Induced Expression of the Male Isoantigen in the Skin of Female Mice.* (32602)

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Grafts can be successfully exchanged between animals which are genetically, and hence antigenically, identical. In 1955 Eichwald and Silmser(1) reported that female mice of the C57 strain rejected male grafts from the same inbred strain. This reaction was soon shown to have an immunological basis, since second-set reactions and specific tolerance were demonstrated(2,3,4). Hence it seems clear that male mice of the C57 strain possess an antigen which is not present in C57 females. In addition, male mice of many other unrelated inbred strains possess this same "male" antigen(5). Males and females, even of a highly inbred strain, are obviously not genetically identical, for male mice have an XY and female mice an XX sex chromosome constitution. It has been tacitly assumed by previous investigators that the gene determining this "male" antigen is located on the Y chromosome. It is possible, however, that the genetic information necessary to produce this antigen is located on some other chromosome, but that the antigen is expressed only in the male due to the influence of the male hormonal environment, or repressed in the female as a consequence of the female hormonal environment. The experiments in this study were designed to explore this latter possibility.

Materials and methods. Mice. C57Bl/6J and B6AF1 (F1 offspring of matings between C57Bl/6J males and A females) mice were purchased from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and maintained on a diet of commercial mouse pellets and water *ad lib*. The experiments to be described included procedures which verified the homogeneity and histocompatibility of these mice, since it was necessary to graft adult male and female C57Bl/6J mice with skin from newborn and adult C57Bl/6J female donors. In addition, the B6AF1 females were grafted with newborn C57Bl/6J female skin. All of these grafts survived permanently. Newborn mice were acquired either by ordering pregnant females from the Jackson Laboratory or by mating C57Bl/6J mice in this laboratory.

Skin transplantation. The technique used for skin transplantation was a modification of the method of Al-Askari, Lawrence and Thomas(6). Ether anaesthesia was used. The grafts were rectangular and measured approximately 1.0 × 0.7 cm. When a mouse received more than 1 graft, the immunizing grafts were placed in the left anterior axillary region, and the test graft on the opposite side. The direction of graft hair growth was reversed in relation to that of the recipient, facilitating observation of the borders of the graft. When retransplantation of grafts was necessary, care was taken to insure that no host tissue was retransplanted with the graft, and that the retransplant was not in a hair growth cycle. Recipients were usually unbandaged after 8 days, except that when second-set rejections were expected the recipients were unbandaged earlier. Mice which unbandaged themselves prior to 8 days postgrafting were included in the study only if the graft appeared healthy at 8 days.

Grafts were examined grossly at least every other day after unbandaging, and the day of rejection was recorded as that day on which complete graft breakdown occurred. A graph of cumulative graft mortality versus days after grafting was plotted, and the median survival time (MST), which is defined as the day after transplantation when 50% of grafts have been rejected, was read directly from the graph.

Hormone administration. Estradiol and testosterone were purchased from K & K Laboratories, Plainview, L. I. Solutions of 0.1 mg/cc and 0.3 mg/cc estradiol and of 2.0 mg/cc testosterons were made by first dissolving the hormone in a small amount of 100% ethanol and then diluting to the desired

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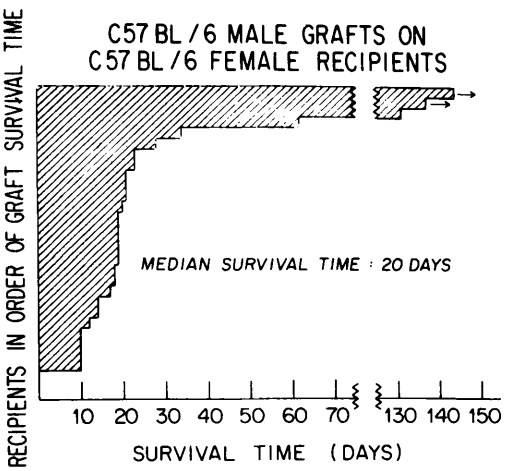


FIG. 1. Each bar represents the survival time of 1 first-set C57Bl/6J male graft on a C57Bl/6J female recipient. The arrows indicate graft survival beyond the observation time charted in this illustration. The survival times of the 27 skin grafts represented varied from 10 to >144 days, with a median survival time of 20 days.

concentration with Mazola corn oil. The above solutions were injected subcutaneously, 0.1 cc/day, in donor mice, according to the schedule shown in the experimental section of this report, and control animals were injected with corn oil.

