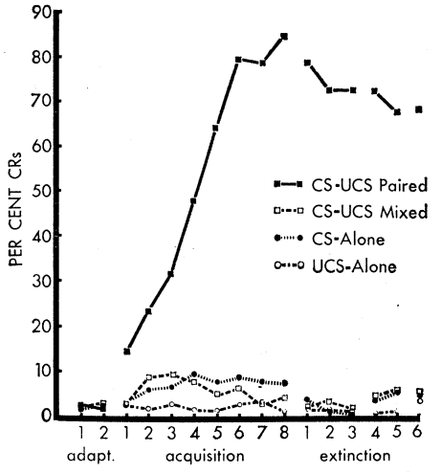

Fig. 1. Mean percentage of responses plotted in 70 trial blocks for adaptation, acquisition, extinction, and spontaneous recovery. CRs = conditioned responses. Blocks of trials are indicated along the abscissa.

The fourth group (group CS-UCS Paired) was the classical conditioning group and it received paired presentations of the CS and UCS at a CS-UCS interval of 500 msec. Twelve rabbits were assigned to the classical conditioning group and four to each of the control groups. Groups CS-UCS Paired, CS-Alone, and UCS-Alone received 70 acquisition trials per day at randomized intertrial intervals of 20, 30, and 40 seconds (mean of 30 seconds); whereas group CS-UCS Mixed received 70 CS alone and 70 UCS alone trials, randomized with the restriction that no more than two CS alone or two UCS alone trials occurred consecutively; these trials were presented at randomized intertrial intervals of 10, 15, and 20 seconds (mean of 15 seconds). During extinction, all groups received 210 presentations of the CS alone on the first day, 140 on the second, and 70 on the third.

In adaptation and acquisition each eyeball retraction was recorded, provided it was at least 1 mm in amplitude and occurred no later than 525 msec after "CS onset"; and in extinction the recording interval was extended to 600 msec. During acquisition the responses of rabbits in group CS-UCS Mixed were recorded only on trials in which the CS was presented, and for group UCS-Alone the responses were recorded in an interval from 500 msec before to 25 msec after onset of the UCS. Figure 1 shows the results of plotting the percentage of eyeball retractions for all groups in the latency range of 55 to 525 msec (defined as the latency range of conditioned responses) for blocks of 70 trials. Examination of the figure reveals that for adaptation the percentage of responses was about 2 percent in all groups. In acquisition, none of the control groups demonstrated a level of responding which exceeded 9 percent on any day, whereas group CS-UCS Paired showed a steady increase in the percentage of conditioned responses and attained a level of 85 percent on day 8. The extinction curves show that the control groups did not demonstrate more than a 5-percent level of responding. On the other hand, group CS-UCS Paired showed considerable resistance to extinction by responding at a level of 79 percent on trial-block 1 and showing only a small decline to 68 percent on trial-block 6.

The performance curves obtained in the investigation clearly indicate that the course of acquisition and extinction of the retractor bulbi response in the classical conditioning group cannot be attributed to changes in the spontaneous frequency of eyeball retractions resulting from UCS presentations (group UCS-Alone); pseudoconditioning or sensitization (group CS-UCS Mixed); nor can they reflect the occurrence of alpha (reflex) responses to the CS (group CS-Alone), particularly since examination of the latency distribution of responses revealed no evidence of alpha responses (that is, the few responses observed were unsystematic in their distribution). Although the rate of conditioning of eyeball retractions does not appear to be appreciably different from that observed for the nictitating membrane (*5*), the response does demonstrate substantially greater resistance to extinction. Whether this increased resistance to extinction is attributable to the retractor bulbi response system or to the differences in methods of detecting the responses has not yet been ascertained (*6*).

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Mosaic Histocompatibility of Skin Grafts from Female Mice

Abstract. *The histoincompatibility determined by one or more genes on the X chromosome of the mouse effects a complete rejection of skin of the (C57BL/6 ♀ × BALB/c ♂) F_1 hybrid male grafted onto the reciprocal type F_1 hybrid male, but only an incomplete rejection of either reciprocal type F_1 hybrid female skin, grafted onto the same type of male host. The resulting mosaic survival pattern of the female graft is interpreted as support for the Lyon hypothesis of X-chromosome inactivation.*

Lyon (*1*) has postulated that either the maternally derived or the paternally derived X chromosome of each somatic cell in the normal female mouse becomes genetically inactivated at the time in embryogeny when it also becomes heteropyknotic, a cytological phenomenon described by Ohno and Hauschka (*2*). Furthermore, the particular X chromosome that is inactivated is initially determined at random but remains inactivated in subsequent cell generations so that cells of the two X inactivation types will be distributed with equal frequency and as clones in adult tissues. Inactivation of X, she hypothesized, is a normal method of gene-dosage compensation, permitting the genes of only one X chromosome to be effective in each cell of the female, as in the male.

