HISTOCOMPATIBILITY IN THE CHICKEN AS AFFECTED BY THE SEX CHROMOSOMES

by

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B.S., Kansas State University, 1961

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE
GENETICS

Department of Dairy and Poultry Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1967

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INTRODUCTION

Chickens are known to vary in their karyotype according to sex, the female being heterogametic ZW and the male ZZ (Shoffner, 1965; Owen, 1965). At present no gene has been assigned to a locus on the female W chromosome.

The present studies evolved from observations on primary skin graft exchanges within inbred lines and their reciprocal F_1 hybrids carried out to determine degree of isohistogenicity. In two lines more ♀ → ♂ grafts were rejected than grafts in other sex combinations, a result explainable on the basis of a W-linked histocompatibility antigen. The presence of some males that accepted female grafts was noted. This result suggested that they might possess a W-chromosome translocation in their genome, or alternatively that they were immunologically less responsive against weak histocompatibility antigens. A third inbred line did not show ♀ → ♂ graft rejections as essentially all grafts were accepted in all sex combinations.

On the basis of these preliminary observations experiments were set up to test the hypotheses that Z- and W-linked histocompatibility antigens exist in chickens. Tests were also conducted to determine whether a gene(s) controlling immunological responsiveness was located on the Z chromosome.

REVIEW OF LITERATURE

Kozelka (1932) grafted secondary sexual structures (comb, spur and wattle) from young chicks, usually 3 days old, to various parts of the body of the same individual and to the bodies of other individuals of the same age. Several substrains of Leghorns were used to produce unrelated chicks, a group of half or full brothers and sisters, and a group produced by back-crossing daughters to their fathers. A significant correlation existed between the coefficient of relationship and the percentage of grafts persisting. Sex was a factor in determining absorption. A significantly larger number of grafts was absorbed when female tissues were grafted on male hosts than in any of the other combinations. This "sex antagonism" was further supported by the great variation of the female spur and comb grafts on male recipients. The female served equally well as a host for either male or female tissues.

Eichwald and Silmser (1955) using inbred mouse strains C57BL and A/Jax and their reciprocal F_1 mice as recipients noted that if skin grafts were from male donors of either parental strain on F_1 females or intrastrain grafts on C57BL females that 0/28 were accepted. In strain A/Jax, 10/18 intrastrain ♂ onto ♀ grafts were accepted. By contrast ♂ to ♂, ♀ to ♀, and ♀ to ♂ grafts almost always succeeded (64/68).

The original data of Eichwald and Silmser were interpreted at their request by Hauschka (1955), who proposed Y linkage of a histocompatibility gene. Endocrine requirements of male skin lacking in female hosts was considered a less probable alternative. Snell (1956) found Y linkage both intriguing and plausible, but doubted if firm proof could ever be obtained.

Prehn and Main (1956) observed the rejection of isologous ♂ to ♀ skin grafts in C57BL mice, but a lack of rejection within strains DBA/2, C3H/He and BALB/C An. Controls were successful ♀ to ♂ isologous grafts. They were unsuccessful in showing that a second-set effect might be a method of demonstrating antigens too weak to be observed by primary grafts alone using BALB/C An, C3H/He and DBA/2.

Eichwald et al., (1957) observed further skin grafts with the strains of mice used in 1955. They tested two hypotheses: (1) The existence of a histocompatibility gene on the Y chromosome, and (2) the assumption that male grafts require an adequate level of male sex hormone which female animals do not supply. Evidence for (1) was the capacity of first male grafts to immunize an isologous female (or their F_1 hybrids) so that second male grafts were more speedily rejected as observed for other histocompatibility antigens. First-set male grafts were rejected in 31 ± 8 days, and second male grafts from genetically identical male donors in 13 ± 0 days. An attempt to rule out (2) was made. Male grafts were placed on three groups of female mice, one of which had been castrated, another injected with testosterone, and another after having received testicular grafts from male mice of the same strain. Also a group of male mice received male skin grafts after having been castrated and injected with estrogen. The results were not clear cut. Several of the female mice rejected while male mice accepted the male skin as if no pretreatment had taken place. In several testosterone-treated females the grafts survived longer than expected. In one of the castrated females the male graft still persisted after 4 months. Graft survival was shortened in all females having received testicular isografts. This probably represented a second-set phenomenon, the

testicular material having served as the first set.

Short and Sobey (1957), using mice, found the sex of donor had little or no effect on grafts in A^W and DBA/Jax strains, but a marked effect in A/Fa and C57BL/Fa strains. They stated that if the hypothesis of androgen dependence is correct the fact that male grafts are not always rejected in some lines may be accounted for by postulating a threshold effect. If the presence of a histocompatibility gene on the Y chromosome was correct, the lack of response in some lines could be due to incomplete penetrance.

Hirsh (1957) used strain C57BL mice of both sexes as recipients of isologous day-old thymus grafts from both sexes and noted that female recipients rejected over half the male thymic grafts, whereas 70 to 90% of grafts in other groups were successful.

