In a hope to obtain information on the nature of the hereditary hairlessness in mice, experiments with ectodermal transplants between hairless and normal animals have been carried out by several investigators. First, Crew and Mirskaia (1932) succeeded in obtaining epidermal grafts from hairy to hairless adult mice which stayed on with no loss of hair for about three weeks. David (1934) succeeded in a graft from a hairless to a normal mouse. According to him, the hairless skin graft behaved autonomously on the normal host, while Fraser (1946) demonstrated in experiments with young mice that the skin of rhino, one of the hairless mutants, transplanted to a normal host showed depilation only in the center of the graft. He assumed that parts of the rhino skin adjacent to normal skin behave non-autonomously, indicating their ability to utilize, though not produce, some substances necessary for the maintenance of normal stratified squamous epithelium.

The present study deals with reciprocal skin transplantations between hairless and normal mice, undertaken in order to throw a light on the action of the genes for hairlessness.

**MATERIALS AND METHODS**

Two hairless mouse mutants, “rhino” (hr\(^h\)) and “furless” (fs), maintained in our laboratory, were employed in the present investigation. At the age of 12 to 14 days the rhino mice begin to lose gradually the hair around the eyes, and the process continues toward the posterior end of the body until around the age of 18 to 20 days all hair of the animals is shed, when appear hyperplastic tendencies of the epidermal derivatives.

The furless (fs) mice begin to lose their first fur coat at the age of about 19 days, and gradually depilation proceeds over the entire body. About the age of six weeks, a new coat is produced but it persists for only a short time. Still later this coat is also shed and, thereafter, the animals remain distinctly deficient for a period of more than a year (Green 1954). Several rhino mice, varying from 1 to 3 months of age,
and about one month old furless mice, which were in the process of the first depilation, were available for ectodermal transplantation.

Each mutant mouse was paired with a normal litter mate. A piece of dorsal skin measuring approximately $2 \times 3 \text{ cm}$ was transferred from the donor to the dorsal region of the recipient mouse and simultaneously the reciprocal grafting was performed. For skin transplantation the modified method of Liebelt (1957) was used. The mice were anaesthetized with ether, their hair was removed with scissors, and the dorsal area was disinfected with 70% alcohol. Grafts were prepared by removing the dorsal skin and preserving it in physiological saline. The transplantation bed of the recipient was prepared by removing the dorsal skin of the same region and the same size as those of the graft. The graft was placed on the bed and joined to it by finger pressure and was sutured around the cut border. The bed and the graft should not be allowed to dry out during the operation.

Some of the grafts were turned around by 180° for the determination of the success or failure of the graft since the hair on a successful graft would grow in the opposite direction to that of the skin of the host. After operation the grafted mice were housed individually for two weeks and later were kept by twos.

Skin pieces for histological observation were removed from the middorsum of animals killed by decapitation. The specimens were fixed with Bouin’s solution, embedded in paraffin, and cut in serial sections at 15 μ in thickness. Sections were stained with Heidenhain’s haematoxylin and counterstained with eosin.

RESULTS

In order to determine whether hairlessness is due to the direct action of a gene on skin cells or is a secondary manifestation of gene effect on some other part of the body.
reciprocal skin transplants between hairless and normal mice were performed.

The experimental procedures and transplantabilities of each group are summarized
Fig. 6. Dorsal skin graft from non-rhino to rhino litter mate, a month after operation.

Fig. 7. Dorsal skin graft from non-furless to furless litter mate, a month after operation. Note the part behind the graft with remaining hair, while the front part of the body is already depilated.

Fig. 8. Dorsal skin graft from furless to non-furless litter mate. One month after operation, the furless graft not yet depilated, while the body hair of the donor had been already shed.

Fig. 9. Rhino skin, at 6 months of age. Showing well developed cysts. ×55.

Fig. 10. Section of a rhino graft on non-rhino host, 4 months after operation. Note that follicle-end cyst or sebaceous gland cyst disappeared but the utriculi remained. Decrease in thickness of the graft by 1/2 of that of the donor's skin. ×130.

