The site of action of the ichthyosis locus (*ic*) in the mouse, as determined by dermal-epidermal recombinations

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This paper is dedicated to Professor H. Grünberg, F.R.S., on his retirement from the Chair of Animal Genetics at University College London.

**SUMMARY**

Skin of 14-day *icjc* and +/+ embryos was separated into dermal and epidermal layers using trypsin, and recombined in the combinations +/+ epidermis with +/+ dermis, +/+ epidermis with *icjc* dermis, *icjc* epidermis with +/+ dermis, and *icjc* epidermis with *icjc* dermis. The recombined tissues were implanted in adult histocompatible testes and allowed to grow for 2 weeks. The type of hair that developed in the grafts corresponded to the genotype of the epidermis, and was not influenced by the genotype of the dermis. We conclude that the *ic* locus acts in the epidermis.

**INTRODUCTION**

The recessive mutant gene ichthyosis (*ic*) in the mouse causes a short sparse coat (Carter & Phillips, 1950). Spearman (1960) found no reduction in number of hair follicles in *icjc* mice, but the hairs were much thinner and shorter than normal and the air spaces were irregular in shape. All hairs were wavy and tended to vary in thickness along their length because of alterations in size of the medulla. In skin from the back both the hair follicles and the epidermis appeared normal, even though the hairs were abnormal, but on the tail the epidermis was thicker than normal and showed hyperkeratinization.

The ichthyosis locus apparently controls some process necessary for the formation of normal hair. Previous results give no evidence on the question of the site of action of this locus, whether in the epidermis, the dermis, or possibly both or neither. The technique for separating skin from mouse embryos into dermal and epidermal components and making recombinations of components of different genotypes (Mayer & Fishbane, 1972; Mayer, 1973) allowed us to

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investigate this question. We found that the primary effect of the *ic* locus occurs in the epidermis, and that *ic/ic* dermis is indistinguishable from normal dermis in its effect on hair development.

**MATERIALS AND METHODS**

Mice homozygous for ichthyosis (*ic/ic*) were obtained from an inbred line maintained by brother–sister matings of *ic/+ × ic/ic* mice for approximately 22 generations. Mutant embryos were obtained from *ic/ic × ic/ic* matings. Homozygous normal mice were derived from the same inbred line by intercrossing *ic/+* mice and testing the +/? offspring for absence of *ic*. In order to produce enough +/+ mice for the experiment the homozygotes (+/+ ) were outcrossed to mice of the C57BL/6J strain, producing +/+ *F*₁ hybrids. Normal embryos were obtained from *F*₁ × *F*₁ matings. Male *F*₁ mice served as histocompatible hosts for tissue from both types of embryos.

Skin was obtained from 14-day *ic/ic* and +/+ embryos, separated into dermal and epidermal layers, and recombined in all four possible combinations according to the methods described by Mayer, Mittelberger & Green (1974). The recombined tissues were grafted into the testes of adult males and allowed to develop for 2 weeks, after which the testes were removed, fixed in 10 % formalin, and cleared in oil of wintergreen. The skin grafts were removed and examined for hair type and structure.

Two types of control procedures were carried out in order to determine the effectiveness of the separation procedures. First, pieces of dermis and epidermis were fixed immediately after separation, sectioned, and examined for presence of contaminating cells. No contaminating cells were found. Secondly, isolated dermis and epidermis were grafted alone into adult testes and allowed to develop for 14 days. No hair follicles developed in six such grafts of epidermis alone or in four of dermis alone.

**RESULTS**

From a total of 121 original grafts, 94 were recovered for study. Two of these had sharply contrasting sections of hair types and apparently were mosaics attributable to incomplete separation of embryonic skin layers. They were not included in the results. The observations on the 92 remaining grafts are shown in Table 1.

All the grafts possessed numerous hairs arranged either parallel or in a ball. Since the inbred ichthyosis line was homozygous for agouti many of the hairs contained much yellow pigment which made them a little more difficult to classify than hairs containing only black pigment. In order to eliminate subjective judgements as much as possible, we scored the grafts on three morphological criteria: (1) overall appearance of the graft, including the degree of crowding as well as the alignment of hair shafts; (2) appearance of the most
<table>
<thead>
<tr>
<th>Combinations*</th>
<th>No. of grafts</th>
<th>Scores†</th>
<th>Significance of differences</th>
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<tr>
<td></td>
<td>1</td>
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<tr>
<td>Overall appearance</td>
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<tr>
<td>A. $+/+$</td>
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<td>B. $+/+$</td>
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<td>C. $+/+$</td>
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<tr>
<td>D. $+/+$</td>
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<tr>
<td>Appearance of individual hair</td>
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<td>A. $+/+$</td>
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<td>Pigment distribution</td>
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<td>D. $+/+$</td>
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* Epidermis
Dermis

† See Results, p. 718.
common type of hair in each graft, judging both the size and shape of the hairs; and (3) pigment distribution in the hair shafts. A scale of 1–4 was used as follows:

Overall appearance of graft:
(1) Few hairs in disorderly arrangement.
(2) Relatively crowded hairs in disorderly arrangement.
(3) Somewhat crowded hairs in only slightly disordered arrangement.
(4) Crowded hairs in parallel arrangement.