Feldman (1958) found if lymph nodes of male C57BL mice immunized by C3H tumor MCIM were transferred to non-immunized C57BL male and female mice that immunity was obtained in all sex combinations when they were challenged with a test graft of MCIM 3 days later. However, when animals were inoculated with MCIM cells 7 days after implantation of the activated cells the lymph nodes transferred from ♂ to ♂ conferred immunity (only 1/10 gave a lethal take), whereas those transferred from ♂ to ♀ did not show transfer of immunity, resulting in 10/11 successful homografts. As lymph nodes transferred from ♀ to ♀ also showed transfer of immunity, the lack of cytotoxic immune response against MCIM in the ♂ to ♀ transfer was attributed to destruction of male lymph node cells by the female host. Using C3H strain male and female hosts preimmunized with spleen or liver cells from males before transfer of male immunized lymph nodes, Feldman found inoculated males' tumors to be significantly smaller than females.

This indicated that spleen and liver as well as lymph nodes contain the Y-determined antigen. Similar results were obtained in mice of BRS, RIII and Swiss strains.

Michie and McLaren (1958) proposed that strain differences in rejection of ♂ to ♀ grafts could be due to three possible causes: (1) Strain differences in a Y-linked histocompatibility gene or genes and hence in Y-controlled antigens, (2) strain differences in respect of the donor's capacity to give effect to male specific antigen, or his skin's capacity to survive the immune response of the recipient, and (3) strain differences in respect of female recipients' capacity to respond to the male specific antigen. To discriminate between the three hypotheses, they suggested using a strain showing the Eichwald-Silmser effect in high degree (A/Fa), a strain not showing the effect (DBA/Jax), and the reciprocal F_1 hybrids between the two. Hypothesis (1) would be tested by grafting skin from two types of F_1 hybrid males to F_1 hybrid females. If the hypothesis was true, grafts from males with DBA/Jax fathers would take, while grafts from males with A/Fa fathers would break down. If not true the two types of grafts would behave alike. To discriminate between hypotheses (2) and (3), they would graft male skin from the two parent lines onto female F_1 hybrids. If hypothesis (2) was correct, grafts from DBA/Jax males would take while A/Fa male grafts would break down. If hypothesis (3) was true, grafts from the two donor strains would behave alike.

Mariani et al., (1958) found that tolerance to isologous male skin could be produced in female mice of A and C57BL strains by injection at birth of living spleen cells taken from isologous male donors. These results provided evidence for the immunological basis of male skin graft rejection by

untreated isologous females.

Sachs and Heller (1958) showed accelerated second-set rejection of all isologous ♂ to ♀ grafts within strains C3H, BRS, C57BL, A/Jax x C3H F_1, DBA/2 and BALB/C. Using C3H mice they demonstrated immunizing effects of spleen and liver cell injections followed by skin grafts. They were unsuccessful in detecting hemaglutinins or cytotoxic antibody following repeated skin grafts, or repeated skin grafts and spleen or liver cell injections.

Eichwald et al., (1958) noted the strength of rejection of ♂ to ♀ grafts varied from strain to strain using strains C57BL, A/Jax, C3H, ST, and BALB/C. Rejection of male skin by F_1 recipient females was universal provided one of the parents was strain C57BL. The male factor was found in all normal tissues of C57BL males tested including lung, salivary gland, blood, spleen and liver. Experiments using F_1 hybrids, second-set phenomena, and tumor 58 indicated that the male factor was not strain specific. Castration (2 weeks before grafting) and administration of hormones (testosterone propionate, 0.05 ml subcutaneously 2 weeks prior to grafting, repeated at grafting or 2 weeks after grafting) did not significantly influence the fate of ♂ to ♀ isografts.

Bernstein et al., (1958) found that castration of females and males 3 weeks prior to grafting had no effect on the survival time of ♂ to ♀ skin isografts in C57BL/6 mice. These data suggested the same gene was present in C57BL/6 and DBA/2 mice, as ♂ to ♂ reciprocal hybrid grafts were accepted.

Krohn (1958) noted the histological appearance of male grafts undergoing destruction does not differ materially from that seen in the ordinary homograft reaction. Dead epithelium was undermined and cast off as a scab

while the dermis was widely infiltrated with round cells and plasma cells. Reaction time was more drawn out than in homograft reaction, making exact time of breakdown very difficult to establish.

Zaalberg (1959) found sex of donor had no effect on isologous skin grafts with CBA mice, but a marked effect when C57BL females were grafted with isologous male tissue. Both types of reciprocal F_1 females rejected either type of reciprocal F_1 male tissue and both types of parental male tissue. The results indicated CBA males possessed the histocompatibility factor. The histocompatibility locus of CBA and C57BL Y chromosomes were assumed to give rise to identical antigens, as F_1 males accepted skin grafts from both parental line males. Also interchanges between reciprocal F_1 males were accepted.