The bulk of evidence supporting the hypothesis comes from sex-linked genes (or normally autosomal genes translocated onto the X chromosome) which through local gene action affect either the structure or color of the coat. The observed effect for such genes in the heterozygous female is a curious mosaic pattern of gene expression. Sex-linked genes with nonlocal action, on the other hand, show variable gene expression in the heterozygous female. Such observations on both local and diffuse acting genes to date have been consistent with the hypothesis. Lyon has recently reviewed the supporting evidence (see *3*) and has extended the hypothesis to cover mammals in general.

A further test of the hypothesis was made possible by a recently reported discovery of one or more histocompatibility genes located on the X chromosome of the mouse (*4*). The existence of these genes was demonstrated by the rejection of grafts exchanged

between reciprocal types of F_1 hybrid males derived from highly inbred strains. Such males carry X chromosomes of different origin. The effect is not observed in grafts between reciprocal types of hybrid females, for they carry both X chromosomes of the two origins.

Ordinarily, one would expect skin of either reciprocal type hybrid female placed on either reciprocal type male to be rejected, for females have one additional and different X chromosome which the males do not have. In contrast, however, if we assume the Lyon hypothesis to be true, somatic cells of such females would have either one or the other X chromosome in a genetically inactive form. Therefore, when F_1 hybrid female skin is grafted onto an F_1 male, half of the grafted cells, on the average, should be incompatible and rejected, and half should survive, provided these genes exhibit local gene action. If the hypothesis is true but gene action is diffuse, then grafted female skin might show a weaker and more variable antigenicity than male skin grafts, reflected by fewer rejections or by a longer mean survival time or both.

All mice used in this study were one-month-old F_1 hybrids derived from the cross of pedigreed strains: BALB/cAnNBy ♀ × C57BL/6JNBy ♂, or from the reciprocal cross. The mice so produced are designated CBF_1 and BCF_1, respectively.

The grafting technique was that of transplanting tail skin (*5*), but modified by not using antibiotics and by substituting several folds of masking tape for the collodion and wound clips to hold the protective glass tubing in place on the tails. All grafts were rotated through an angle of 180° so that grafted tissue could be recognized by hair and tail-scale direction. The area of the grafts was 6 to 12 mm^2; the grafts were of dorsal tail skin so that pigmentation was of maximum density.

The CBF_1 male was the only host used in the experiment. The BCF_1 male was not used as a host, for its rejection of the CBF_1 male graft, 60 percent of the time (*4*), was too inconsistent for effective use in the present tests. Three grafts were placed linearly on the dorsum of each host tail. One was always a CBF_1 male control graft; the other two were different types according to the design of the experiment. These various donors-on-host combinations, as well as the numbers of hosts used in each, are presented in detail in Table 1. The order of the three grafts on the tail was varied to eliminate any fault in the results arising from relative position.

Grafts were observed once a week with a magnifying hand lens (×14) throughout 15 weeks. To avoid biased recorded observations, the type of hybrid donor of every graft was kept

