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RABBIT AS A MODEL FOR CHLORPROMAZINE-INDUCED HYPERPIGMENTATION OF THE SKIN

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Abstract—Hyperpigmentation of exposed skin areas, comparable to that seen in less than 1 per cent of patients chronically dosed with chlorpromazine after intensive long-term therapy, has been produced in sixteen out of sixteen chronically dosed pigmented rabbits, receiving between 20–30 mg/kg per day. Thirty-min u.v. irradiation of a clipped or shaved area produced clear-cut hyperpigmentation of naturally pigmented skin areas in about 4 weeks. The characteristic occurrence of granular pigment in the dermis which is normally free of pigment was also observed. Hyperpigmented rabbits did not develop any concomitant ocular pathology, as seen in some patients on long-term, high-dosage chlorpromazine therapy.

7-HYDROXYCHLORPROMAZINE was previously established as an important metabolite of chlorpromazine in human drug detoxication.[1] It is normally stored in form of its unconjugated derivatives along with nonphenolic drug metabolites in the tissues of chronically dosed patients without any side effects of drug therapy.[2] In the urine of such patients it occurs in form of its conjugates (mostly *O*-glucuronides) as the major group of chlorpromazine metabolites.[3, 4] Searching for a metabolic correlate in the urines of a small number of chronically dosed patients with the late side effect of hyperpigmentation of exposed skin areas, first observed by Ey *et al.*[5] a slight elevation over the normally minimal amounts of unconjugated 7-hydroxychlorpromazine derivatives was observed.[6] As very few such patients were available for study, a suitable animal model appeared desirable. McDonald *et al.*[7] had succeeded in producing ocular opacities, another late side effect of chronic high-dosage chlorpromazine therapy, in guinea pigs. However, they could not induce hyperpigmentation of the skin in this species, despite continuous u.v. irradiation of animals maximally dosed for periods of 12 months.

According to studies of the urinary chlorpromazine excretion patterns in man, dog, cat and rabbit[8] and albino v. normally pigmented individuals of the same species,[9] all these mammals had the drug detoxication pathways necessary for sulfoxidation, *N*-oxidation and demethylation. Major differences were seen primarily in the pathways for hydroxylation and conjugation with glucuronic acid. Rabbits were found to be the species with the greatest potential for forming excess, unconjugated 7-hydroxychlorpromazine, and appeared to be the most likely candidates for hyperpigmentation of the skin, if the scheme for the interaction of 7-hydroxychlorpromazine with melanin

was correct. Data *in vivo* and *in vitro*[6] suggested that melanin was instrumental in accelerating the oxidation of 7-hydroxychlorpromazine to its positive ion radical which ultimately formed charge-transfer complexes with peripheral melanoprotein. These presumably accounted for hyperpigmentation.

MATERIALS AND METHODS

Flemish Giant black rabbits, Dutch Belts with combined albino and pigmented genetic features were used, and white New Zealands also served as controls. Young adult females weighing from 1·3 to 2 kg each were chronically dosed by stomach tube with 20 or 30 mg chlorpromazine hydrochloride per day, for periods of 3–6 months. A steady state of chlorpromazine metabolism in this species is established in about 3 weeks.[8] The urinary drug excretion rate thereafter remains reasonably stable in the same animal. An area of approximately 7 × 11 cm of the dorsal skin was clipped or shaved when necessary, two to four times per week, and one-half of the area was exposed to increasing daily u.v. irradiations until a 30-min period was reached. Either a 20 W Westinghouse sunlamp FS 20 or a 275 W General Electric sunlamp, with their respective broad ranges of wavelengths, was used at a distance of about 21 in. from the dorsal skin. The Westinghouse lamp has a range of 290–310 mμ, and the General Electric lamp from 320 to 400 mμ, with a maximum around 360 mμ. The other half of the shaved area served as control and was at all times covered by a strip of aluminum foil held in place with tape during the irradiation period.