Hauschka et al., (1959) found ♂ to ♀ skin grafts in several inbred mouse strains gave three types of response: (1) Consistent rejection in C57BL/Ha and A 129/Ha, (2) varying frequencies of compatibility in C3Hf/Ha and Y/HeHa, and (3) complete compatibility in DBA/2 and Y/HeHa subline 2. The male factor was present in all strains of mice as shown by suitable outcrosses. Female nonreactivity was attributed to the presence of the Y antigen in male-compatible females, resulting from translocation or non-disjunction during spermatogenesis. Occasional rejection of female skin by females in group (2) fitted this hypothesis. Male tolerance was experimentally induced in females by X-irradiation, injection of male cells at birth, and (in only 1 case) by foster nursing a female from a refractory strain on a male-tolerant nurse. They showed that isologous female anti-male sera contained weak but definite leucoagglutinins specific for male leucocytes and leucocytotoxins specific for male spleen cells; they did not

agglutinate male red blood cells. Five tumors originating in C57BL/Ha male mice contained the male antigen as shown by their transient growth inhibition in females.

Mariani et al., (1960) using ♂ to ♀ skin isografts with strain A mice of different ages found age of recipient made a difference in acceptance. Older females rejected significantly more grafts than younger females. Younger male skin had a greater growth potential on old females than old male skin, but old and young male skin was accepted equally well on young females.

Billingham et al., (1960) found tolerance induction of male skin isografts in C57BL/6 females could be achieved in over 90% of females inoculated by 12 days of age with 5-10 million male spleen cells. Only 300,000 leucocyte cells would confer tolerance in all neonatal subjects injected intraperitoneally, and 10,000 would confer tolerance to 25%. Tolerance was induced in 20% of females using an extract prepared from isologous male cells. These authors studied the antigenic constancy of the Y factor in different strains using the principle of tolerance induction. Newborn C57BL/6 females were injected intraperitoneally with 4-8 million bone marrow cells from male mice of the strain investigated. When adult the mice were first challenged with a skin homograft from the donor strain of the neonatal inoculum, followed 3 weeks later by a male skin isograft. Controls were newborn C57BL/6 females injected with homologous bone marrow. No mouse was tolerant of its homologous test graft. However, donor strains A, C3H, or Au consistently induced tolerance in C57BL/6 female to male isografts. The results were considered proof that all male mice of the 5 strains had the same antigen determined by their Y chromosome.

Billingham et al., (1960) also attempted to show that females which failed to reject male isografts in line AU had in their genetic constitution the histocompatibility factor determined by the Y locus in the male. Ten newborn C57BL/6 female mice were injected with 5 million bone marrow cells from untreated AU females that bore healthy male skin isografts 150 days. None of the neonatally inoculated females was tolerant to male isografts which made a translocation hypothesis seem implausible. Tests were conducted to abolish tolerance of the male antigen by injecting sensitized lymph node cells from C57BL/6 females that had rejected male grafts into tolerant females. Tolerance was abolished in females that had been made tolerant by isologous male donors, but not in females made tolerant by homologous male donors. Females made tolerant by isologous male donors were chimeras, whereas in females made tolerant by homologous male donors the only source of Y antigen must have been the male test graft they bore. A graft vs. host reaction was not elicited when 23 C57BL/6 newborn males were inoculated with 5 million (and 12 more with 20 million) lymph node cells prepared from the pooled brachial and auxillary nodes of C57BL/6 female mice that had rejected male skin. If disparity existed only at the Y locus between host and graft, the antigenic stimulus was considered too weak for a graft vs. host reaction. An attempt to transfer tolerance passively by injecting 1/6 of an adult donor equivalent lymphoid cell prepared from the pooled spleens and nodes of adult C57BL/6 female mice (that had been injected at birth with male A strain bone marrow cells) into normal C57BL/6 females whose ages were a few hours to 50 days was unsuccessful.

Klein and Linder (1961) used strain C57BL mice that consistently

rejected ♂ to ♀ skin grafts and strain CBA which consistently accepted them to analyze the reactivity of C57BL females. All F_1 females rejected male CBA grafts. Of 110 backcross (C57BL ♂ x CBA ♀) ♂ x CBA ♀ females grafted with CBA male skin, 25 grafts survived 140 days and 85 were rejected. The median survival time was longer as compared to C57BL intrastrain or CBA to (CBA x C57BL) F_1 grafts. Nine of the backcross females that had rejected CBA male skin after 29 days were regrafted 50 days later and rejected the grafts in an average of 17 days. The 77% rejection ratio of the backcross hybrid females was attributed to the segregation of two dominant autosomal genes, each by itself being capable of determining reaction against the Y antigen. The increase in graft survival time in backcrosses that rejected the transplant as compared with F_1 and hybrid grafts was interpreted to indicate that the presence of both reactivity genes would cause a more rapid rejection than if only one was represented.

Linder (1961) attempted to induce tolerance of ♂ to ♀ grafts in C57BL adult mice by injecting 3×10^7 bone marrow cells in three doses given at 10-day intervals. Only 33% of the females still accepted male grafts after 140 days.

