The estimation of the number of histocompatibility genes controlling the successful transplantation of normal skin in mice

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[Plates 40 and 41]

The success or failure of a transplant of tissue from one animal to another depends on the action of a number of independently acting histocompatibility genes which are present in the donor’s tissues and absent from those of the host. An estimate of the number of such genes in mice can be made from the proportion of grafts which survive transplantation from either of the two parent strains to members of their F₁ generation. If \( n \) is the number of genes, the proportion of successful grafts is \( (\frac{1}{2})^n \). Most previous work of this sort has used tumour tissue to provide material for grafting. A normal tissue, such as skin, has several advantages over malignant tissues which may kill the host and are difficult to observe; it is also probably more exacting in its genetic requirements. We have used single 7 mm diameter full-thickness pieces of skin transplanted orthotopically to the side of the chest as test grafts.

Taking survival in an autograft-like condition 100 days after grafting as the criterion for judging success or failure, two of 120 A strain and one of 154 CBA strain grafts survived transplantation to F₁ generation mice. Assuming that each separate antigen is capable of causing breakdown of the graft, these figures imply that certainly not less than fifteen independently segregating genes control the fate of a transplant. But since breakdown of such ‘successful’ grafts was observed as late as 180 days after grafting, the estimates represent minimal values only.

The survival times for the grafts are distributed widely from 10 or 11 days (the normal survival time for interstrain homografts) to more than 100 days. Both frequency distributions (for A and CBA donors) are quite unlike the theoretical distribution for the frequency of occurrence of 0 to 15 homozygous gene pairs in the F₂ generation. They also differ between themselves and suggest that the alleles in the CBA strain are less potent sources of antigens than those in the A strain. It is not possible to equate numbers of gene differences with survival for any given number of days, but clearly the individual genes have widely differing powers of forming antigens.

The process of destruction, once begun, is soon complete and resembles the breakdown process in normal interstrain homografts more closely than it does the slower, vacillating process found in mice that have acquired tolerance to foreign skin by virtue of an inoculation during embryonic life.

Second set grafts are usually thrown off more rapidly than the first set but anomalous results occur occasionally.

Immunoparalysis was not found even when five sets of grafts were transplanted in succession.

INTRODUCTION

The general results of much detailed work on the transplantability of tissues (both normal and malignant) can be set out in a series of propositions as follows:

1. Iso-transplants (within a given inbred strain) and a fortiori auto-transplants are always successful. Work on iso-transplants of skin in mice (Billingham, Brent, Medawar & Sparrow 1954) suggests that it may be necessary to add the proviso that signs of incompatibility (even extending to destruction of the graft) may

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occur if host and donor, although both members of the same inbred strain, come from separate lines within the strain. Such results are presumably due to mutations at one of the loci controlling incompatibility and there is now plenty of evidence that such mutations have occurred at the H-2 locus. Huseby & Bittner (1951) on the other hand, reported that ovaries could readily be exchanged between two lines of A strain mice which had been bred as separate diverging lines for more than fifty generations.

Recent work by Eichwald & Slitmser (1955), which we have confirmed, requires another proviso. Eichwald & Slitmser have shown that male skin is not permanently accepted by a female recipient, even when both host and donor are members of an inbred strain of mice. Grafts of other combinations of skin from the two sexes succeeded without difficulty.

(2) Homo-transplants (between two separate inbred strains of the same species or between randomly selected members of a species which has not been inbred) are all destroyed after a short initial period of acceptance, which seems to be relatively constant in the wide variety of species that have been tested. The inevitability of rapid destruction certainly holds good for homografts of skin (Medawar 1944), kidney (Dempster 1953), vaginal stroma and epithelium (Krohn 1955a) and many other tissues, including a variety of tumours. There is still some doubt about the universal validity of the proposition when parts of endocrine organs are transplanted (Billingham & Parkes 1954; Krohn 1955b; Ingram & Krohn 1956) (see also Strong 1936; Nordholt 1940).

(3) $F_1$ hybrids of any two inbred strains readily accept grafts from both parent strains, from each other, or from their progeny (i.e. they are universal recipients within the breeding system), but grafts from the hybrid offspring are not accepted by either parent strain (Little & Johnson 1922; Bittner 1936; Kaliss & Robertson 1943; Loeb 1945).

(4) Transplants from either parent strain to $F_2$ mice or from one parent strain to the offspring of the $F_1$ hybrid backcrossed to the other parent strain only succeeded in a proportion (usually very small) of cases.

A tissue can, it would appear, be transplanted successfully only if all of a number of alleles which the donor cells possess are also present in the host. Other alleles, possessed only by the host and absent from the donor’s tissues, have no influence on the outcome of the grafting operation. Any exceptions to this general rule depend on previous treatment of the host or on special properties (e.g. of lymphatic drainage or vascularity) inherent in the chosen site of grafting.

The development of most of these propositions had naturally to await the introduction of genetically homogeneous inbred strains of animals, which were soon exploited by those engaged in research on tumour growth to show that the control of susceptibility to a tumour is effected by the action of multiple independent genes (‘histocompatibility genes’) (work summarized by Snell 1953).

Work on the transplantability of normal tissues from one animal to another has been largely concerned with descriptions of the breakdown process, with its time relations and with an analysis of the factors which modify the responses of either the host or donor tissues. Much less attention has been paid to the genetic
Histocompatibility genes in mice

analysis of the reaction. There are, however, considerable advantages in the use of a normal tissue, like skin, for such tests, rather than the tumours with which most work has been done. First, there is no risk that the test animal will be killed by rapidly growing tumour tissue before a reaction against it has had time to develop adequately. Secondly, the grafts can be made orthotopically; they are clearly visible and the normal pattern of the response to their presence is very well documented. Thirdly, the behaviour of the graft is apparently a very sensitive indicator of minor differences in compatibility. The main difficulty is that the number of histocompatibility genes controlling the transplantation of normal tissues appears to be much larger than the number which determines whether a tumour is rejected or not. Larger numbers of experiments are, therefore, required.

A method for estimating the number of such genes can be based on proposition 4 (above), for, on the assumptions that the genes are segregating independently and that they each determine antigens which are singly sufficient to cause breakdown of the graft, the proportion of successful grafts will vary with the number of genes involved and can be shown to be equal to \((\frac{1}{2})^n\) (where \(n\) = the number of controlling genes) or \((\frac{1}{2})^{2n}\), depending on whether \(F_2\) or \(R_2\) backcross mice are used for the test.