Results. I. First-set and second-set C57Bl/6J male grafts on C57Bl/6J female recipients. Fig. 1 shows the survival of grafts from C57Bl/6J male donors on C57Bl/6J female recipients. While the usual survival of grafts across major histocompatibility barriers is only 9 to 11 days, the rejection of male skin by females of the same inbred strain is a slower, more chronic reaction, in which some grafts are rejected relatively quickly while others survive for long periods of time, sometimes permanently. The MST for male grafts on females of the C57Bl/6J strain in this laboratory was 20 days. The MST for this reaction in other strains is even longer(7). C57Bl/6J females which have already rejected one C57Bl/6J male graft reject a second graft in an accelerated fashion, as shown in Fig. 2. The MST for this reaction is 11 days. These observations were in good agreement with those of previous investigators.

II. Newborn C57Bl/6J male grafts on C57Bl/6J female recipients. In order to investigate the possibility that the hormonal environment of the adult male plays a role in the expression of the male specific antigen, newborn C57Bl/6J males were used as donors of skin grafted onto mature C57Bl/6J female recipients. These grafts had a MST of 56 days (Fig. 3) as compared with the MST of 20 days when grafts from adult donors were used. All of these grafts of skin from newborns were eventually rejected.

Medawar(8) has provided quantitative data demonstrating that the survival time of a graft is inversely proportional to its size, so that a large graft (containing more antigen) is rejected more quickly than a smaller graft (containing less antigen). The grafts from newborn and adult C57Bl/6J male donors were the same size, and the prolonged survival time of the newborn male grafts on C57Bl/

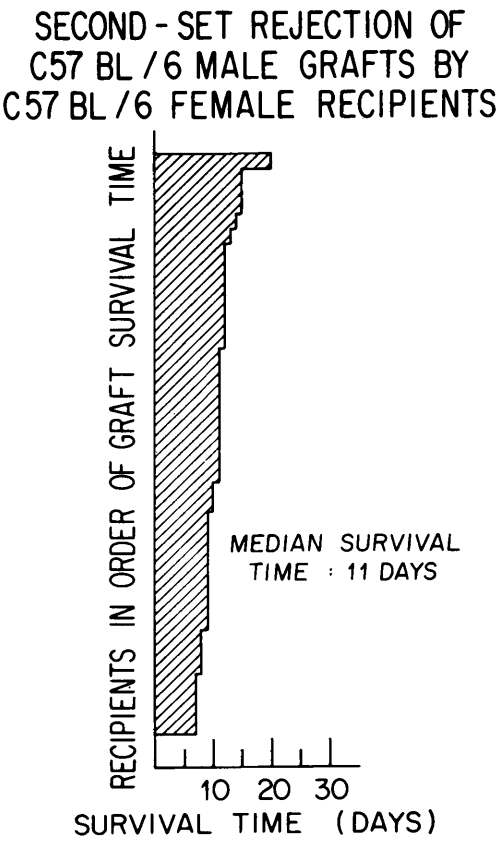
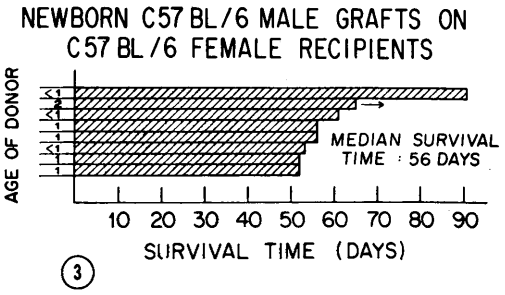


FIG. 2. Each bar represents the survival of one C57Bl/6J male skin graft on a C57Bl/6J female which had already rejected 1 male graft. The survival times of the 39 skin grafts represented varied from 7 to 20 days, with a median survival time of 11 days.