Howard (1961) observed that (C57BL x CBA) F_1 female mice bearing apparently healthy C57BL male skin grafts could still be sufficiently anti-male immune to prevent injected C57BL male spleen cells from initiating graft-vs.-host reactions. Thus he illustrated the relative insensitivity of skin graft rejection for detecting the onset of immunity to the weak Y-linked histocompatibility antigen.

Zeiss et al., (1962) reported a male histocompatibility antigen in rats of inbred hooded and black strains, but not in an albino strain.

Miller (1962) demonstrated that every male in one strain of platyfish produced an immunogen which elicted a homograft reaction in most female platyfish of the same strain. Two other highly inbred strains of platyfish were tested but failed to exhibit the ♂ to ♀ incompatibility. However, females of one of the two closely related strains rejected male homografts from the other strain more rapidly than they rejected female homografts.

Solomon (1962) revoked his earlier suggestion that a female specific transplantation antigen might be associated with a W chromosome in female chick embryos. His results showed that the sex difference (larger female spleens) in splenomegaly was produced regardless of donor sex, and had no immunological basis unless one postulated a W chromosome in females, and that the possible transplantation antigen associated with such a W chromosome was individual specific.

Cock (1962a) using an assortment of F_1 back- and 3-way crosses between 6 lines of inbred Brown Leghorn chickens where female and male pairs were picked at random, studied the immunological status of ovarian homografts. Fifteen of 22 orthotopic homografts of ovary survived over 280 days in genetically heterogeneous males. The presence of testicular tissue prejudiced survival and normal development of grafts, but genetic disparity did not. Erythrocyte elimination and hemagglutination tests on three birds with successful ovary grafts demonstrated an immune response against the ovary donors, but skin grafts from the ovary donor survived indefinitely (225 days) in 2 of 3. Cock suggested that ovary grafts survived by a process of self enhancement due to release of a high ratio of humoral to transplantation antigens, and that this also tended to protect a subsequent skin graft from the same donor. Cock (1962b) using Line C White Leghorn

and B x In F_1 Brown Leghorn chickens was unable to transfer ovaries successfully using the same procedures, although testis graft exchanges were successful. Skin grafts on 5 hosts in which testis grafts had survived showed substantial, though variable, prolongation of graft life. The failure of ovary grafts was thought not due to a sex-linked antigen since ovary grafts had previously survived in males (1962a). Also skin grafts from I line White Leghorn on I and F_1 (C x I and M x I) hosts survived without apparent deterioration for 160 to 400 days in all sex combinations.

Hauschka and Holdridge (1963) working with mice presented evidence for translocation of a piece of Y chromosomal material to male compatible females. They reasoned that if the translocation in a given mouse strain was of fairly recent origin there would be two classes of females: male compatible (MC) and male incompatible (MI). The Y translocation would soon become homozygous in a sib-mated stock, or be quickly eliminated, or persist awhile in a heterozygous state. Their yellow strain had MC females which accepted second male skin grafts and MI females which gave a second-set response to male grafts. When skin isografts were exchanged between MC and MI females, 4/14 MC to MI grafts were accepted compared to 8/9 MI to MI, MI to MC, and MC to MC skin grafts. Further evidence for a Y translocation was observed when male C57BL grafts to F_1 females (C57BL x MC-C3H or C57BL x MC-DBA/2) were accepted in 82 and 100% of recipients. A few broke down gradually but second male grafts persisted. Male C57BL skin grafts on backcross females (C57BL x DBA) ♂ x C57BL ♀ were rejected 45% of the time. All daughters of this backcross had inherited one X chromosome from their DBA/2 grandmother, but only half of the backcross females

had inherited the questionable autosome If all grafts had taken, one could assume that a piece of Y was translocated to DBA/2 X chromosomes. The 45% actual takes agreed with the hypothesis of antigenic Y substance contaminating a DBA/2 autosome. They also presented evidence for immunoselection against isologous male tumor cells containing the Y chromosome with strain C57BL females.

Katsch et al., (1964) showed that C57BL/6J female mice given an intraperitoneal injection of 1 to 8 million isologous epidermal sperm cells would exhibit either delayed or accelerated rejection of isologous male skin, depending on both the number of sperm cells injected and the time of application of the graft (5 to 21 days after injection). They suggest from their data that a long time interval or a large dose of sperm cells resulted in maintenance or delayed rejection of the male graft, while a small dose and short time interval produced an accelerated rejection. Results indicated that sperm were richer in Y chromosome antigen than spleen cells.

Kelly et al., (1964) showed with adult C57BL/1 females that isologous males' spleen, liver and kidney cells would induce tolerance to male skin grafts. Two injections were made weekly for 5 weeks, and grafts were applied 8 days after the first injection. The ability of the cells to induce tolerance was explained on the basis that the antigenic material involved in Y-linked histocompatibility was present in nucleated tissue distributed throughout the body and was present in the liver and kidney in amounts comparable to that found in the spleen.