This paper reports a series of such experiments designed to provide an estimate of the number of histocompatibility genes influencing the transplantation of normal skin from the \(A\) and \(CBA\) strains of mice.

**Materials and Methods**

**Mice used**

The mice used in this study were all derived from the \(A\) and \(CBA\) strains. The \(CBA\) strain was originally derived from a cross between Bagg Albino and \(DBA\) strains. The \(A\) strain was originated by a cross between the Cold Spring Harbor and Bagg Albino mice. According to Snell (1954), mice of the \(A\) strain have the \(H-2^a\) allele (formerly \(H-2^{dk}\)) and \(CBA\) strain mice the \(H-2^k\) allele at the \(H-2\) histocompatibility locus. Pure-bred and hybrid litters were obtained as required from original breeding pairs, which were provided by Professor P. B. Medawar, F.R.S., University College London. A single litter of the \(F_1\) hybrid (\(CBA\) \(\varphi \times A\) \(\varphi\)) provided the parents (7 \(\varphi\) and 3 \(\varphi\)) of the \(F_2\) generation [(\(A \times CBA\)) \(\times (A \times CBA)\)]. The \(F_2\) generation was chosen to provide hosts for the grafts, in preference to the backcross generation (\(R_2\)) because the likelihood of obtaining compatible homografts of skin from the parent strains was higher [(\(\frac{1}{3})^2\) instead of (\(\frac{1}{2}\))^2].

Throughout the study an attempt was made to keep closely within one line of the inbred strain since Billingham et al. (1954), as mentioned earlier, have shown that mild incompatibility reactions occur when host and donor, even though both come from the same strain, diverge by as few as 10 to 12 generations. The \(A\) and \(CBA\) animals used as donors to the \(F_2\) generation were closely related to the parents of the \(F_1\) generation.

Mice of both sexes were used; their ages ranged from about 40 days to about 12 months. Younger animals were too small to handle satisfactorily; older
animals were difficult to anaesthetize safely. The usual age at grafting was about 60 days.

**Selection of donors**

In the control series grafts were exchanged between pairs of animals. In the experimental series one donor provided skin grafts for a number of $F_2$ hosts so that material of identical antigenic make-up was tested on groups of animals. Skin for transplantation was taken as required from both the dorsal and ventral surfaces of the body. Areas of active hair growth were avoided.

Donors for second set grafts were either the mouse used to provide the first graft or a close relative (sib, first cousin, or parent).

**Technique of grafting**

In general, the method of grafting followed closely that described by Billingham & Medawar (1951) with only a few minor modifications. Single, full-thickness grafts, about 7 mm in diameter, were transplanted to sites on the side of the chest which had been prepared by removing the host integument down to the level of the panniculus carnosus muscle, over either the lateral thoracic or internal mammary vessels. Bands of active hair growth in which the hair follicles penetrated deep in the panniculus carnosus sometimes made it necessary to choose intermediate graft positions. The direction of the hairs of the grafts relative to the surrounding host skin was reversed and, wherever possible, ventral skin was placed over the lateral thoracic vessels and dorsal skin over the mammary vessels. These manoeuvres helped greatly to identify the grafts when host and graft had the same coloured hair. The grafts were usually described as 'good fits', which meant that the graft-host junction was expected to heal by first intention.

A control autograft was placed on the side opposite to the homograft in all the early experiments and at random in later experiments.

**Modification of technique**

Avertin (0·01 ml./g b.w. of a freshly prepared 1·67 % solution of Bromethol in normal saline) was used as anaesthetic. When dressing the area after grafting it was found useful to bring the plaster bandage tightly into apposition with the chest wall by taking up the slack bandage with a pair of Spencer Wells forceps applied along the dorsal surface of the dressing. The firm ridge of plaster thus formed also provided a useful means of catching hold of the mouse.

**Inspections**

The first post-operative inspection in early experiments was carried out 7 days after the operation. In the experimental series, however, the first inspection was delayed until the 10th or 11th day, i.e. until about the time that breakdown of a normal homograft would ordinarily be expected to occur. Further dressings of the wound were not needed.

**Histology**

Many of the grafts, or parts of them, were removed as biopsies. They were fixed in Bouin's fluid and embedded in paraffin wax. The blocks were cut at 7 μ
and representative sections at different levels were stained with haematoxylin and eosin or with haematoxylin and Van Gieson.

**Criteria of permanent survival**

It was necessary to define arbitrarily what one should consider to be 'permanent' acceptance of the graft by the host, both to give a reasonable time limit to the experiments and because very little was known about how long it might take for a slight immunity reaction to make itself apparent.

Regeneration of hair by the graft, which might be regarded as the restoration of full function, proved to be an entirely inadequate criterion, for many grafts regenerated full crops of hair only to break down later.

In their work on acquired tolerance Billingham, Brent & Medawar (1956a) used grafts of not less than 50 days' standing when they wished to demonstrate that tolerance could be destroyed by the transfer of lymph nodes from a normal to a tolerant mouse. Since this interval represents the median survival time + 40 standard deviations, and since the graft's expectation of life increased with its age, naturally occurring breakdown was a remote contingency. An interval of 100 days was used by Bittner (1936) in his experiments on the survival of spleen transplants. We finally adopted an autograft-like state for 100 days after grafting as the criterion for survival. Our experience has shown, however (see below), that grafts that have survived even for so long may yet be the victims of a very delayed reaction and be cast off as long after grafting as 180 days.

The time of breakdown of each graft was determined by repeated inspections at intervals (after the first inspection) of not more than 3 days during the first 25 days after grafting and then at rather longer intervals of up to 7 days, together with histological observations of the biopsy specimens.

**Results**

**Autografts**

Apart from occasional technical failures, all these grafts were, as expected, entirely acceptable to their hosts and survived satisfactorily. None of those which were examined microscopically showed any cellular infiltration with round cells. Most were observed until the death of the mouse, in many instances for over 1 year.

**Isografts**

The homozygosity, for practical purposes, of the parent strains and successive generations was demonstrated by the successful exchange of grafts between members within each generation and between generations. In no instance, with up to six generations separating host and donor, was more than a minor incompatibility reaction observed, as shown by coarseness of the dermis and sparseness of the hair follicles. Otherwise, autografts and isografts were treated identically by all hosts. Some grafts were removed for histology and others were observed until the death of the animals, 6 months or more later.
In view of the recent work of Eichwald & Silmser (1955), suggesting that the male Y-chromosome may carry a histocompatibility gene not present in the homologous female X-chromosome, it is unfortunate that none of the ♀ → ♂ combinations were observed for more than about 25 days, an interval too short for the delayed reaction described by Eichwald & Silmser to have become apparent.