Fig. 11. Non-rhino skin, at 3 months of age. ×85.

Fig. 12. Section through the edge of a non-rhino graft on rhino host. Non-rhino graft tissue on the right, rhino host tissue on the left. ×115.
SKIN TRANSPLANTATION BETWEEN NORMAL AND HAIRLESS MICE

in Figure 1. Reciprocal transplants between hairless mutants and their normal litter mates were almost all successfully incorporated into the host, as was expected, since these animals have been inbred for many generations, so that histocompatibility between the graft and the host’s tissue should have been sufficient. There was, however, no such closely related genetical background between rhino and furless strains, hence it was difficult to obtain long enough surviving grafts between them for arriving at a conclusion (Table 1).

Table 1. Results of skin transplantations between normal and hairless mice

<table>
<thead>
<tr>
<th>Donor</th>
<th>Host</th>
<th>Successful “takes”</th>
<th>Graft grew hair but fell off within one month</th>
<th>Graft fell off without growing hair</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhino</td>
<td>non-rhino</td>
<td>12</td>
<td>0</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>non-rhino</td>
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<td>10</td>
<td>1</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>rhino</td>
<td>rhino</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>furless</td>
<td>non-furless</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
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<td>furless</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>furless</td>
<td>rhino</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>rhino</td>
<td>furless</td>
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<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>haired</td>
<td>haired</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>43</td>
<td>3</td>
<td>10</td>
<td>56</td>
</tr>
</tbody>
</table>

Rhino skin grafted on non-rhino hosts formed a thin, transparent scab on the surface layer within a few days after the operation (Fig. 2). This scab consists of the cornified layer. After the scab dropped off, the graft appeared on the surface as a reddish and smooth slough about one week after operation. Later, the grafted skin became thick and developed skin folds as pronounced as those occurring in donor mice. The grafts showed the characteristic rhino follicles and epidermal hyperkeratosis within a month (Fig. 3).

In the course of grafting experiments some incidental observations were made concerning the effects of the operation itself on hair regeneration. It was interesting to note that a few thin hairs appeared on the border around the edges of the rhino graft on normal non-rhino hosts 10 to 20 days after operation (Fig. 4), but were shed again about one month later. This indicates that to the sparse hair regeneration of rhino skin grafted on normal mice the following factors could have contributed; (1) tendency to rejuvenescence arising in graft tissue, caused by the operation, (2) invasion of host hair into the outer border of the graft and (3) ability of fully adult rhino grafts to utilize the substance which is produced by normal skin cells and to maintain a normal stratified squamous epithelium.

To decide why thin hair regenerates on adult rhino grafts, several rhino skins were grafted on other rhino hosts or retransplanted to the same host, turned around by 180°
from their original position. Similar results were obtained with rhino grafts on rhino hosts as on normal hosts as mentioned above (Fig. 5); thin, curly and entangled hairs were developed on the border of the graft after two weeks and complete depilation took place before the donors characters appeared on this graft.

Successful grafts of normal non-rhino skin to rhino host grew a normal coat of hair and showed no signs of the characters of the recipient. They behaved autonomously until the mouse was discarded. Figure 6 shows a successful graft of the skin from a normal hairy mouse transplanted to the dorsal region of a rhino brother.

In a similar fashion, ectodermal skin exchanges between the furless mutant and normal litter mates were performed, and some "takes" were obtained (Table 1). The development of the graft after operation resembled that of rhino skin grafts, but the furless graft was depilated completely when the scab dropped off a week after the operation. However, the depilation of the body of the recipient furless mice was always incomplete and a few hairs remained scattered over the whole surface of the body.

Furless skin grafted to normal mice was shed repeatedly but not in coordination with the periodicity of the donor (Fig. 8). In some cases, for example, the hair in a furless graft regenerated after transplants was no more shed. Non-furless skin grafts on furless mice developed a normal hair coat a month after operation and no effect of the host tissue on the graft could be recognized (Fig. 7).