Eichwald and Wetzel (1965) conducted tests with 4 different DBA sublines. In each subline isologous ♂ to ♀ skin grafts were accepted. The

sublines were crossed with C57BL males. All F_1 female mice rejected DBA and C57BL parental male skin. This proved the presence of the male-specific antigen in the DBA male mice and argued against the presence of a Y chromosome translocation in DBA female mice. The failure of the DBA sublines--whose F_1 females were tested--to reject isologous male grafts was explained by the absence of the specific property to react to male specific antigen, a deficit corrected in F_1 hybrids by a contribution from the C57BL/6 parent. Sixteen of 27 backcrossed (C57BL x CBA/2b) ♂ x DBA/2b ♀ females rejected male DBA/2b grafts. This result ruled out the X chromosome as carrier of the "reactivity" gene, and was consistent with the assumption of one or two independently segregating autosomal genes. The mean rejection time of the backcross females, as in the data of Klein et al., exceeded that of the F_1 females; most of them rejected early, but 5 of 16 rejected after two months. This was consistent with the assumption of Klein et al., that the presence of both reactivity loci results in quick rejection, while the presence of only one "reactivity" locus causes late rejection.

McLaren (1965) used C57BL females that had been immunized by rejecting either two successive male C57BL skin grafts, or a graft of C3H male skin, or a male graft of C3H followed by a male graft of C57BL skin, to see if immunization would affect male embryos. On day 18 the foetuses were removed, sexed and weighed. Results gave no indication that immunization against Y antigen would affect growth or development of male foetuses. The number of implants, embryonic mortality, and sex ratio were similar in immunized and control females.

Burbenik et al., (1966) used line C57BL/6 to study the effect of

humoral antibodies against Y-linked histocompatibility antigen on tumor grafts containing the antigen. They found immune serum had opposite effects in males and non-tolerant females. A direct effect was detected in transfer to males and was characterized by inhibition of growth of the tumor. An indirect effect, the enhancement of tumor growth, was detected in transfer to non-tolerant females. The indirect effect was distinctly stronger than the direct effect which it overlapped.

Zeiss (1966) using a strain of rats isohistogenic except for the Y antigen showed a second-set response if second grafts were applied 21 days following first grafts, or after complete destruction of first grafts. When second grafts were applied before rejection of first grafts was complete they outlived their forerunners in 18/33 grafts or reached end points in consonance with them (5/33).

Eichwald et al., (1966) studied the histologic appearance of second-set skin grafts from male donors to isologous female mice using C57BL/6 x BALB/C F_1 mice. When sensitization was with skin grafts or by intraperitoneal injection of 1 to 20 million male spleen cells 8 to 30 days prior to grafting most test grafts were rejected fairly weakly as characterized by a marked lymphocytic infiltration and a well preserved or hyperplastic epithelium. If the same number of spleen cells were injected subcutaneously and the sensitizing interval was 8 to 15 days a fairly strong host response characterized by engorgement, hemorrhage, epithelial necrosis, and absence of lymphocytic infiltration was noted. A longer sensitizing interval gave results as found following skin graft sensitization.

Kelly et al., (1966) found tolerance to male skin grafts was induced

in adult C57BL/1 female mice in most instances by the injection of cell-free tissue preparations obtained from spleen, liver, kidney, lung and heart of males. The potency of disrupted tissue preparations was generally well preserved by storage of tissue at deep-freeze temperatures for periods of 2-6 months. An exception was stored spleen. Tolerance to male skin grafts 1.8 x 2 cm could be induced by a single injection of 200-400 mg of cell-free antigenic material prepared from liver, spleen, kidney, heart and lung tissue 7 days prior to grafting as well as by a series of injections which involved in aggregate 900 mg wet weight tissue equivalent. In the case of liver tissue, the administration of as little as 100 mg of cell-free antigenic material in a single injection produced a high incidence of tolerance and a perceptible effect was noticed at doses as small as 10 mg.

Polley (1960) used noninbred White Leghorn and Rhode Island Red chicks of both sexes as hosts for full thickness skin grafts from White Leghorn and Rhode Island Red parental line chicks of both sexes, and from female chicks of each of the reciprocal F_1 crosses. Chicks were 3-4 or 10-11 days of age. Grafts were visually scored for 15 or 20 days after grafting. In all cases the crossbred female donor tissue carrying the sex chromosome (Z chromosome) of the breed other than that of the host was rejected more rapidly than the tissue from the reciprocal crossbred female donor. This difference in severity of reaction between the reciprocal crossbred female donor tissues was in the direction to be expected from sex-linked effects and was significant in two of four age and breed host groups. If W-linked or maternal effects were present they were of relatively minor importance since they did not prevent expression of

differences in severity of homograft reactions as hypothesized on the basis of sex linkage. Furthermore the absence of sex of host effects indicates that W-linked antigen(s) was not detected even though, on the average, 3 of the 4 grafts on each male were from females.

