratocytes (× 144).

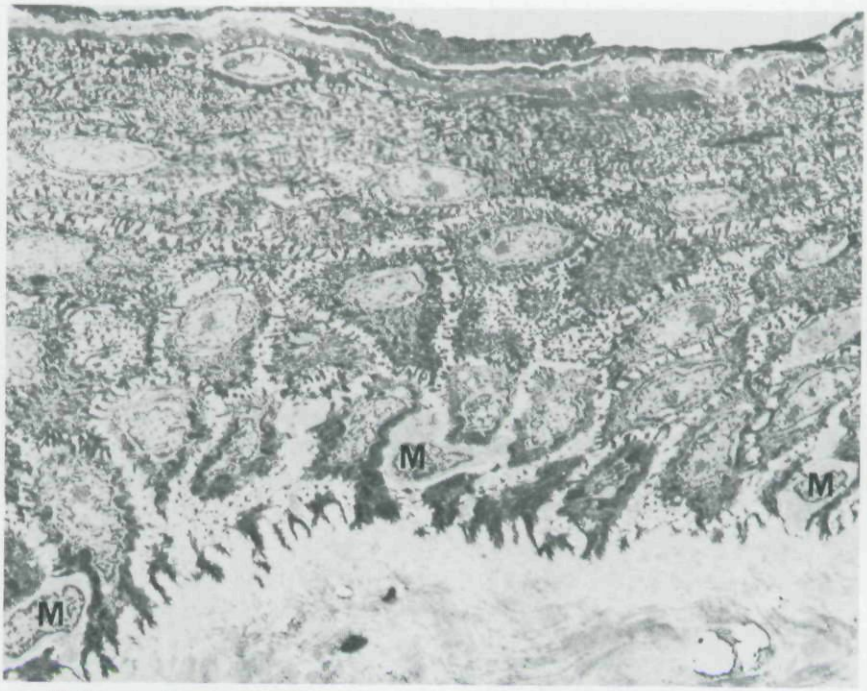

FIGURE 4. Epidermis of depigmented skin 16 years after application of thorium-X. Basal melanocytes (M) are present in apparently normal numbers (× 1280).

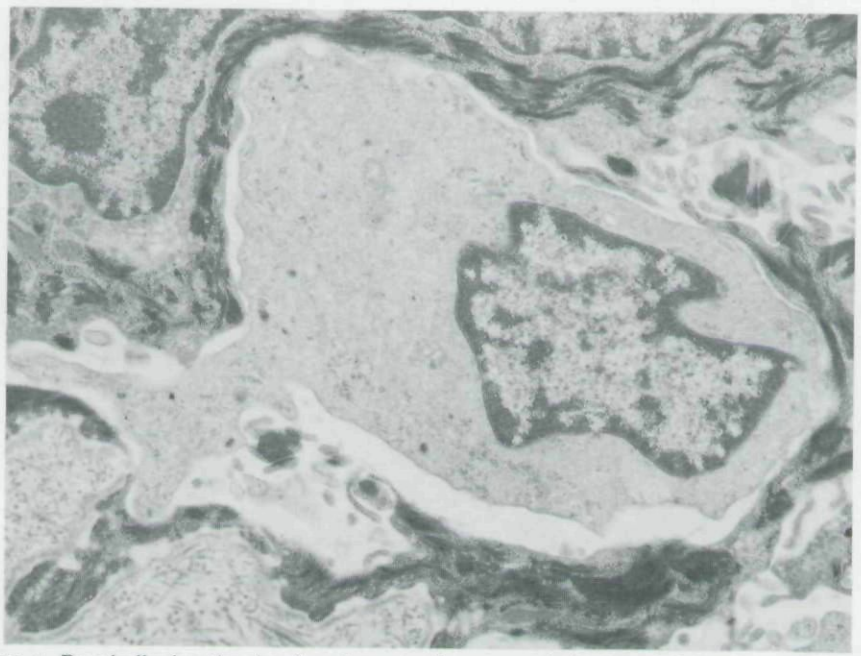

FIGURE 5. Practically inactive basal melanocyte from area depigmented following application of thorium-X 16 years previously. Of several hundred melanocytes observed in thin sections, this was the most active seen in terms of numbers of melanosomes (× 11,200).