RESULTS AND DISCUSSION

Within 3 weeks after the maximum 30-min irradiation period was instituted, all sixteen pigmented experimental animals (two black Flemish Giant and fourteen Dutch Belt) showed distinct areas of hyperpigmentation, either in patches or contiguous. Albino skin, whether from control white New Zealand rabbits (one untreated, three chronically dosed with chlorpromazine), or from the white portions of twenty Dutch Belt rabbits (fourteen dosed and six controls), showed only mild to pronounced erythema. Both sunlamps produced essentially the same results, but the 275 W General Electric sunlamp did so somewhat more rapidly.

Figures 1 and 2 illustrate the procedure and the results. Figure 1 shows two pigmented rabbits, a Dutch Belt and a black Flemish Giant, with the shaved dorsal skin areas, one-half of which is covered with foil for u.v. irradiation in their restraining boxes. Figure 2 shows a Dutch Belt rabbit with induced skin pigmentation, after 4 weeks of u.v. exposure, and a total period of 5 months on 20 and finally 30 mg/kg chlorpromazine.

In addition to albino New Zealand control animals, and the white skin portions of the Dutch Belt rabbits, further controls were set up, by daily irradiation of the shaved dorsal skin areas of eight Dutch Belt rabbits, not dosed with chlorpromazine. Ultraviolet irradiation produced some patchy darkening of the exposed areas, but these required longer periods of irradiation of at least 7 weeks of daily 30-min exposures, and no contiguous pigmentation was ever produced. Biopsied and autopsied skin samples of the white and pigmented areas, either shielded from or exposed to u.v. irradiation, in control and experimental rabbits, were obtained from a total of seventeen animals, eight of which were controls (one New Zealand each, dosed and un-

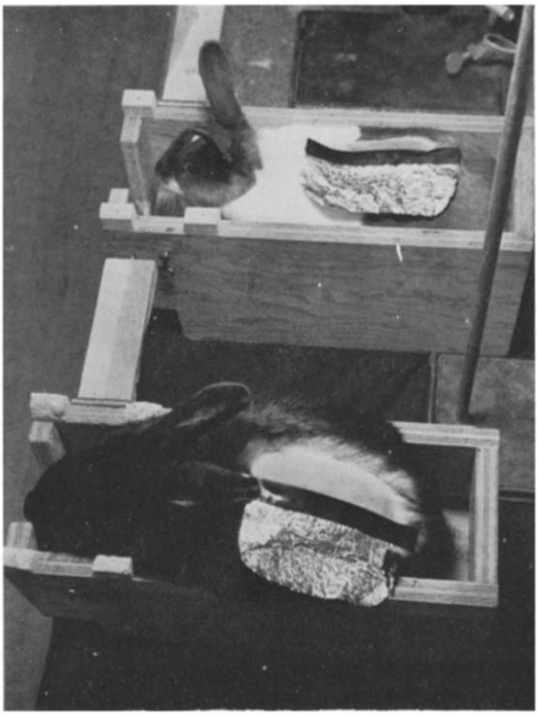

FIG. 1. Rabbits in restraining boxes for u.v. irradiation of shaved dorsal skin. One-half of the shaved area is covered with aluminum foil held in place by plastic tape.

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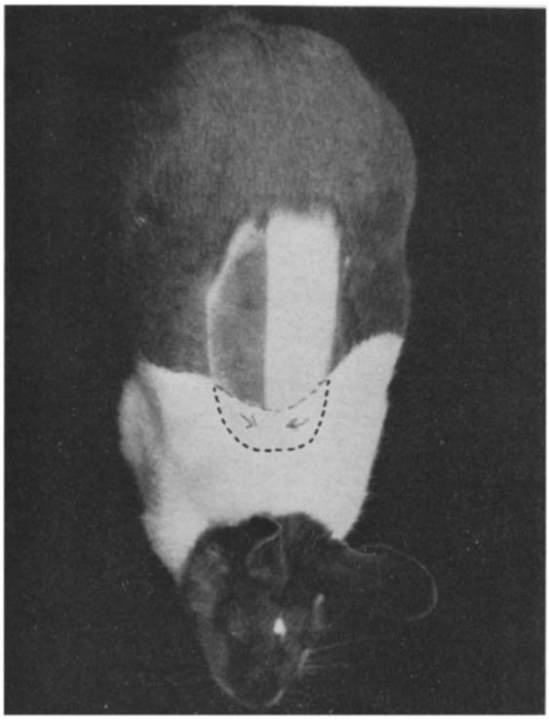

FIG. 2. Chronic chlorpromazine-treated Dutch Belt rabbit showing hyperpigmentation of naturally pigmented area on the u.v. exposed portion of shaved skin. The albino area of shaved skin shows no difference between exposed and unexposed areas (arrows in area within dotted lines). Hyperpigmentation was produced with a 275 W General Electric sunlamp. Two small spots, each on the hyperpigmented and control sides, are the result of skin biopsies.