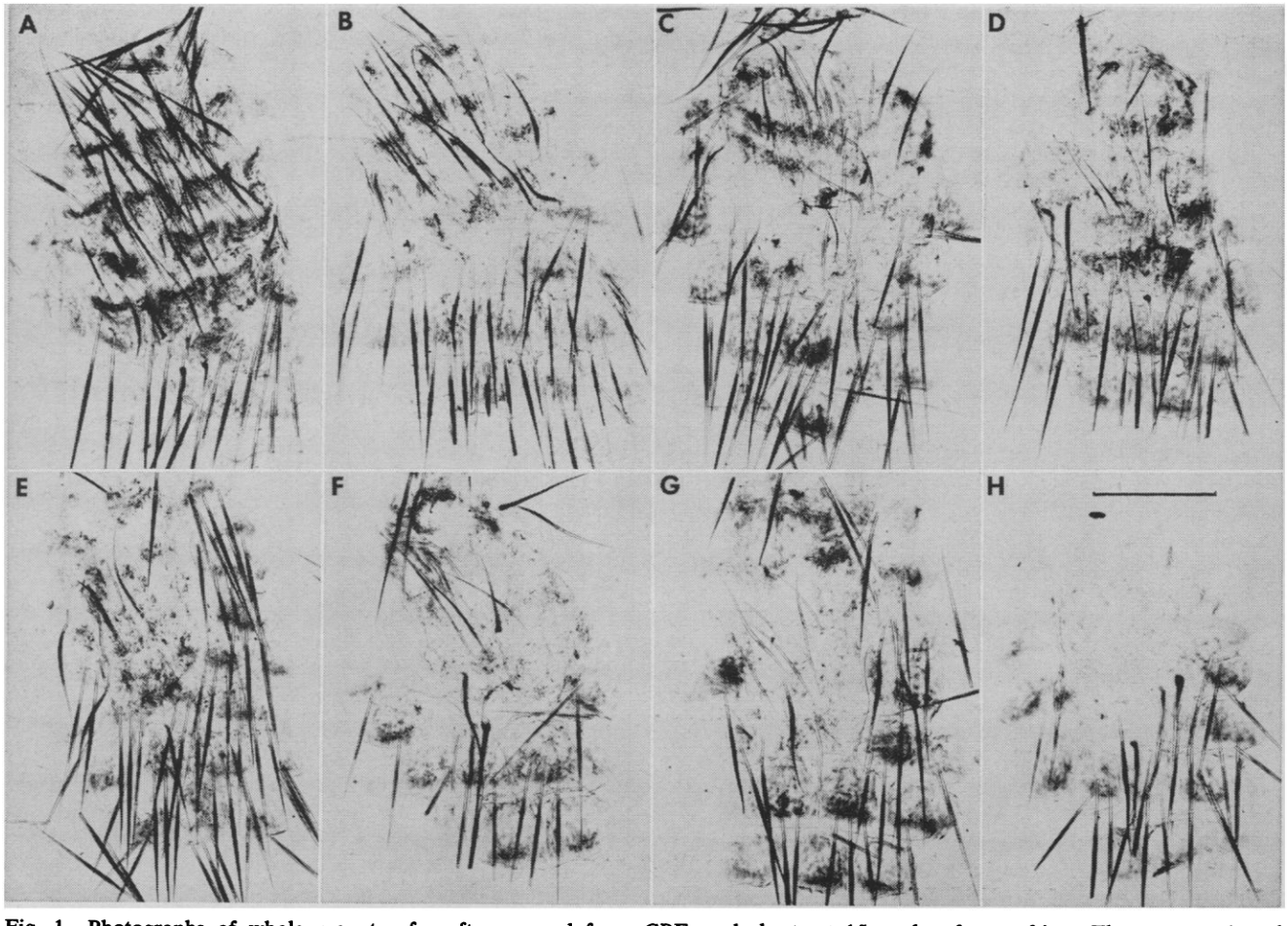

Fig. 1. Photographs of whole mounts of grafts removed from CBF_1 male hosts at 15 weeks after grafting. These were selected to illustrate the range within which mosaic graft survival was observed. Host hairs are pointing downward; graft hairs are pointing upward. *A*, A CBF_1 male control graft; *B* through G, CBF_1 female grafts; and *H*, a BCF_1 male graft. The straight line drawn in *H* represents 1 millimeter.

unknown to the observer until the end of the experiment.

Graft rejection was of the slow type; the graft was evaluated on the condition of hair, pigment, and scale pattern. The criterion which we termed "first sign of rejection" (FSR) permitted us to compare graft survival in the earlier stages of rejection. This, in effect, was the time when a graft first showed either pigment loss, scale-pattern loss (usually seen as scaling and flaking of the epidermis), or stubbled hair (ends of hairs broken off just above the surface of the skin).

After the gross observations were complete at 15 weeks, each graft was removed, pressed to a slide, dried completely, and then covered with a mounting medium and cover slip. From these slides we examined each graft under the microscope for surviving graft tissue. The donor of each graft on the slide was unknown to the observer.

We used three categories of survival: (i) Complete survival indicated that the condition of pigment, scale pattern, and hair was equal to that of the surrounding host tissue; (ii) partial survival indicated an intermediate condition; and (iii) no survival indicated no evidence of grafted tissue.