Grafts from either of the parent strains to the first hybrid generation behaved in the same way and have been observed for well over a year, at which time albino A skin was still growing a vigorous crop of white hair on the pigmented F₁ host. Here again, it is unfortunate that none of the donors was male.

**Table 1. Summary of control and experimental series of grafts**

‘Compatible’ grafts are those which were indistinguishable from autografts 100 days after grafting.

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**Homografts (table 1)**

None of the interstrain grafts had been totally destroyed by the 9th post-operative day, but all showed signs that a vigorous specific reaction was in progress and were invariably destroyed by the 13th day. The process of destruction took 3 or 4 days. These figures are closely similar to those provided by the much larger series of experiments of Billingham et al. (1954).

Grafts from the first cross generation to the parent strains were treated similarly. No graft survived for more than 14 days and none for less than 10 days.

**Homografts from A strain to F₂ hosts**

120 F₂ mice received technically satisfactory grafts from A strain donors. The grafts were rejected at intervals which ranged from 10 to 180 days (see tables 2, 3, 5a, 6 and figure 1 for the distribution of breakdown times).

In most instances the behaviour of such grafts was indistinguishable from that of autografts for the greater part of their life span until a visible reaction suddenly developed, proceeded at the normal pace and within 3 to 6 days had destroyed the graft. In some cases, especially when the grafts survived for a long time, the details of the reaction were somewhat obscured by host tissue replacing the dying graft. Instead of undermining the graft dermis, ingrowing host epidermis covered

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<td></td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>171-180</td>
<td>2 (175 180)</td>
<td>54</td>
<td>12</td>
<td>17</td>
<td>5</td>
<td>120</td>
<td>39</td>
<td>67</td>
<td>24</td>
<td>21</td>
<td>3</td>
<td>154</td>
</tr>
</tbody>
</table>

* (32, 33, 34, 35, 37).
over the dermis and dipped into the follicular pits. Later, the native ingrowth gave way in places and small areas of the graft dermis were subsequently shed from time to time.

If one accepts survival for 100 days as an arbitrary dividing line, only two grafts survived permanently. The detailed progress of the grafts in these two mice (expts. 78 and 80) is given below and indicates that survival in one (expt. 78) was

Table 3. The survival of A skin grafted to F₂ hybrid mice, classified according to coat colour of the recipient

<table>
<thead>
<tr>
<th>coat colour of recipient</th>
<th>survival time (days)</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₂ mouse</td>
<td>10-13 14-20 21-30 31-40 41-50 51-60 61-70 71-80 171-180</td>
<td></td>
</tr>
<tr>
<td>Agouti</td>
<td>21 21 10 1 — — — 1 —</td>
<td>54</td>
</tr>
<tr>
<td>White</td>
<td>10 13 6 1 — — — — —</td>
<td>32</td>
</tr>
<tr>
<td>Black</td>
<td>5 4 2 1 — — — — —</td>
<td>12</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>7 5 4 1 — — — — —</td>
<td>17</td>
</tr>
<tr>
<td>Brown</td>
<td>3 1 1 — — — — —</td>
<td>5</td>
</tr>
<tr>
<td>total</td>
<td>46 44 23 4 — — 1 2</td>
<td>120</td>
</tr>
</tbody>
</table>

Table 4. The survival time of CBA skin grafted to F₂ hybrid mice, classified according to coat colour of the recipient

<table>
<thead>
<tr>
<th>coat colour of recipient</th>
<th>survival time (days)</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₂ mouse</td>
<td>10-13 14-20 21-30 31-40 41-50 51-60 61-70 71-80 141-150</td>
<td></td>
</tr>
<tr>
<td>Agouti</td>
<td>12 29 17 6 1 — 1 1</td>
<td>67</td>
</tr>
<tr>
<td>White</td>
<td>7 18 13 1 — — — —</td>
<td>39</td>
</tr>
<tr>
<td>Black</td>
<td>4 9 9 1 1 — — —</td>
<td>24</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>4 8 8 — 1 — — —</td>
<td>21</td>
</tr>
<tr>
<td>Brown</td>
<td>— 2 1 — — — — —</td>
<td>3</td>
</tr>
<tr>
<td>total</td>
<td>27 66 48 8 2 1 1</td>
<td>154</td>
</tr>
</tbody>
</table>

Table 5 and 6. The proportions of homografts surviving for more than 13, 20, 30, 40 days classified according to coat colour of host F₂ mouse

Donor material: Table (a) A strain (white), Table (b) CBA strain (agouti).

<table>
<thead>
<tr>
<th>coat colour of host</th>
<th>no. of expts.</th>
<th>13 days</th>
<th>20 days</th>
<th>30 days</th>
<th>40 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) White</td>
<td>32</td>
<td>22 69%</td>
<td>9 28%</td>
<td>3 9%</td>
<td>2 6%</td>
</tr>
<tr>
<td>Agouti</td>
<td>54</td>
<td>33 61%</td>
<td>12 22%</td>
<td>2 4%</td>
<td>1 2%</td>
</tr>
<tr>
<td>Black</td>
<td>12</td>
<td>7 58%</td>
<td>3 25%</td>
<td>1 8%</td>
<td>—</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>17</td>
<td>10 59%</td>
<td>5 29%</td>
<td>1 6%</td>
<td>—</td>
</tr>
<tr>
<td>Brown</td>
<td>5</td>
<td>2 40%</td>
<td>1 20%</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>total</td>
<td>120</td>
<td>74 62%</td>
<td>30 25%</td>
<td>7 6%</td>
<td>3 3%</td>
</tr>
<tr>
<td>(b) White</td>
<td>39</td>
<td>32 82%</td>
<td>14 36%</td>
<td>1 3%</td>
<td>—</td>
</tr>
<tr>
<td>Agouti</td>
<td>67</td>
<td>55 82%</td>
<td>26 39%</td>
<td>9 13%</td>
<td>3 4%</td>
</tr>
<tr>
<td>Black</td>
<td>24</td>
<td>20 83%</td>
<td>11 46%</td>
<td>2 8%</td>
<td>1 4%</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>21</td>
<td>17 81%</td>
<td>9 43%</td>
<td>1 5%</td>
<td>1 5%</td>
</tr>
<tr>
<td>Brown</td>
<td>3</td>
<td>3 100%</td>
<td>1 33%</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>total</td>
<td>154</td>
<td>127 83%</td>
<td>61 40%</td>
<td>13 8%</td>
<td>5 3%</td>
</tr>
</tbody>
</table>
certainly not permanent. The exact survival time of the other is in doubt, but may represent permanent survival.