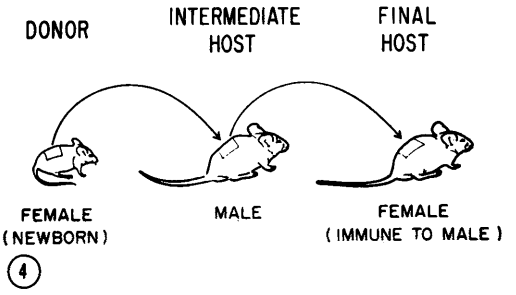


FIG. 3. Each bar represents the survival of 1 newborn C57Bl/6J male skin graft on a C57Bl/6J female recipient. The age of the donor is given in days. The 2 day old donor was between 48 and 72 hours old, the 1 day old donors were between 24 and 48 hours old, and the <1 day old donors were less than 24 hours old. The survival times of the 8 grafts represented varied from 52 to 91 days. The arrow indicates graft survival beyond the observation time charted in this illustration. The median survival times of these grafts was 56 days.

FIG. 4. In experiment IV skin from newborn C57Bl/6J females was grafted onto sexually mature C57Bl/6J male intermediate hosts, allowed to remain in residence for various periods of time, and then excised and retransplanted to adult C57Bl/6J female final hosts which had already rejected two C57Bl/6J male skin grafts. Modifications using intermediate female hosts, non-immune final hosts, and adult female donors were also performed. In experiment V the intermediate host was a B6AF1 female, and the final host was not pre-immunized. The data for experiment IV are in Table II, and the data for experiment V are in Table III.

6J females (MST = 56 days), as compared to the survival time when adult male donors were used (MST = 20 days), is therefore probably indicative of a lower concentration of male antigen on newborn male skin. Basch and Stetson(9) have demonstrated that H-2 antigens in C57Bl/6 mice, although present at birth, do not reach adult levels until about 20 days of age. The low concentration of male antigen on newborn C57Bl/6 skin may be just another example of this general phenomenon, or may be indicative of the hormonal immaturity of these newborn mice.

III. Exogenous hormonal treatment of C57Bl/6J male and female donors. Attempts were made to induce the male antigen in females and repress it in males by long-term exogenous treatment with testosterone and estradiol respectively. These treated animals were then used as donors of skin grafts to normal C57Bl/6J female mice (Table I). The grafts from testosterone treated females survived on female recipients, indicating that no new antigen had been induced by the treatment. In contrast, the MST of estradiol-treated male donor grafts was prolonged, but the survival of the grafts from oil-treated control animals was also prolonged, for reasons which are not apparent at present. These negative results were not surprising, since the simple treatments given would not be expected to mimic the complex hormonal differences between male and female mice.

IV. Newborn C57Bl/6J female grafts on adult C57Bl/6J male intermediate recipients retransplanted onto immune C57Bl/6J female final recipients, and various controls. Since it was judged to be impossible to reproduce accurately a male humoral environment in a female mouse, newborn C57Bl/6J female skin was grafted onto adult C57Bl/6J males, allowed to remain in residence for

TABLE I. Hormonal Treatment of C57Bl/6 Skin Graft Donors to Female C57Bl/6 Recipients.

No. of mice	Sex	Treatment	Age of donor at start of treatment (days)	Days treated	Median survival time (days)
23	Male	Estradiol	11–13	17–55	36
14	Male	Oil	12	17–55	58
26	Male	None	—	—	20
20	Female	Testosterone	13–16	30–60	Permanent
12	Female	None	—	—	Permanent

TABLE II. The Survival of Skin Grafts from Newborn C57Bl/6J Female Mice on Adult C57Bl/6J Female Recipients After Residence on Sexually Mature C57Bl/6J Male Intermediate Hosts, and Various Controls.