On the other hand, subsequent depilation of the furless host on which non-furless skin was grafted showed an interesting behavior; namely hair of the posterior part of the body, behind the graft, was not shed, while the anterior area, in front of the graft, was depilated in regular fashion (Fig. 7). It seems highly possible that the depilation of the furless mutant develops in a definite direction, from head to tail, so that the depilation of the posterior area was suppressed, the grafted normal tissue obstructing the path of depilation.

The morphological changes that occurred in the rhino skin grafted on non-rhino mice, were characterized by thinning of the whole ectodermis especially the follicle-ends or sebaceous gland cysts in the lower dermis (Figs. 9 and 10). The utriculi and hyperkeratosis of upper dermis do not only remain unchanged but eventually become more elongated.

No apparent effect of non-rhino graft on rhino host could be recognized histologically in this experiment. There were no widening of the hair canal, shortening of the follicles and hyperplastic changes (Figs. 10 and 11).

DISCUSSION

From the results of reciprocal skin transplantations with rhino mice and their normal litter mates we may conclude that the fate of rhino character had already been decided before 24 days of age so that the rhino grafts on normal hosts were not affect-
ed by the host's skin. On the other hand, Fraser (1946) had demonstrated in young newly born or one day old mice, that the rhino skin on a normal host was affected by the host only in the region of close proximity to normal skin cells. It seems reasonable to assume that rhino skin cells are influenced by the host at a very young stage only but adult rhino skin cells are no more affected by the normal host.

The histological observations have also confirmed our finding that rhino grafts on normal hosts maintained the donor's characteristics autonomously for about four months until the mouse was discarded.

A remarkable decrease of cysts in the lower dermis was observed in rhino grafts but other characteristics of rhino mutation, namely widening of follicle necks and formation of utriculi (Montagna et al. 1952) were rather intensified, while the normal grafts on rhino host were unchanged either morphologically or histologically. In the case of transplants of rhino skin grafts to normal hosts, a slight temporary regeneration of hair was observed on their outer border, due to tissue rejuvenescence. A similar result was obtained in the case of transplants from rhino to rhino. These phenomena might have been rather the effect of temporary rejuvenescence of graft tissue than produced under the influence of normal host skin cells.

Reciprocal transplants of furless mutant and normal litter mates were done during the period of first coat shedding. The furless graft had completely shed hair a few days after operation, although depilation of the donor's skin was usually incomplete. After the operation, hair of the furless graft was regrown once in the donor's periodic time, but the second depilation was not always according to the donor's periodicity, for instance, in one case no more depilation occurred. The non-furless skin on furless hosts was not affected by the host cells at all even as to the periodicity of the host. However, the hair of the area behind the non-furless skin graft of the furless host did not shed and remained without change through the depilation period. From these facts, it may be also considered that the phenomenon of hair loss of furless mice is concerned with a diffusible agent moving from head to tail.

We may assume in conclusion that the two types of hairless mutants used in the present experiments deal with two different genes whose action is expressed in different ways. The rhino character of the rhino skin cells was already decided before the age of three weeks and remained unchanged. On the contrary, the depilation of furless skin cells even if they are in adult stage is affected by a diffusible factor given forth by the normal host.

**SUMMARY**

1. Reciprocal transplants of dorsal ectodermis between adult hairless mice and their normal litter mates were investigated in two hairless mutants, rhino and furless.
2. Of a total of 56 grafts in which all eight combinations survived the operation
(Table 1), 40 cases were completely successful.

3. Successful grafts of non-rhino skin to rhino host behaved autonomously and also rhino grafts on non-rhino hosts behaved autonomously except for a temporary hair growth on the outer border of the graft observed about three weeks after the operation.

4. Furless skins grafted on non-furless hosts were depilated once immediately after operation and regrew after a month, but the second depilation occurred irrespective of the donor’s periodicity. The non-furless skin on furless host was not affected by the host, on the contrary the body hair of furless host behind the graft remained unchanged, while the area in front of the graft was depilated in regular fashion.

LITERATURE CITED