The results of all tests are summarized in Table 1. The average interval prior to the first sign of rejection was very similar for the different graft types except that female grafts did not show these signs as soon as the BCF_1 male grafts did. When all female results were pooled, the differences were statistically significant ($P < 0.05$) by the t test. Differences of FSR in other group comparisons were not significant.

All control CBF_1 male grafts in all tests showed complete survival: hair, pigment, and scale pattern were maintained. All BCF_1 male grafts showed complete rejection (Table 1). The grafts from both types of females, on the other hand, showed survival ranging from almost complete survival to no survival.

The photographs in Fig. 1 depict some of the characteristic types of graft survival encountered. A few approached the appearance of the control CBF_1 male graft (Fig 1*A*), but pigment was always a bit less dense in these cases. In some grafts pigment was diluted and only a patch of the graft was still present with its scale pattern evident; the hair was nearly normal but a few scattered pigmentless hairs were usually present (Fig. 1*B* and *C*). In other grafts pigment was diluted to varying degrees; scale pattern, if any, was barely visible, and the hair was sparse and variably pigmented (Fig. 1*D*, *E*, and *F*). In still other grafts, pigment was entirely missing and the hair was mostly of a fine, pigmentless type (Fig. 1*G*). Over a quarter of the female grafts resembled the BCF_1 male graft (Fig. 1*H*) by having no surviving tissues.

Table 1. Time of first sign of rejection (FSR) and graft survival condition at 15 weeks after grafting for both male and female grafts with different kinds of accompanying grafts. CBF_1 male hosts were used in all tests.

Accompanying grafts	No. hosts*	No. grafts	FSR ± SE (weeks)	Graft survival		
				Complete	Partial	None
		BCF_1 ♂				
BCF_1 ♂ + CBF_1 ♂	6	12†	3.17 ± 0.26	0	0	12
BCF_1 ♀ "	6	6	3.00 ± 0.34	0	0	6
Pooled	12	18	3.11 ± 0.20	0	0	18
		BCF_1 ♀				
BCF_1 ♂ "	6	6	3.33 ± 0.40	0	4	2
BCF_1 ♀ "	6	12†	4.00 ± 0.31	0	10	2
CBF_1 ♀ "	6	6	3.67 ± 0.75	0	6	0
Pooled	18	24	3.75 ± 0.26	0	20	4
		CBF_1 ♀				
BCF_1 ♀ "	6	6	4.33 ± 0.70	0	4	2
CBF_1 ♀ "	6	11†‡	3.59 ± 0.25	0	5	6
Pooled	12	17	3.85 ± 0.30	0	9	8
		CBF_1 ♂				
Varied	30	30	—	30	0	0

* Note that the roles of "test" graft and "accompanying" graft were reversed for certain entries of the table. This means that the number of hosts was sometimes entered twice. The number of individual host mice actually used in the experiment was 30, as reflected by the number of hosts bearing CBF_1 male control grafts. † Data on both "test" and "accompanying" grafts were pooled when of the same type. ‡ One graft was lost within 1 day after grafting due to technical failure.

The difference between survival of the male and female grafts was highly significant ($P < 0.01$) in a χ^2 test. The difference between survival of the reciprocal type hybrid female grafts was not significant.

Over 75 percent of the female grafts in all tests reached a condition in which all pigment was lost, the scale pattern was difficult to discern, and only stubbled hair, if any, remained. This period of quasi rejection began from 4 to 6 weeks after grafting and lasted for about a month before a limited recovery of pigment, hair, or both was evident. Control grafts never went through such periods, and BCF_1 male grafts never exhibited recovery with reappearance of indications of graft survival.

The results of this experiment offer strong support to the hypothesis presented by Lyon. The various types of partial graft survival are consistent with what might be expected from an early random determination of X-chromosome inactivation.

We also conclude that the pertinent genes have local action. This is not only indicated by the mosaic survival pattern of the female grafts, but, also, the first sign of rejection of female grafts was observed at the same time (the difference not being statistically significant), whether or not grafts were on the same hosts with BCF_1 male grafts. This indicates that antigenicity is not any weaker in female than in male tissues as nonlocal gene action might require.

The recovery of recognizable characteristics of grafted cells after the appearance of nearly complete rejection can be interpreted as an initial rejection of a critical number of supportive cells in the graft followed by eventual replacement by host cells and by regenerated donor cells.

The slightly longer (but statistically significant) time taken by female grafts to show the first sign of rejection compared to that of the male grafts indicates a possible masking or protective action of the compatible female cells, which interferes with early signs of rejection of the incompatible cells (*6*).

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