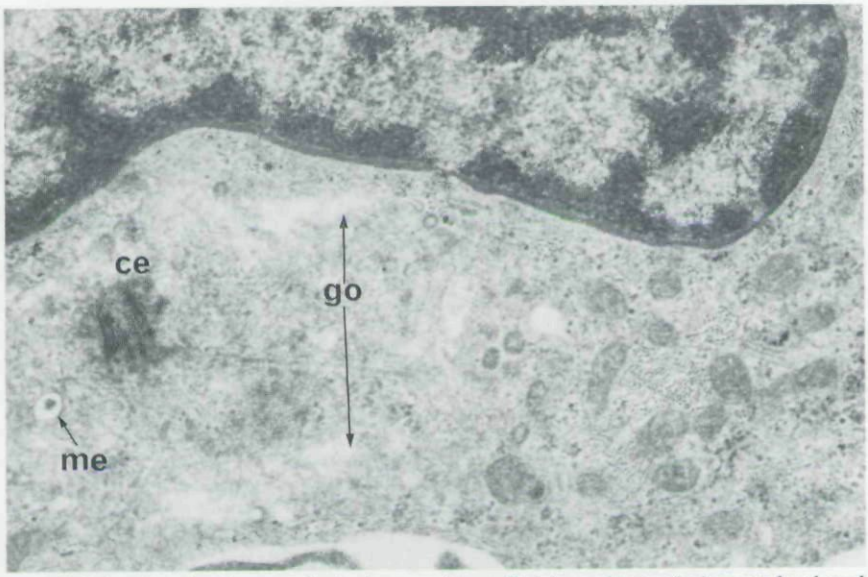

FIGURE 6. Golgi region of melanocyte in depigmented area. Golgi membranes (go) are undeveloped: ce— centriole; me— stage 1 melanosome. Mitochondria are moderately numerous, and a few coated vesicles are also present (× 25,280).

Electron microscopy

Low-power survey micrographs of epidermis revealed the presence of basal melanocytes in numbers consistent with a normal density distribution (Fig. 4). These melanocytes presented an appearance of almost complete absence of melanogenic activity, with only a very occasional stage 1 or stage 2 melanosome present and numerous cytofilaments (Figs 5 and 6). Rough endoplasmic reticulum and Golgi membranes and vesicles were very poorly developed, though mitochondria were moderately numerous. The basal melanocytes presented a much less active appearance than control, non-freckle melanocytes of the same subject, than albino melanocytes, and than fetal melanocytes that had never been exposed to UV irradiation.

The epidermal keratocytes and Langerhans cells presented a completely normal appearance, as did the general arrangement of the dermis which contained what appeared to be a normal complement of cells, vessels, and nerves, and which in no way resembled that of a scar.

Re-examination of skin taken at 7 and 14 days following the original application of thorium-X revealed considerable destruction of cells, both melanocytes and keratocytes, in the basal layer of the epidermis, and highly-active melanocytes were present suprabasally. Melanin-containing macrophages, monocytes, and lymphocytes were numerous, and there was much intercellular exudate. The basal lamina was intact, except where it was being traversed by cells of the latter two types which were also present in the dermis in numbers obviously greater than normal.

DISCUSSION

The original application of thorium-X led to a reaction in which, certainly, large numbers of melanocytes were destroyed but whether all those present disappeared is impossible to say. Sixteen years afterwards it appears that the area affected contains a normal number of melanocytes—normal, that is, for pale skin of the freckled subject. These melanocytes could be

derived from cells which escaped damage, or their descendants, or some may have migrated in from surrounding untreated epidermis. Whatever the respective numbers, the overwhelming majority present are in an almost totally inactive state, and this condition has persisted for at least 16 years with complete absence of freckles, despite exposure to UV. The area of skin in question has, so to speak, reverted to the condition of the entire skin of the subject before he started to manifest the freckling trait, and is apparently incapable of producing freckles now. How might this be explained?

The absence of freckling and the general hypopigmentation is strictly limited to the area treated with thorium-X, and is evidently due to depression of melanogenic activity of the melanocytes present within it, and not to their absence or diminution in number. Cause and effect, certainly, but why has this state of affairs persisted for so long? The general depression of melanogenesis seems unlikely to be due to failure of delivery of circulating systemic controlling factors, such as hormones, or whatever, and it is difficult to conceive of any local environmental change brought about by thorium-X, either in epidermis or dermis, which could account for such long-standing inhibition, though the sharp localization of the phenomenon to the area treated does suggest an environmental effect. The weak Dopa-reaction indicates a very low level of tyrosinase activity which could be due either to excessive inhibition of the enzyme (normal melanocytes are known to contain tyrosinase inhibitors) or to a low level of synthesis. The poor development of rough endoplasmic reticulum and Golgi apparatus and the sparsity of melanosomes, indicating a low level of general protein synthesis, makes the latter possibility the more likely. The absence of macroscopic freckling could be seen as a further manifestation of the general low level of melanogenesis, though the occurrence of the occasional larger, more active freckle-type melanocyte in the Dopa preparation could be said to represent abortive attempts to produce freckles. If so, why have they not succeeded?

It must be concluded that no satisfactory explanation can be advanced to account for the appearances illustrated in Fig. 1, though the present examination has illuminated them to some extent. It would be of interest to transplant whole freckled skin or, better still, a freckled epidermal sheet from the same subject, into the depigmented area and see if the freckles faded or not after a period.

Have we here a practical method for removing freckles? For some people, the presence of freckles in certain situations is a matter of concern and distaste, and their complete removal in a one-off operation might be considered worth some temporary discomfort. The irritation caused by the thorium-X solution applied in the present instance was not great, and the same result might be produced by a solution of lower concentration. Apart from the pigmentary changes there have been no long-term after-effects; sensation is normal in the area, and the Geiger counter is silent when passed over it.

ACKNOWLEDGMENTS

This study was supported by grants from the Wellcome Trust and the Cancer Research Campaign.

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