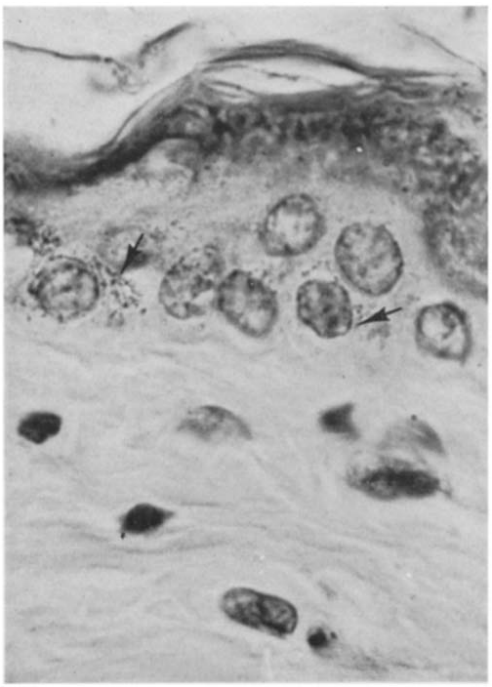

FIG. 3. Ultraviolet irradiated pigmented skin area of control rabbit, not dosed with chlorpromazine. H & E stain, ×1200. Pigment in epidermis only.

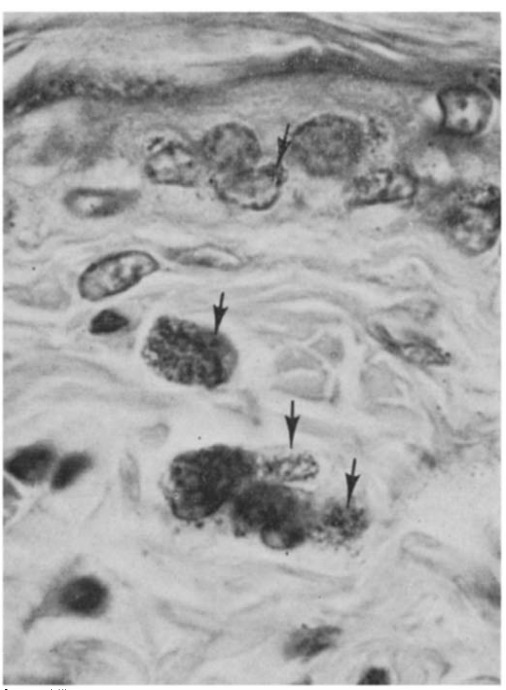

FIG. 4. Chlorpromazine-treated rabbit, pigmented skin area after u.v. irradiation, H & E stain, × 1200 Pigment in both epidermis and dermis.

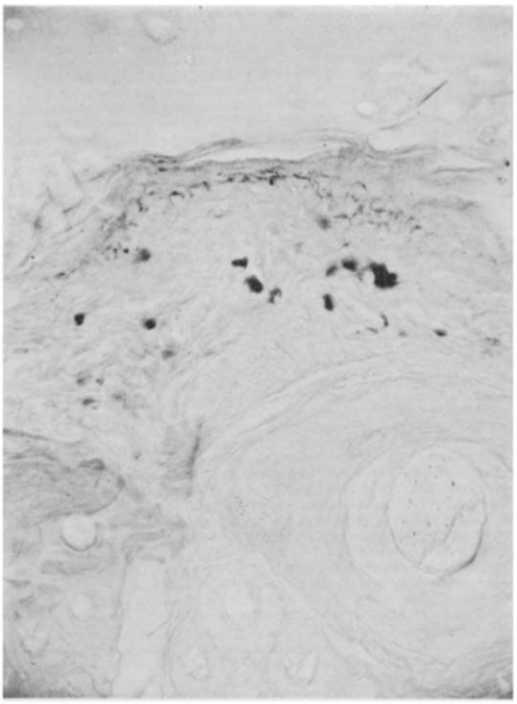

FIG. 5. Chlorpromazine-treated rabbit, pigmented skin area after u.v. irradiation, Prussian blue stain ×256. Brown pigment, Fe negative. Pigment abundantly present in both epidermis and dermis.