**Homografts from CBA to F₂ hosts**

154 $F₂$ mice received technically satisfactory homografts from CBA strain donors and rejected them at intervals of between 11 and 155 days (see tables 2, 4, 5b, 6 and figure 2 for the distribution of breakdown times). Only one mouse tolerated the graft for more than 100 days, and none permanently tolerated a skin homograft from the CBA strain (see section below).

Calculating the value $n$ in $(\frac{3}{2})^n =$ proportion of successful grafts, the results from these two series indicate that not less than fourteen independently segregating

\[ n \]

homozygous gene pairs

\[ 10 \quad 15 \quad 20 \quad 25 \quad 30 \quad 35 \quad 40 \quad 45 \quad 60 \quad 70-80 \quad 120 \quad 170-80 \]

**Figure 1.** Distribution of breakdown times. A strain grafts $\rightarrow F₂$ hosts. 120 grafts. Inset is the theoretical frequency of 0 to 15 homozygous gene pairs in the $F₂$ progeny.

**Figure 2.** Distribution of breakdown times. CBA strain grafts $\rightarrow F₂$ hosts. 154 grafts.

$H$-genes are responsible for the incompatibility of A strain grafts and that not less than seventeen genes are concerned when CBA skin is grafted. Since compatibility was not absolute even in these ‘successful’ grafts, but relates only to survival for 100 days, the actual number of $H$-genes must be in excess of this figure.

The frequency distributions of the breakdown times for the two series of experiments are given in figures 1 and 2. For the combination $A \rightarrow F₂$ the maximum frequency occurs at a breakdown time of about 11 days, i.e. at about the time
when grafts between two different strains break down. The shape of the distribution is quite different from the frequency distribution in the $F_2$ generation of 0 to 15 pairs of homozygous genes (shown as an inset to figure 1). If one attempts to equate numbers of gene differences with survival time it is therefore necessary, at least, to assume that differences between host and donor of five or more genes lead to a reaction which is indistinguishable from that which occurs when the genetic differences are maximal.

The shape of the distribution of the $CBA \rightarrow F_2$ survival times differs from that for the $A \rightarrow F_2$ distribution. The evident differences between the two distributions seem to imply differences in the quality of the histocompatibility genes belonging to each strain.

**Table 6. Mean survival times and expectation of life of grafts from $A$ and $CBA$ strain mice to $F_2$ mice**

<table>
<thead>
<tr>
<th></th>
<th>$A \rightarrow F_2$</th>
<th>$CBA \rightarrow F_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean survival time (days)</td>
<td>19.8</td>
<td>21.3</td>
</tr>
<tr>
<td>Excluding survival for &gt; 150 days (2+1 mice)</td>
<td>17.1</td>
<td>20.4</td>
</tr>
<tr>
<td>Excluding survival for &gt; 60 days (3+2 mice)</td>
<td>16.6</td>
<td>20.1</td>
</tr>
</tbody>
</table>

The shape of the theoretical curve can be considerably modified by varying the proportions of the total complement of genes which are taken to have unit or less than unit effect—an assumption which accords better with the known facts than that each has the same effect. The result of such alterations is to produce steps in the distribution such as we have found experimentally.

Table 6 shows other differences between the two series. More grafts from the $CBA \rightarrow F_2$ combination survived to the longer intervals, but their mean survival time was shorter and the expectation of life, though increasing as survival time increased, was less.

**Second-set grafts**

Second-set grafts were used (a) to determine the reaction to a second dose of ‘antigen’ in immunized hosts who had displayed varying degrees of acceptance to the first graft and (b) when the first graft had been a technical failure.
Histocompatibility genes in mice

(a) Second-set graft to specifically immunized $F_2$ hosts (table 7)

Twenty-two mice (other than those described in detail below) in which homografts had previously broken down received a second-set graft from the original donor or from one as closely related to the original as possible. Periods of 15 to 90 days separated the two operations.

<table>
<thead>
<tr>
<th>Survival time of 1st graft (days)</th>
<th>Survival time of 2nd graft (days)</th>
<th>Interval between grafting (days)</th>
<th>Survival time of 1st graft (days)</th>
<th>Survival time of 2nd graft (days)</th>
<th>Interval between grafting (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-13</td>
<td>&lt;10</td>
<td>15</td>
<td>19</td>
<td>&lt;10</td>
<td>31</td>
</tr>
<tr>
<td>11-13</td>
<td>&lt;10</td>
<td>31</td>
<td>20-25</td>
<td>&lt;11</td>
<td>31</td>
</tr>
<tr>
<td>12</td>
<td>&lt;13</td>
<td>35</td>
<td>20-25</td>
<td>&lt;12</td>
<td>51</td>
</tr>
<tr>
<td>12</td>
<td>13</td>
<td>34</td>
<td>22</td>
<td>&lt;10</td>
<td>34</td>
</tr>
<tr>
<td>13-16</td>
<td>25</td>
<td>26</td>
<td>24-28</td>
<td>&lt;10</td>
<td>34</td>
</tr>
<tr>
<td>15</td>
<td>&lt;10</td>
<td>36</td>
<td>28</td>
<td>&lt;8</td>
<td>32</td>
</tr>
<tr>
<td>16-20</td>
<td>7</td>
<td>26</td>
<td>30-34</td>
<td>&lt;12</td>
<td>44</td>
</tr>
<tr>
<td>17</td>
<td>&lt;10</td>
<td>30</td>
<td>30-35</td>
<td>&lt;10</td>
<td>90</td>
</tr>
<tr>
<td>17-20</td>
<td>&lt;13</td>
<td>35</td>
<td>45</td>
<td>&lt;10</td>
<td>76</td>
</tr>
<tr>
<td>19</td>
<td>&lt;10</td>
<td>34</td>
<td>45</td>
<td>&lt;16</td>
<td>76</td>
</tr>
<tr>
<td>19</td>
<td>&lt;10</td>
<td>34</td>
<td>60</td>
<td>20</td>
<td>64</td>
</tr>
</tbody>
</table>

Accelerated breakdown was seen in all but three trials, and the mean survival time of the second-set grafts (omitting these three grafts) was less than 10 days. Two of the three unusual grafts broke down soon afterwards. The unexpected longevity of these two may have been due to incomplete immunization by the first-set graft, which was accompanied by infection. In the remaining case, the second set graft may have been overgrown by native epithelium early on and this giving way later, to allow the rejection of graft dermis, may have given the impression of retarded breakdown.