	Intermediate host*		Final female host	
Age of female donor (days)	Sex	Time in residence (days)	Pre-immunized†	Survival (days)
1,1,1,1	Male	8,8,8,8	+	>182,>182,>182, >182
1	Male	15	+	>175
<1,<1,<1,<1	Male	25,25,25,25	+	17,>189,>189, >189
3,3,<1,1,2,2,2,1,<1,4	Male	40,41,40,43,41,41,41, 41,40,41	+	9,9,10,11,16,17,17,23, >163,>239
<1,1,<1,<1	Male	46,46,47,47	0	33,36,>234,>234
77,73,73,73,73	Male	40,40,41,41,41	+	>165,>164,>164, >165,>165
1,1,<1	Female	62,62,63	+	>171,>171,>171

* The intermediate hosts were all sexually mature.

† The survival time of the 2 immunizing grafts, the time allowed to elapse between immunizing grafts, and the time between immunization and retransplantation had no effect on the final outcome.

various periods of time, and then excised and retransplanted onto C57Bl/6J female recipients already immune to male skin. In this manner female skin remained healthy and viable for long periods of time in the tissue environment of a male mouse. The experimental plan is illustrated in Fig. 4. Modifications using non-immunized final recipients, adult female donor grafts, or intermediate female hosts were also performed, and the results are shown in Table II.

8 out of 10 newborn female donor grafts that had been in residence on an adult male for 40-43 days and then excised and retransplanted to an immune female recipient were rejected. The MST for these grafts was 16.5 days. When the intermediate recipient was female rather than male, the retransplants survived permanently, showing that grafts could survive the retransplanting procedure. When the final recipients were not pre-immunized, 2 out of 4 retransplants survived and 2 were rejected in 33 and 36 days respectively. This prolonged survival, compared with a MST of 16.5 days with pre-immunized recipients, is consistent with the hypothesis that the antigen induced on newborn female skin and the antigen of normal males are identical. When adult female donor skin was used instead of newborn, the retransplants survived permanently. When the period of residence of newborn female donor grafts on the intermediate male recipient was as short at 8 to 15 days, all of the retransplants survived, and 3 out of 4 grafts retransplanted at 25 days also survived.

These experiments seem to indicate that newborn female skin which has been in residence on an adult male for sufficient time can be shown to possess "male" antigen. One possible explanation for this finding is that male antigen had been adsorbed onto the cell surfaces of the graft. However, adsorption of antigens, if it occurred, would be expected to occur prior to 15 days post-grafting; yet the 8 and 15 day retransplants and most of the 25 day retransplants survived permanently on the final hosts. Another possible explanation is that cells from the intermediate host containing male antigen had infiltrated the graft, the rejection being essentially a reaction against these cells. However, in the short term retransplants (8 days) the inflammatory and wound-healing responses, originating in the host, were still occurring, so that these retransplants contained many intermediate host cells. Yet these retransplants survived perfectly well on the final hosts. In addition, adult C57Bl/6J female skin, treated in the same manner as newborn female skin, and subject to the same potential host cell infiltration (and antigen adsorption), survived permanently on the final host. It is unlikely that host cell infiltration or antigen adsorp-

TABLE III. The Survival of Skin Grafts from Newborn C57Bl/6J Female Mice on Adult C57Bl/6J Female Final Recipients After Residence on B6AF1 Female Intermediate Hosts.

Age of female donor (days)	Time in residence on intermediate B6AF1 host (days)	Survival on final host (days)
1	41	10
1	41	>181
1	41	>181
<1	41	>187
<1	41	>187
<1	41	>187
1	40	>189
1	40	>189
1	40	>189
1	40	>189
1	40	>189

tion would occur to a strikingly different degree in newborn and adult grafts. Instead, it is more reasonable to assume that the cells of a newborn are not yet fully differentiated, and can therefore still be induced to produce antigens which adult cells have lost the ability to produce. Hence the evidence accumulated thus far favors the hypothesis that in a male environment newborn female cells can be induced to produce male antigen.