Bailey (1963) detected histoincompatibility associated with the X chromosome in mice. His work involved two unrelated highly inbred strains, C57BL/6JNBy and BALB/cAnNBy and their reciprocal F_1 hybrids. Tail skin grafts 4 to 12 mm^2 in area were exchanged within groups of 4 to 13 mice of like sex. All skin grafts in males of BC F_1 on CB F_1 hosts and half of the grafts exchanged between F_1 males in the reverse direction were sloughed by 9 weeks after grafting. In contrast none of such exchanges were sloughed in females. When sensitized F_1 males received second-set grafts, the survival time was much reduced. When sensitized F_1 males were challenged with parental strain male grafts, the paternal but not the maternal strain skin was sloughed.

MATERIAL AND METHODS

The chickens used were of two inbred lines, RPL-6 and R, and their reciprocal F_1 hybrids. RPL-6 White Leghorns were obtained from the U.S.D.A. Poultry Research Laboratory at East Lansing, Michigan, where the line has been under intensive inbreeding and selection for lymphoid leucosis resistance since 1939 (see Crittenden et al., 1964). Line R Brown Leghorns imported from the Poultry Research Center, Edinburgh, Scotland, had an inbreeding coefficient of >.76; having been selected for over 30 years for increased red in the plumage (see Craig and McDermid, 1963).

In one experiment the Cornell randombred population of White Leghorn (WL) was used for controls (see King et al., 1959).

Artificial insemination was used to obtain fertile eggs. These were held in egg coolers until a two-week supply had accumulated and were then incubated. All chicks were pedigree hatched and wing banded for identification. Chicks were placed in battery brooders until 3 weeks of age. They were then transferred into colony batteries, each compartment containing 10 to 15 chicks. At 8 to 10 weeks of age (longer for smaller R line chicks) they were transferred to the Avery Research Center and vaccinated for bronchitis and Newcastle disease. Prior to 6 months of age they were vaccinated for fowl pox.

Three grafting techniques were used. Whole thickness skin graft exchanges were made between 3 to 4 week-old chicks using an adaptation of the Polley et al., (1960) technique. Flexible collodion was applied to the back several minutes prior to grafting to stiffen the down and skin

of both donor and host. Immediately preceding skin grafting anesthetization was accomplished by injecting intraperitoneally 0.03 ml sodium pentobarbitol (commercial "Halatol" containing 1 grain sodium pentobarbitol per cc which was diluted 1:5 with physiological saline) solution per 10 gm body weight. An ink line was made at the anterior border of each skin graft prior to excision for each of 4 grafts removed from the host's back. Approximately square grafts, 1 x 1 cm, were excised using 3/4 inch angular straight edge scissors and blunt forceps. After removal, and prior to application to its assigned host, the graft was placed ventral side down on filter paper moistened with physiological saline in a watch glass. Four grafts were removed from each of four donors in a group prior to transplantation to the same birds which were then prepared hosts. Each host received tissue from each of the other 3 donors in the group and an autograft. Donor tissue was assigned at random to the 4 sites. When transplanted the grafts were reversed (the ink line was posterior) so that feathers would grow in reverse. A drop of glue (Methyl-2-Cyanoacrylate) was applied to the median edge of the graft and then a Johnson and Johnson plastic strip band aid was applied after being coated with petroleum jelly. As faulty technique in using the glue was the apparent cause for mechanical loss of some grafts in an early experiment, it was not used subsequently. With proper bandage placement it was unnecessary. Chicks were checked daily to assure that bandages were in place. Bandages were removed at 4 days after grafting. The grafts were scored daily for 2 weeks, and at regular intervals thereafter.

A wattle-to-shank grafting technique was used for adults over 2 1/2 months of age. A holding device described by Crittenden (1963) restrained

the hosts while shanks were prepared for wattle tissue. Four wattle grafts were transferred to each host. A quantity of wattle tissue was excised from each donor sufficient to graft hosts for which it was donor. Each graft was approximately 3/4 x 1 1/4 cm in size. After removal of the wattle it was split by tearing and slitting with a scalpel blade and then placed on a filter paper soaked with physiological saline in a numbered petri dish. Two graft sites were prepared on the anterior surface of each recipient's shank. Sites were prepared by excising to a depth so that blood vessel anastomosis could be established. The upper site on each shank was prepared by removing two scales below the first full scale. The lower site was prepared in like manner by removing the third and fourth scales below the top graft, so that two intact scales remained between grafts. Donor tissue was cut to fit the site using angle scissors and placed on the graft bed with forceps. The grafts were bandaged with 3/4 inch x 3 inch Johnson and Johnson "plastic strips" after vaseline had been applied to prevent sticking. In later experiments, Johnson and Johnson "Curads" bandages were used making application of vaseline unnecessary and thereby facilitating the procedure. Bandages were removed at 5 or 6 days after grafting and grafts were observed at regular intervals thereafter.

A third grafting technique was developed using adult wattle as donor tissue for 3-week-old hosts. The hosts were prepared by removing whole thickness skin from their backs as previously described, except that 5 instead of 4 sites were prepared. Wattle tissue was prepared as described in the second technique, but applied to the backs of the hosts as were whole thickness skin grafts. No glue was used. Curads bandages used to

protect the grafts were inspected daily and removed 6 days postoperative. Scoring was done daily for 2 weeks, and at regular intervals thereafter.

