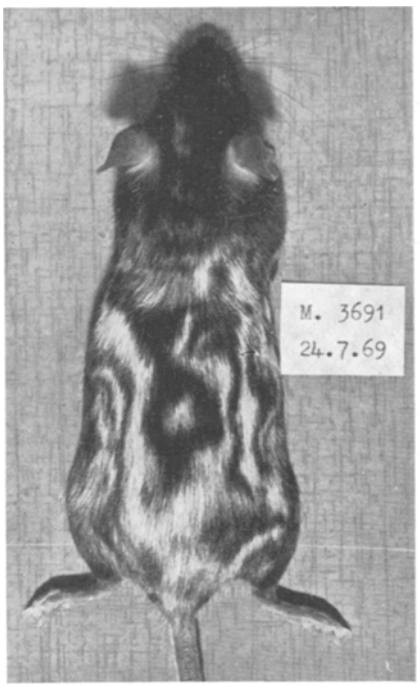
Depigmentation and Coat Colour Variegation in Mice Treated with 8-Hydroxyquinoline

It has been found that topical application of 8-hydroxyquinoline (oxine, 8-HQ) to the skin of female mice of several pigmented strains strongly inhibits pigmentation of the subsequent growth of hair. The effect shows remarkable selectivity, follicles in some areas of treated skin producing completely depigmented hair throughout a growth phase, while in identically-treated adjacent areas pigmentation of hair occurs normally. This results in pronounced patterns of depigmented and normal hair which differ markedly in appearance from those types of coat colour variegation known to be determined genetically in various ways.

Induction of variegation by 8-HQ is reversible on cessation of treatment, while with continued applications the patterns on individual mice change markedly with time. The effect is thought to be closely associated with the waves of hair growth as worked out by BORUM[1], and it bears at least a superficial resemblance to the production of coloured coat patterns on albino rats by injections of an isoalloxazine dye[2].

8-HQ is a chelating agent which has found considerable use in analytical chemistry and as an antibacterial and antifungal agent. In some animal tests it has shown evidence of carcinogenic activity, while other tests have given



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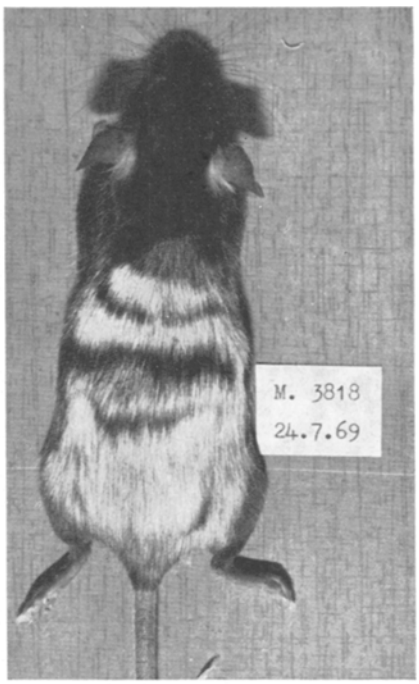

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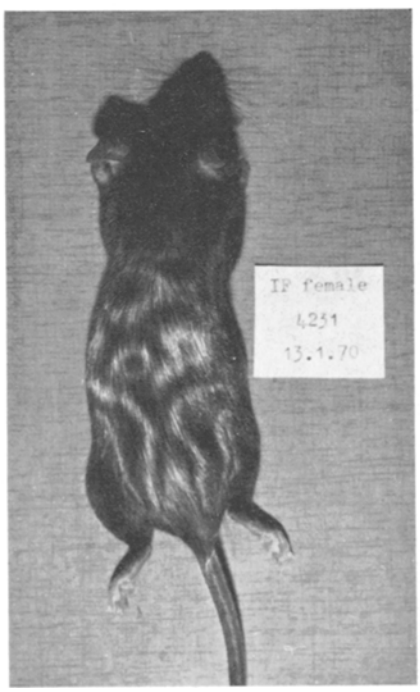

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Patterns of depigmentation on female mice treated topically with 0.5% 8-hydroxyquinoline in acetone. Fig. 1 and 2, C57BL. Fig. 3, IF; Fig. 4, CBA.

doubtful or negative results. Its selective depigmenting action was first observed within a few weeks of starting experiments on its carcinogenicity in which applications (0.3% w/v in acetone, twice weekly) were made to the clipped dorsal skin of C57BL × IF F_1 hybrid mice from about 8 weeks of age.