That there is very little overlap between the histocompatibility genes of $A$ and $CBA$ grafts is shown by the fact that the mean survival time (of $CBA$ grafts) for seven $F_2$ animals which received $A$ skin first and $CBA$ skin subsequently was 14-5 days. The reaction was of the first-set type.

(b) Second-set grafts to $F_2$ hosts following technical failure of first-set grafts

Thirty animals were included in this group, 12 to 51 days separating the first and second operations. Eighteen grafts showed definite accelerated breakdown (mean survival time < 10 days). Six grafts behaved like first-set grafts (mean survival time 19-5 days). The remaining six animals all rejected their grafts, but it was not certain whether a first or second-set reaction was taking place. Evidently it is sometimes possible for an unsatisfactory first-set graft to immunize the host.
Successive sets of grafts

Fourteen third-set, ten fourth-set, and two fifth-set grafts were observed, and all, except the third-set graft in expt. 14, whose second set also behaved anomalously, broke down in under 10 days, the reaction resembling an accelerated second-set reaction. Another anomalous result was provided by expt. 224 (see below). The intervals between graftings ranged from 13 to 43 days.

Descriptions of first-set homografts surviving for over 50 days and of anomalous second-set grafts

Expt. 14

This agouti F₂ female received an autograft and a homograft (CBA strain) when 52 days old. The homograft was supple and pink on the 6th day and lost a complete ‘ghost’ epithelium 10 days after grafting. At 21 days maloriented agouti hair of normal density was growing from the graft, which maintained an active hair pelt until the 59th day, when it had shrunk considerably in size. The graft was removed on the 64th day (figure 3, plate 40). Interpretation of the histological picture was difficult, and differentiation of host from donor epithelium was uncertain because both were pigmented. It was the consensus of opinion that the surface epithelium was probably derived from overgrowth by the host, while the epithelium deep in the follicles might well have been the epithelium of the original graft. Foci of round-cell infiltration were conspicuous around the follicles, but not beneath the surface epithelium.

The site of the original graft was filled by another homograft from a brother of the original donor. To begin with this graft behaved like the first graft and had grown new hair by the 20th day. On the 26th day, however, the graft had become harder and lost most of its hair. The graft was removed on this day and a third graft was transplanted. Histologically, the second graft presented a very unusual form of second-set reaction (figure 4, plate 40), for it showed an acute cellular infiltration much more resembling an ordinary first-set reaction. Some of the epithelium of the graft was probably still alive, but once again the difficulties of interpretation made it impossible to be certain.

EXPLANATION OF PLATES 40 AND 41

All figures (except figure 11) are of homografts of skin removed as biopsies and photographed at a magnification of ×37.

PLATE 40

Figure 3. Expt. 14. First set graft 64 days after grafting. The epithelium lining the deep follicles is probably of donor origin.

Figure 4. Expt. 14. Second set graft 26 days after grafting. Acute cellular infiltration.

Figure 5. Expt. 14. Third set graft 24 days after grafting. The graft had been completely destroyed and undermined.

Figure 6. Expt. 78. First set graft 179 days after grafting. Total destruction of graft with undermining by ingrowing native epithelium.

Figure 7. Expt. 78. Second set graft 36 days after grafting. Total destruction of graft.

Figure 8. Expt. 144. First set graft 159 days after grafting. Total destruction of graft with undermining. Note similarity in appearance to figure 6.
The third-set graft progressed like the two preceding grafts until the 17th day, but by the 24th had become hard and scabby and had not grown any new hair. Half was removed as a third biopsy (figure 5, plate 40), which revealed an obviously dead graft.

Expt. 78

This albino F₂ male received an autograft and a homograft (A strain) when 37 days old. Both grafts healed normally and showed downy pelts 16 days after grafting. An active hair pelt, demonstrated by regrowth after regular clipping, was retained by the homograft until the 176th day, when a small red scab was seen along its ventral edge. Two days later the graft was shrunken and more scabby; the hairs could easily be pulled out. At 179 days the graft was removed. The sections showed small round cell infiltration and replacement of the graft by host tissue (figure 6, plate 40).

Seven days later a second graft was placed anterior to the biopsy site. The graft behaved in an unusual fashion. It lost a complete 'ghost' at 9 days, but was rather blotchy at that time. On the 17th day there was a scab along the graft-host boundary, but there had been no contraction of the graft. Two days later the graft itself was scabby. At 21 days the graft was growing albino hair, and was covered with transparent scales. At 33 days the graft was carefully examined under anaesthesia and the hair on it trimmed. It was not appreciably harder than an autograft and appeared to be viable. At 36 days the graft had become scabby and harder and was removed. From the serial sections it was concluded that the graft had broken down (figure 7, plate 40). The survival time was considerably less than that of the first-set graft and the cellular reaction was confined to the graft bed, but as the graft had undoubtedly grown a fresh crop of hair, it did not succumb to a typical second-set reaction.

Expt. 80

This albino F₂ male received a homograft (A strain) when 39 days old. The graft behaved like a normal autograft until 176 days after grafting. On the 178th day a thick, active crop of hair was still present, but a pin-head-sized scab had newly developed centrally among the hairs of the graft. Unfortunately, it is not known whether this small scab represented a specific reaction or not. The animal was found dead 191 days after grafting, in a condition which precluded any useful histological observations.

Plate 41

Figure 9. Expt. 224. First set graft 81 days after grafting. Total destruction of graft and replacement by ingrowing native epithelium.

Figure 10. Expt. 224. Third set graft 59 days after grafting. Virtually normal looking graft.

Figure 11. Expt. 224. Second set graft 66 days after grafting. Actively growing hairs.

Figure 12. Expt. 224. Second set graft. Healthy appearance.

Figure 13. Expt. 224. Third set graft. Healthy looking graft.
Expt. 87

This agouti $F_2$ male received a homograft (A strain) when 37 days old. To begin with, the graft behaved like an autograft and by the 20th day was still growing new albino hair. After 60 days the graft was still like an autograft in all respects. At 72 days the hair pelt was still intact, but a few scabs were noticed on the surface of the graft. At 80 days the changes had progressed further and recent destruction was obvious to the naked eye. Microscopical examination of the biopsy confirmed this opinion.