Grafts were scored numerically in the same way for all techniques. A scoring system based on that of Polley et al., (1960) was used as presented in Table 1.

F_1 chickens produced by RPL-6 ♂ x R ♀ matings are abbreviated hereafter as $6RF_1$ and from the reciprocal mating as $R6F_1$.

Blood typing of RPL-6 for B locus antigens was done using techniques as described by Fanguy (1961). Line R was assumed homozygous for one allele at the B locus based on skin grafting results.

Table 2 summarizes the type of grafting procedure used and the genetic stocks involved for each of 7 experiments that comprise this study.

Table 1. Macroscopic numerical scoring system used to estimate the severity of the homograft reaction.

Score	Description
6	Smooth, bright, healthy appearing.
5	Smooth, but some discoloration and/or inflammation apparent.
4	Moderate discoloration and may be slightly shrunken.
3	Discolored and shrunken.
2	Discolored, much shrunken, crusty, and becoming detached at edges.
1	Graft sloughed.
X	Graft missing but not sloughed (faulty operative technique or accidental loss).

Table 2. The genetic stock and type of grafting procedure used in the experiments.

Experiment number	Type of graft	Donors				Recipients			
			Sex and number				Sex and number		
		Line	Males	Females	Age (days)	Line	Males	Females	Age (days)
1	Skin on back	R	20	23[1]	16-17	Donors were recipients			
		WL	2	3	16-17				
2	Wattle on shank	6 $B^{13}B^{13}$	10	14	75-120	Donors were recipients			
		6 $B^{12}B^{13}$	3	5	75 120				
3	Skin on back	$6RF_1$ $B^{13}B^{R}$	6	8	28-34	Donors were recipients			
		$6RF_1$ $B^{12}B^{R}$	2		28-34				
		$R6F_1$ $B^{13}B^{R}$	8	8	28-34				
4	Wattle on shank	$6RF_1$ $B^{13}B^{R}$	11	14	108-116	$6RF_1$ $B^{13}B^{R}$	22	4	108-116
		$R6F_1$ $B^{13}B^{R}$	5	4	108-116				
		6 $B^{13}B^{13}$	11	10	195-240	$R6F_1$ $B^{13}B^{R}$		4	108-116
		R $B^{R}B^{R}$	5[2]	11	420-730				
5	Wattle on back	6 $B^{12}B^{13}$		2	258-265	$R6F_1$ $B^{13}B^{R}$	4	6	21
		6 $B^{13}B^{13}$		2	244-265				
		R $B^{R}B^{R}$		4	696-710	$6RF_1$ $B^{13}B^{R}$	4	6	21
		$6RF_1$ $B^{13}B^{R}$		1	167				
		$R6F_1$ $B^{13}B^{R}$		2	167				

Table 2. (Continued)

Experiment number	Type of graft	Donors				Recipients			
			Sex and number				Sex and number		
		Line	Males	Females	Age (days)	Line	Males	Females	Age (days)
6	Wattle on shank	6 $B^{13}B^{13}$		1	105				
		R $B^{R}B^{R}$		1	133	$R6F_1$ $B^{13}B^{R}$	3[3]		122
		$6RF_1$ $B^{13}B^{R}$		1	98	$6RF_1$ $B^{13}B^{R}$	1[3]		122
		$R6F_1$ $B^{13}B^{R}$		1	98				
7	Wattle on shank	6 $B^{13}B^{13}$	4[4]	6	413-470	6 $B^{13}B^{13}$	12[5]		400-470
		6 $B^{12}B^{13}$	1[4]		443	6 $B^{12}B^{13}$	6[5]		400-470

[1]The sex of 8 additional birds which died early in the experiment were not determined.

[2]The 5 male R donors had each retained previous female grafts over 200 days.

[3]The $6RF_1$ male and 1 $R6F_1$ male had rejected all female grafts in Experiment 5.

[4]The $B^{12}B^{13}$ and 2 $B^{13}B^{13}$ male donors had rejected all female grafts in Experiment 2, i.e., FIn. The other 2 $B^{13}B^{13}$ male donors had accepted all female grafts, i.e., FC.

[5]Two $B^{12}B^{13}$ and 2 $B^{13}B^{13}$ male recipients were FIn, and 2 $B^{13}B^{13}$ male recipients were FC from Experiment 2. Four $B^{12}B^{13}$ and 8 $B^{13}B^{13}$ males were previously ungrafted controls.

RESULTS

The Success of the Grafting Techniques

A measure of the success of the grafting techniques is given by the frequency of initial acceptance of all grafts and the continued acceptance of autografts. Table 3 summarizes the initial acceptance for all grafts by experiment and type of graft. Initial acceptance was not affected by graft type. There were some differences in initial acceptance between experiments. The initial loss of grafts was primarily due to slippage of the graft or adherence of the graft to the bandage. There was better initial acceptance of wattle grafts on adult shanks in Experiments 2, 4, 6 and 7 than of skin on chick back or wattle on chick back in Experiments 3 and 5. The better initial acceptance of wattle tissue on adult shanks is probably due to better bandage placement over the graft and isolation of the host following grafting. Chicks were not caged separately following grafting and the activity of other chicks could account for slippage of some grafts on hosts' backs. Only grafts initially accepted were scored for homograft reactions. Only 1 of the 109 autografts initially accepted was lost and it was due to mechanical damage to the graft site 100 days after grafting.