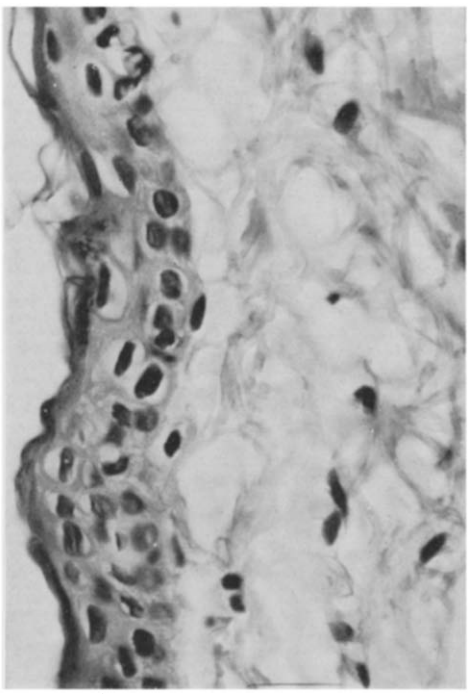

FIG. 6. Albino skin area of chlorpromazine-treated rabbit after u.v. irradiation. H & E stain, ×640. No pigment in either epidermis or dermis.

treated, and six untreated Dutch Belt), and nine experimental rabbits (one Giant Flemish, eight Dutch Belt).

After u.v. irradiation alone, there were increased numbers of heavily pigmented melanocytes in the naturally pigmented areas of all six biopsied pigmented control rabbits (Fig. 3). However, the dermis was free of pigment in all four albino rabbits and in five of the six biopsied Dutch Belt controls. One Dutch Belt control showed occasional dermal melanophores in all of its samples. This animal, as well as the first of the experimental rabbits, had been treated with a depilatory cream which caused some erythema and skin irritation, but neither scaling nor blistering were seen. An electric hair clipper and occasionally a razor were used thereafter. When u.v. irradiation was combined with chronic chlorpromazine therapy, however, there was profound hyperpigmentation of the previously pigmented epidermis, and very large numbers of pigment stuffed melanophores throughout the upper dermis (Figs. 4 and 5), in all sixteen pigmented experimental rabbits. According to Greiner and Berry[10] the accumulation of granular pigment in the dermis was characteristic of chlorpromazine-induced hyperpigmentation of exposed skin in patients.

In the albino areas of skin, neither u.v. irradiation alone nor combined with chlorpromazine administration, produced microscopically appreciable epidermal or dermal pigmentation (Fig. 6). Ultrastructure of the epidermal melanocytes was normal.

With regard to the hyperpigmentation of the skin, it appears that in the patients of our hospital and in the rabbits, this was a cosmetic problem rather than a medical one. Neither the patients nor the rabbits appeared physically sick. Discontinuation of chlorpromazine resulted in a slow but perceptible bleaching of the hyperpigmented areas, over a period of several months. Discontinuation of u.v. irradiation only, or other therapeutic measures at maintenance of chlorpromazine therapy which proved clinically feasible in patients, have not been studied in the rabbits to date. Attempts to induce additional conjugation of 7-hydroxychlorpromazine and its derivatives with glucuronic acid are in progress.

The chemical nature of the adduct between melanoprotein and 7-hydroxychlorpromazine presumably formed *in vivo* is under investigation. The rabbits with hyperpigmentation of the skin showed no lenticular or corneal pathology whatsoever by slit-lamp examination. This lends support to our theory[11] that the late ocular side effects of chlorpromazine therapy are not due to the same genetic or metabolic conditions giving rise to hyperpigmentation of the skin.

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