Twenty days later a second graft was placed on a bed anterior to that of the first homograft. This graft had broken down by the 13th day.

Expt. 144

This agouti $F_2$ male received a homograft (CBA strain) when 38 days old. The graft healed in well and behaved like an autograft for 145 days. On the 159th day the animal died. Microscopical examination of the graft showed recent breakdown of the type seen in expt. 78. Survival time of the graft was estimated at 155 days (figure 8, plate 40).

Expt. 224

This agouti $F_2$ female received a homograft (CBA strain) when 34 days old. The graft had grown new hair 21 days later and still survived with an active hair pelt until 72 days after grafting, when it had become harder and scabby. It had been completely replaced by host tissue, as shown by biopsy (figure 9, plate 41) on the 81st day. A second graft was transplanted 37 days later. After 66 days the graft was photographed and examined under a binocular microscope using liquid paraffin to render the skin translucent (figure 11, plate 41). The blood vessels of the graft were indistinguishable either in density, calibre, or tortuosity from those in the surrounding host skin. The graft continued to behave like an autograft for more than 200 days and grew several crops of hair after shaving. 260 days after the first graft and 132 days after the second graft, two third-set grafts were transplanted to see whether a fresh antigenic stimulus might affect the earlier graft. Both sets grew normal crops of hair and showed no signs of failure. Biopsy of one of the third-set grafts after 59 days revealed a virtually normal autograft-like structure, though there were a few round cells around the bases of the follicles (figure 10, plate 41). The grafts maintained this general character throughout the rest of the life of the animal. During the next months a large mammary tumour developed rapidly and the mouse died during an attempt at removal. The grafts were removed and appeared to have undergone no further change (figures 12 and 13, plate 41).

Relationship of coat colour of $F_2$ hosts to the incompatibility to A and CBA skin homografts

Phenotypes of five coat colours were observed in the $F_2$ generation and were called white, agouti, cinnamon, black and brown. Some of the pigmented
individuals had non-pigmented patches on their tails. The distribution of the five coat colours was insignificantly different from expectation (White 71, Agouti 121, Cinnamon 38, Black 36 and Brown 8, compared with expectations of approximately 68, 116, 39, 39 and 13).

Tables 5a and 5b set out the numbers of grafts which survived for more than 13, 20, 30 and 40 days, according to the coat colour of the host.

On the whole the data are remarkably homogeneous and could be taken to suggest that the compatibility of coat colours between host and donor does not influence the survival time of the transplant. If one groups the data on survival time into 10 to 13, 13 to 20 and >20 days, and classifies them according to coat colour (black and brown being tabulated together), the $\chi^2$ values are 1:932 and 0:753 respectively. There is some indication, however, that the longest survival (>100 days) occurs only in the combination white-white or agouti-agouti. No graft of different coat colour survived for more than 70 days. In coming to this conclusion, one must take into account the facts that observations may not have been so reliable when host and donor tissues have the same coloured hairs and that the numbers of observations are inevitably small. With 20 chromosome pairs and at least fifteen histocompatibility genes, some linkage between coat colour and $H$-genes would hardly be surprising, but the effects become difficult to demonstrate.

**Regeneration of hair in grafts**

The day on which new hairs could first be seen by naked eye was recorded for both the autografts and the test grafts to $F_2$ mice. One in three of all test grafts and two in three of those which survived for 18 days or more, grew new hairs. No graft survived for more than 22 days without showing hair growth. The mean intervals between grafting and the appearance of new hairs for the sample of sixty-two test grafts was $17.9 \pm 0.27$ days, a figure which is insignificantly different from the figures for 108 autografts of $17.1 \pm 0.10$ days. White grafts always grew white hairs; occasionally autografts on pigmented mice grew a few white hairs.

**Discussion**

It has been usual to base any discussion of the sort of results which we present here on the assumptions that (1) each independent factor is responsible for the formation of a single antigen and that each antigen by itself is capable of causing breakdown of the graft and (2) that each $H$-gene is fully antigenic while the recessive $h$-allele is not. In these terms the results indicate that probably at least fifteen independent factors are concerned in determining the transplantability of normal skin in $A$ and $CBA$ mice. The estimates, which are based on prolonged but not, as it turned out, permanent survival of homografts, give a lower limit only. The true number must be larger. The estimates are also based on very few surviving grafts and would be considerably altered by small changes in the number of successes. Whatever the exact number may turn out to be, it is clear that very many factors are involved in the transplantability of a normal tissue such as skin.

The possibility that it may be incorrect to talk of fully active and inactive
genes in the histocompatibility system was suggested by Gorer (personal commu-
nication to Snell 1948). At the time there was no evidence for the antigenicity of
the H-genes and some against it (Snell 1948), but the general similarities between
histocompatibility and blood-group systems, where each allele is effectively
antigenic, lead one to expect that such differences will be found, and it is therefore
interesting that none of the alleles revealed so far by the analysis of the H-2 locus
has proved to be recessive. If all the recessive alleles are antigenic the number of
loci will be considerably reduced from the figure which is implicit in the present
findings, though the number of antigens will not be affected.

These, and other assumptions, which make the estimated number of histo-
compatibility genes even more inexact, have been discussed by Snell (1948).

There is little evidence that skin from a mouse which contains a double dose of
the dominant H-gene is more antigenic than one which contains only a single dose
of the particular gene. Prehn & Main (1954) attempted to show this by comparing
the survival time of grafts from the F₁ hybrid to the two parent strains, with the
survival time of interstrain grafts. In one of three trials they found a significant
increase in survival time for skin from the F₁ hybrid. Our own scanty evidence,
however, supports the findings of Billingham et al. (1954), who were unable to
demonstrate such differences.

The only other extensive genetically controlled experiment of the type we have
reported, in which normal tissues have been used, is that of Bittner (1936) who
made subcutaneous transplants of spleen in a large series of inbred and hybrid
mice. The mice were autopsied at about 100 days after transplantation, and any
mouse in which graft tissue could be found was considered to be susceptible.
5·1% of 156 F₂ animals accepted grafts from the C₃H strain and 11·9% of 193 F₂
mice accepted grafts from the N strain, figures which, in terms of dominant/reces-
sive genes, indicate that ten or eleven factors are concerned in determining the com-
patibility of C₃H grafts and seven or eight different factors in N strain grafts.
Bittner’s figures for smaller groups of backcross animals were less consistent and
more contradictory. Thus, no graft succeeded in sixty-one experiments using
N backcross animals, and two out of fifty-seven when C₃H backcross mice were
used. This latter proportion indicates the presence of five factors.