Appearance of individual hairs:
(1) Short, narrow, curled.
(2) Medium long, narrow, somewhat wavy.
(3) Short, narrow, straight, and tapering, or long, broad, slightly bent, and tapering.
(4) Long, broad, straight, and tapering.

Pigment distribution:
(1) No pigment.
(2) Granular, unevenly distributed pigment.
(3) Slightly more even distribution of pigment.
(4) Evenly distributed pigment.

All scoring was done blind. Table 1 gives the frequencies and mean scores of the four combinations for the three criteria. Typical grafts of the four combinations are shown in Figures 1–4. The recombination grafts with +/+ epidermis and ic/ic dermis resembled the grafts in which both layers were +/+ (Figs. 1, 2). The recombination grafts with ic/ic epidermis and +/+ dermis resembled grafts in which both layers were ic/ic (Figs. 3, 4).

The significance of the difference in frequency of scores between control and recombination grafts was tested by $\chi^2$ (Table 1). There were no significant differences between the combinations with the same type of epidermis for all three criteria. However, when combinations with +/+ epidermis were combined and compared with those with ic/ic epidermis (A + B vs. C + D, Table 1), the differences were highly significant for all three criteria. Thus the genotype of the epidermis determines the type of hair produced.

**DISCUSSION**

Our results demonstrate that the gene at the ic locus acts in the skin and more specifically in the epidermal portion of the skin. However, they do not demonstrate conclusively that the activity of the locus is confined to the epidermis. The defect caused by the ic allele is demonstrable in our system only when that allele is present in the epidermis. But we have not ruled out the possibility that
the wild-type allele has some function in the dermis also, and that the loss of such a function might be revealed by a mutant allele more severely defective than \textit{ic}. In a similar experiment designed to determine the site of action of the agouti locus, Mayer & Fishbane (1972) found that the substitution of nonagouti (\textit{a}) for white bellied agout (\textit{A}^{w-\textit{j}}) was effective in determining the colour pattern
of hair only when present in the dermis, but Poole (1974) found that when the alleles compared were lethal yellow (A\textsuperscript{v}) and a, an effect of gene substitution on pigmentation could be demonstrated in both dermis and epidermis. Possibly the agouti locus is active in both parts of the skin. While some pairs of alleles at this locus differ in their effect on both layers, other pairs differ in effect only on dermis.

Mutant genes at well over 30 loci cause abnormal hair form or texture in the mouse (Green, 1966). For most of them it is not known whether the site of action is in the skin or elsewhere. In principle this information could be rather easily obtained by reciprocal skin transplantations if suitable congenic strains were available. We are aware of only two mutants for which this experiment has been performed. Reed (1938) made reciprocal skin transplants between newborn waved-2 (wa-2/wa-2) mice and their normal sibs and found that the grafts produced hair according to their own genotype, thus demonstrating that the site of action of the wa-2 locus was in the skin. Fraser (1946) made reciprocal skin transplants between newborn rhino (hr\textsuperscript{r}h/hr\textsuperscript{r}h) and normal litter-mates and similarly found that the centres of the grafts behaved according to their own genotype in all cases. Moreover, in grafts of rhino skin, which is normally hairless, to normal hosts, some normal hair the colour of the rhino donor developed around the edges of the grafts, and in grafts of normal skin to rhino hosts a narrow hairless strip developed around the edges. A possible interpretation of this result is that the hr locus acts in the dermis and that host dermis growing in under the graft epidermis supported normal growth of hair when the invading dermis was normal and suppressed it when the invading dermis was rhino. Of the other hair-defect mutants, two in addition to ic have been investigated by the dermal–epidermal recombination technique (downless, dl, Sofaer, 1973; fuzzy, fz, Mayer et al. 1974), and all three have been shown to act in the epidermis.

The use of the dermal–epidermal recombination technique is restricted to those mutants that produce an abnormality of the hair recognizable in the resulting grafts. Grafts can be grown in the embryonic chick coelom as well as in the adult mouse testis, but in both these sites the conditions of hair growth may be somewhat abnormal and not suitable for the detection of moderate differences in waviness, texture or length of hair, or in abnormalities of the molting cycle. In a small experiment to test the suitability of naked (N), a mutation that causes the hairs to break off a little above the surface of the skin beginning at 10–14 days of age, we grafted intact pieces of skin from eight 14-day embryos to the testes of adult males and allowed them to remain for 2 weeks. Half of the grafts should have been naked (N/+ ) and half normal (+/+ ), but we were unable to detect any differences among them. The probability that all would have been N/+ or that all would have been +/+ is 1/128. Possibly an effect of N might have been demonstrable if the grafts had been maintained longer. Undoubtedly, a more extensive search would reveal mutants.
other than the three so far studied to which the dermal–epidermal recombination technique could be applied for determining the site of gene action.

For research aimed at discovering how genes act in controlling morphogenesis, genes affecting hair growth may be a favourable system. There are many such genes, the skin is readily accessible for experimentation, and the hair follicles undergo cycles of activity and regression. Determining the site of action of the genes involved is a necessary step preliminary to further study of this system.

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