Subsequent comparative tests showed that depigmentation occurred more readily in pure line C57BL mice than in the hybrids, but less readily in IF mice. Male mice were always much less affected than females of the same strain.

Female C57BL mice have therefore been used in most experiments. In these mice, 0.1% 8-HQ in acetone applied twice weekly caused just noticeable depigmentation. At 0.5%, however, new hair growth consisted to a major extent of non-pigmented hair, giving rise to a variety of coat patterns as illustrated in Figures 1 and 2. Further increase to 1.0%, or increased frequency of application, made little apparent difference and complete depigmentation has not been observed.

The lesser effect of 0.5% 8-HQ on female IF mice is shown in Figure 3. Several strains of brown mice (C3H, CBA, DBA, MP) given similar treatment also developed patterns of depigmentation (Figure 4).

Applications at 0.5% in acidified aqueous solution or in liquid paraffin similarly induced selective depigmentation in C57BL females. Administration in the drinking water at strengths up to 0.5% had no effect, but this is probably not surprising as 8-HQ is inactivated by intact red blood cells[3] and is unlikely to reach the follicles in an active form.

In contrast to the striking effect of 8-HQ on young adult female mice, applications to C57BL or C57BL × IF mice from 4 days of age had no effect on pigmentation of the first coat of hair. Maximum variegation appears to occur in growth cycles G3 and G4 at approximately 3 to 9 months of age, after which treated mice develop less distinct patterns of depigmentation apparently correlated with the more patchy growth of hair which occurs in older animals.

No report has been found of 8-HQ as an inhibitor of melanogenesis in vivo, though it has been reported to inhibit potato tyrosinase in vitro[4]. Many other substances have long been known to interfere with some stage of melanogenesis, which involves the copper enzyme tyrosinase, but applications of various other copper reagents (2-mercaptopyridine, 8-mercaptoquinoline hydrochloride, cupron, 2,2′-biquinolyl, 2,9-dimethyl-1,10-phenanthroline and zinc dibenzyldithiocarbamate) all failed to inhibit pigmentation in female C57BL mice.

This suggests that the depigmenting action of 8-HQ, like its antibacterial and fungistatic action[3], may depend on the toxic action of a metal complex rather than to direct combination with tyrosinase copper. Although it is suggested that the selective action against follicles in the treated area is probably related to the hair growth cycles, the mode of action of this selectivity is not known.

Zusammenfassung. Die Melanogenese wird bei Mäusen durch 8-Hydroxychinolin selektiv gehemmt. Besonders bei behandelten weiblichen Tieren des Stammes C57BL entwickelt sich ein aussergewöhnliches Haarmuster mit abwechselnden schwarzen und weissen Streifen oder Kreisen. Diese reversible Wirkung wurde bei neugeborenen Mäusen oder nach Behandlung mit Cu-reaktiven Substanzen nicht beobachtet.

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Heterogeneity of Erythrocyte Catalase: Variability of the Isoelectric Point

Human as well as horse erythrocyte catalase can be separated into three enzymatically active fractions designated as A, B and C, according to the molarity of the buffer required for elution from DEAE-cellulose at pH 6.8 (A < B < C)[1–3]. In the absence of oxygen and heavy metal catalysts, fraction A is found exclusively[2–4]. In order to elucidate whether purified fraction A corresponds to the native form of catalase, the isoelectric point (IEP) of this fraction was compared with the one of catalase in a fresh hemolysate.

Fraction B and C of erythrocyte catalase are formed out of fraction A by irreversible oxidation of sulfhydryl groups in the presence of oxygen and heavy metal catalysts[4]. Evidence for the formation of acidic groups during this transition was obtained by comparing the IEP of these fractions.

Materials and methods. Purified fractions A and C of human and horse erythrocyte catalase, as well as hemolysates, were prepared as described previously[4]. Catalase activity was determined spectrophotometrically (Beckman DB) at 240 nm and 25 °C[5]. The number of sulfhydryl groups, disulphide bridges and irreversibly oxidized sulfhydryl groups present in the fractions used for isoelectric focusing was determined by titration with *p*-Chloromercuribenzoate (pCMB)[4,6]. The results are summarized in the Table.

The isoelectric focusing experiments were performed in a Uniphor apparatus (LKB-Produkter AB, Stockholm-Bromma, Sweden) according to the method of Vester-

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