The technical details of Bittner’s experiments must be judged unsatisfactory in
some respects. The transplants consisted of small fragments of spleen inserted
subcutaneously by means of a trochar. No day-to-day observations of the grafts
were possible, and the criterion for survival seems to have been the presence of
macroscopic fragments of tissue at autopsy. Survival of the grafts was not con-
firmed microscopically, and the fact that the spleen normally contains many cells
that are characteristic of the specific homograft reaction would make it difficult, in
any case, to study the reaction against the spleen transplant histologically. The
true proportion of successful grafts is also obscured by the fact that about 10% of
what should have been compatible grafts could not be found at autopsy. On
all counts, therefore, one would expect skin grafts, which can be observed more
regularly and in more detail, to provide better material for this type of experiment.
Billingham et al. (1954) found that no graft (out of fifty trials) survived more than 17 days when skin was transplanted from one strain to the backcross generation of its \( F_1 \) hybrid and the other strain. Fewer successes are to be expected in this type of experiment with \( R_2 \) hosts than with \( F_2 \) hosts. Thus, if ten antigenic factors were acting the expectation of success would only be about 1 in 1000 as compared with 6 in 100 for \( F_2 \) hosts.

The numerous reports on experiments designed to study the genetic control of tumour transplantability have been reviewed by Snell (1948, 1953) and need not be considered in detail here. The least specific tumours seem to require no more than two or three histocompatibility genes. The most specific one was studied by Little & Tyzzer in 1916 and grew in only three of 186 \( F_2 \) hosts. The figures for this tumour indicate that fourteen or fifteen genes were responsible for the incompatibility between graft and donor and are, therefore, very similar to those which we have obtained for normal skin. The strains of mice used were not, however, inbred to the degree that is now required. It has been pointed out elsewhere that results with tumour transplants are likely to give underestimates of the number of responsible genes.

It is evident, that, with so many histocompatibility factors, the number of possible separate skin genotypes, even within the progeny of an \( A \times CBA \) mating, is almost astronomical in magnitude. We have only estimated the number of genes which are not common to both strains. If the combined total is at least twenty and if they are simple, independent, fully dominant genes, then they can be combined in \( 3^{20} \) ways to give \( 2^{20} \) phenotypes. But if each allele is antigenic, a belief which would bring the histocompatibility system into line with the blood-group systems, the number of phenotypes would be closer to \( 3^{30} \). Extending the calculations to all mice and taking into account the fact that ten alleles have now been described (Snell, Smith & Gabrielson 1953; Allen 1955) at the only locus (\( H-2 \)) which has been closely studied, gives an unimaginable figure. Should the situation in human beings be comparable to that in mice, the number of skin groups would certainly be sufficient to confer individuality of skin antigens on all except identical twins. The various refinements in blood-grouping techniques are clearly leading in the same direction.

The wide distribution of breakdown times found in the \( F_2 \) generation resembles that found by Billingham et al. (1956a) for mice which had acquired a semi-tolerance to skin of another strain as a consequence of the injection of a suspension of cells from the donor strain into the host while it was still in the uterus. Such mice show all grades of tolerance, from complete and apparently permanent acceptance of the foreign graft (survival > 400 days) to a state where the graft only survives for a few days longer than an ordinary homograft.

Whereas the normal breakdown of a graft, once it has begun, is rapid, the regression of a semi-tolerated graft may be long drawn out during a period 20 to 30 days (Billingham et al. 1956a) and is characterized by scabs, blemishes and eczematous patches on a contracted graft before breakdown is complete. Our impression from the study of the type of tolerated graft which we have produced does not accord entirely with this description. Rather, it is our belief that, once it has begun, the
reaction is often as rapid as in an ordinary homograft. Whether this indicates that the circumstances in which the two states of partial incompatibility are dissipated are different or not remains to be seen. Certain differences in origin are clear. In one, the tolerance is due to a decrease in the capacity of the immunity producing tissues to react to a full stimulus; in the other a fully reactive system is confronted by a partial stimulus.

As far as can be determined at present, the rejection of any graft depends on the prior development of some form of antigen-antibody reaction. There are, therefore, two phases in the whole process, one of immunization, during which antigen from the graft must reach the immunity producing tissues and find them in a fit state to respond and one of transmission of antibody to the site of the graft where it acts to produce local rejection of the graft.

If, as we believe, the process of graft destruction in our genetically tolerant mice is more rapid once it has begun and more comparable with the normal process than Billingham et al. (1956a) find it to be for their mice with acquired tolerance, it must follow that variations in the length of time for which these homografts survive depends on the time taken to build up immunity. There are several ways in which the graft antigens may elicit immunity.

(1) The graft antigens (foreign H-genes or their products) may induce the formation of individual antibodies which upon reaching a 'critical level' act separately upon the graft. This sort of reaction would account for the protracted niggling type of rejection found by Billingham et al.

(2) The 'critical level' may be produced by the summation of 'subcritical levels' of individual antibodies produced as in (1), virtually no change taking place in the graft until this level has been reached.

Variations in the survival time of homografts, as a measure of the degree of incompatibility between host and donor, would be determined by the number of different types of foreign H-genes possessed by the graft and would stretch as we have found, from the mean survival time of interstrain homografts virtually to infinity. Our data cannot give any useful estimate of the contribution which each H-gene makes to the rate of breakdown. Some clearly act very slowly and feebly; others are much more rapid and potent.

It is interesting to note that the survival time of CBA skin grafts to A hosts is less (10-2 days) than that of the reciprocal graft (A → CBA = 11-0 days) (Billingham et al. 1954), for we have found that fewer grafts from CBA donors survive for over a hundred days on F₂ hosts than from A donors, but that the mean survival time of CBA grafts on F₂ hosts is greater. If this difference is other than a chance one, the apparent contradiction may be resolved by supposing that, whereas the proportion of permanently surviving grafts in the F₂ hybrid is determined by the number of foreign H-genes lacking in the host, the rate of breakdown of incompatible grafts is determined both by quantity and quality of the foreign H-genes. If we assume that CBA tissues contain a greater number of active H-genes (quantity) than A tissues, then the number of permanent survivals on the F₂ hosts will be fewer for CBA grafts. The possession by A strain grafts of more 'strong' H-genes than CBA grafts might balance the extra number of active
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H-genes in CBA grafts, so that there was little difference in the mean survival time of interstrain grafts, but precocious breakdown between the 12th and 19th days after grafting to F2 hosts.