V. Newborn C57Bl/6J female grafts on adult B6AF1 female intermediate recipients retransplanted onto adult C57Bl/6J female final recipients. In this experiment the intermediate recipient was an F1 female from a mating of a C57Bl/6J female and an A male. The C57Bl/6J newborn female graft was in residence on this F1 intermediate host for 40-41 days before excision and retransplantation to a C57Bl/6J female. If ingrowth of host cells or adsorption of antigens were the correct explanation of the experimental data in part IV, the newborn C57Bl/6J female grafts should acquire "A" antigens from the F1 intermediate host and subsequently be rejected by the C57Bl/6J female final recipients. These final recipients were not pre-immunized against "A" antigens because the histoincompatibility between A and C57Bl/6J is much greater than between C57Bl/6J male and female, and the use of a non-immunized final recipient makes the situation more nearly comparable to that in which C57Bl/6J male intermediate recipients were used and the final C57Bl/6J female host was pre-immunized against male antigen. As Table III shows, 10 out of 11 retransplants from F1 intermediate hosts survived on the final host. These data seem to rule out the possibility that host cell infiltration and antigen adsorption could account for the data in experiment IV. Instead, the best explanation of the experimental results is that newborn female skin in an adult male environment can be induced to produce "male" antigen, and that skin cells from adult females have lost the capacity to be so induced.

Discussion. The apparent induction of "male" antigen in newborn female skin cells would indicate that both male and female mice have the genetic information necessary for the production of this antigen. The fact that this antigen normally appears only in the male may be only secondarily dependent on the Y-chromosome in its role as the carrier of information leading to "maleness"(10). Differences between male and female sex hormonal patterns may very well be of prime importance in determining the activity of the "male" histocompatibility locus. The unsuccessful attempts to induce or repress the antigen by exogenous hormonal treatment may only reflect the difficulty of administering the proper combinations and concentrations of hormones, or the failure to begin treatment before significant sexual differentiation has occurred.

Many studies have demonstrated that the total antigenic capabilities of an organism are never simultaneously expressed. Among the best studied examples are the changes from fetal to adult hemoglobins(11). Perhaps the best example of humoral control over the expression of a cell surface antigen is the TL (thymus leukemia) antigen described by Boyse, Old and Stockert(12). In the present study, the male antigen appears to be another example of a transplantation antigen whose expression can be controlled, or "modulated," by humoral factors. In view of the presence of the male antigen in so many inbred strains, the antigen may be a common biochemical entity which is either induced or structurally altered in a male hormonal environment, causing it to be antigenic in a female of otherwise identical genetic constitution.

A great deal of work remains to be done in this system. It would be interesting to study

other inbred strains under the same experimental conditions, and to see whether F1 males from different strain combinations have the same effect when used as intermediate hosts as C57Bl/6J males. Even more important is the task of localizing the source of the inducing substance, and then characterizing the inducer itself. It should be pointed out that the data obtained in the present study are also consistent with the hypothesis that the gene involved has been derepressed as a consequence of removal from a female environment, rather than induced to function as a consequence of transfer to a male environment. Intermediate male hosts which have been adrenalectomized, gonadectomized, hypophysectomized, or subjected to a combination of these procedures may help to supply answers to some of these questions.

Finally, it may be worth suggesting that antigenic alterations controlled by hormones may play an important role in the pathogenesis of certain human diseases, suspected of being autoimmune in nature, which occur in a population undergoing acute hormonal changes; examples are systemic lupus erythematosus occurring in females just past puberty, and eclampsia occurring in pregnant women.

Summary. It has been previously assumed that the "male" isoantigen of mice is determined by a gene located on the Y chromosome. This study seems to indicate that newborn female skin, nourished in a male environment, can be induced to express the male antigen. Hence the expression of this antigen seems to be influenced by the Y chromosome only in its "male-determining" role, and the gene responsible for the production of this antigen may be present in both sexes.

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Role of β-Receptors in Sympathetic Regulation of Electrolytes in Rat Submaxillary Saliva.* (32603)

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Previous work on rat parotid gland demonstrated that pilocarpine evokes a saliva that has sympathetic-like characteristics and that these characteristics result from pilocarpine stimulation of sympathetic pathways(1). This sympathetic component is inhibited by pro-

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