(3) The antigens of the graft may act together, as a combined antigen and call forth the production of a single antibody.

The description of the H-2^kr (now H-2^a) allele in A strain mice makes it clear that one histocompatibility gene may be responsible for the formation of more than one antigenic factor. Similar proposals of multiple antigens, dependent on the action of one gene, have derived from experiments on the blood group antigens in cattle (Stormont 1950; Stormont, Owen & Irwin 1951) and poultry (Briles, McGibbon & Irwin 1950). But the lack of absolute specificity in the serological methods used and the fact that a single synthetic antigen of known chemical composition may give rise to several distinct antibodies on the one hand and on the other may also react with several different antibodies, makes it impracticable to consider the relation of antigen and gene in detail.

There are clearly many opportunities for variations in the rate of development of either phase of the total response, but until we know more about the validity of the one gene/one antigen concept, the nature, or even the separate identity, of the antigens concerned and about the way in which the graft is destroyed, there is little advantage to be gained by further discussion.

Of the many histocompatibility loci that must exist, only one (H-2) has been closely studied. This locus is known to have a very strong influence on the outcome of a transplantation. Counce (1956) for example, has shown that skin exchanged between two isogenic strains, which differed at this locus only, will break down and be rejected just as quickly as skin exchanged between two entirely different strains. In contrast to this observation, she has also shown that, if the difference is at the H-1 or H-3 locus, breakdown occurs much later on, although it is ultimately complete. Some other loci are evidently as effective, singly or in combination, as the H-2 locus, since an equally rapid breakdown may occur where there are other multiple incompatibilities, even when the same allele is found at the H-2 site.

It is also true that compatibility at the H-2 locus will permit a certain proportion of tumours to survive in an otherwise foreign environment long enough to kill the host. Such does not seem to be the case where normal tissues are used (i.e. CBA skin does not survive in C3H mice, though both strains have the same H-2 allele).

Generally speaking, breakdown of successive grafts was accelerated whenever it had been shown that immunity to the first graft was established. Such immunity lasted for at least 100 days and was able to act fully on the second-set graft even when it came from a donor which was six generations removed from the original donor.

Billingham et al. (1956a) have shown that the secondary response is weakened in semi-tolerant mice, though their figures do not demonstrate the correlation between survival times of the first and second grafts which might be expected. Our own results are not sufficiently precise to establish the point firmly but they tend rather to show that, even when the first reaction has been slow to develop the secondary reaction is as vigorous as in normal mice. Differences between the
findings of Billingham et al. and ourselves, in this respect, may be due, as mentioned above, to the different circumstances in which semi-tolerance is achieved. Neither in these experiments nor in other experiments on rabbits (Krohn, unpublished) have we been able to demonstrate an 'immunoparalysis' even when as many as five sets of grafts have been transplanted in succession. But some conspicuous anomalies have occurred; in particular, the responses of expt. 224 (see above) cannot be fitted into any known pattern. It seems unlikely that the finding is related to the development of cancer in the mouse or that an immunoparalysis has been induced in this single instance, even if one believes that the production of an immunity which depends on minor antigenic differences only can be more easily paralysed.

In any case, it might be imagined that once the systemic immunity was sufficient to destroy one graft, it should rapidly destroy a second graft which was transplanted soon afterwards. Perhaps in these conditions, where one is dealing with threshold rather than overwhelming responses, the capacity to reject a graft becomes almost entirely 'used up' in the process of rejection, though the residues can accumulate with each successive graft.

Apart from the role it plays in determining histocompatibility, the H-2 locus is concerned with the simultaneous presence of two or more different antigens which can be detected by red-cell agglutination (Hoecker, Counce & Smith 1954; Amos, Gorer & Mikulska 1955). Thus a series of antigenic factors D, E, K can be defined by serological reagents, anti-D, anti-E, anti-DEK, etc. In this way, the H-2a histocompatibility group is shown to contain the several factors DE and K which are inherited as a unit.

But do these factors, demonstrated by red-cell agglutination, and the haemagglutinins, play any part in the rejection of skin homografts? Apparently not, as Mitchison & Dube (1955) have shown on several grounds. The time relations of the concentration of the agglutinins in the blood are quite out of phase with the development of resistance to the graft. Similarly, the titre of agglutinins in the blood bears no correlation to the fate of the graft. And finally, the grafts cannot be destroyed by passive transfer of serum but only of cells.

Tolerance to homografts can be obtained by the administration of whole blood to foetuses and this is presumably achieved by the immunity-producing tissues of the foetus coming into premature contact with the antigens contained in the inoculum. The active component of the blood seems, however, to be the leucocytes and not the erythrocytes. It can be suggested, therefore, that the histocompatibility substances (which are nucleoprotein in nature, as Billingham et al. (1956b) have shown) are direct products of the nuclei of cells and therefore not present in mature non-nucleated mammalian erythrocytes, while the agglutinogens—equally dependent on a genetic mechanism and developed in the earlier nucleated stages of the erythrocytes’ existence—are only found fixed to the surface of the cell.

One of the reasons why antibodies responsible for the homograft reaction have been so difficult to demonstrate may be that the amount of antibody required to cause graft destruction is much smaller than the amount which is induced against a heterologous protein. This would be especially so if graft destruction depends on
the independent action of the graft antigens and the summation of separate induced antibodies, the amount of each individual antibody being much smaller, though the total antibody material might be of the same order. When, however, the number of separate antigens is presumably reduced (as in some of our long surviving homografts) the amount of each of the fewer individual antibodies present when destruction finally takes place may well be raised to a more easily detected level. The $F_n$ generation animals, referred to below, may be a valuable source of serum from which to isolate homograft induced antibodies.

Many other problems relating to the acceleration of the immune response have not been amenable to study because the reaction ordinarily proceeds at its most vigorous, and only factors which slow down the rate of progress, such as cortisone, pregnancy or X-irradiation can be examined. The isogenic strains of mice which have been developed by Snell, and which have single allelic differences at a histocompatibility locus, should provide one source of material for such studies. Another way of developing a strain of mice with a consistently prolonged breakdown time of about 30 days, is to breed from the surviving semi-tolerant $F_2$ mice which have been produced during the course of this experiment ($F_n$ generation mice).

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