B Locus Incompatible Graft Rejections

B locus incompatible grafts were used as controls in Experiments 1, 2, 3 and 5. The rejection time (for a score of 1) was recorded for each graft, and the results are summarized in Table 4. All B locus incompatible grafts were rejected. There were no significant differences in times

Table 3. Initial acceptance of all types of grafts.

	(Number of grafts initially accepted)/(Total number of grafts)								
	Experiment number								
Type of graft	1	2	3	4	5	6	7	Total	%
Autograft	50/50	29/32[2]	30/32[2]					109/114	95.61
B locus compatible	124/125[2]	67/72[2]	60/78[2]	117/120[3]	72/80[2]	16/16	71/72[2]	527/563	93.60
B locus incompatible	29/29	24/24	16/18[2]		19/20[2]			88/91	96.70
Total	203/204[1]	120/128	106/128	117/120	91/100	16/16	71/72	724/768	94.27
%	99.50	93.75	82.81	97.50	91.00	100.00	98.61	94.27	

[1]In Experiment 1, 20 grafts are not included due to the death of 5 host chicks from over-anesthesia.

[2]Losses due to slippage of graft or adherance of graft to bandage. Poor results in Experiment 3 attributed to excessive use of glue.

[3]Loss due to dehydration of bird from initial water starvation.

Table 4. Time for complete rejection of B locus incompatible grafts.

Experiment number	Donor	Recipient	Type of graft	Number of grafts	Average time of rejection ± s.d.
1	WL	R	Skin on back	13	10.85 ± 1.41
	R	WL	Skin on back	15	13.00 ± 1.65
2	RPL-6 $B^{12}B^{13}$	RPL-6 $B^{13}B^{13}$	Wattle on shank	24	20.42 ± 3.08
3[1]	6RF_1 $B^{12}B^{R}$	F_1 $B^{13}B^{R}$	Skin on back	16	11.19 ± 2.45
5	RPL-6 $B^{12}B^{13}$	F_1 $B^{13}B^{R}$	Wattle on back	19	14.84 ± 1.70

[1]Grafts were reciprocally exchanged between 8 $B^{12}B^{R}$ and 8 $B^{13}B^{R}$ chicks in Experiment 3.

of rejections between different sex combinations in any experiment. Therefore, the average time of rejection for all grafts for a given B locus incompatibility are combined in each experiment.

The prolonged time of rejection for wattle on shank grafts in Experiment 2 as compared to wattle on back grafts in Experiment 5 is possibly due to age of host and/or site differences. However, it may also take longer to reject adult wattle than chick skin as suggested from a comparison of Experiments 5 and 3. These comparisons are confounded with differences in donor and host genotypes.

Evidence of W-linked Histocompatibility Antigen within Inbred Lines

Sex of donor and host did not affect graft survival within line R chickens. When grafts were exchanged between 2 1/2-week-old chicks in all sex combinations, 98% (93/95) were accepted until 330 days postoperatively as reported by Bacon and Craig (1966), Table 5. This suggests that line R is nearly isohistogenic, with little segregation at minor histocompatibility loci. Line R results were later confirmed with adult chickens when 29/30 wattle on shank grafts in all sex combinations were accepted (see Table 5).

The first evidence for a female specific, i.e., presumably a W-linked histocompatibility antigen, was with Reaseheath line C adult White Leghorns in an experiment reported by Bacon and Craig (1966). Of ♀ → ♂ grafts only 33% were accepted, whereas 95% of grafts made in other sex combinations survived for the 200-day observation period ($P < .005$), Table 5. Skin grafting among adult RPL-6 chickens had been conducted a month prior to the

Table 5. Summary of intra-line B locus compatible graft survivals grouped by sex combinations.

		Survival to 200 or 330[1] days postgrafting							
		♀ → ♂		♂ → ♂		♀ → ♀		♂ → ♀	
Line	Age at grafting	Number[2]	%	Number	%[3]	Number	%	Number	%
R	3 week[4,5]	18/18	100	17/17	100	25/26	96	20/21	95
R	Adult[6]	14/14	100	4/5	-	8/8	-	3/3	-
C	Adult[5]	5/15	33	4/5	-	8/8	-	3/3	-
RPL-6	Adult[5]	5/17	29	1/3	-	13/19	68	13/20	65

X^2 Test for Heterogeneity

Line	Combinations compared	d.f.	X^2	P
C	♀ → ♂ vs. others	1	14.46	< .005
RPL-6	♀ → ♂ vs. others	1	5.93	.017

[1]Line R 3-week-old chick data only.

[2]Fraction indicates: (number surviving)/(total grafts).

[3]Percentage survival not calculated where < 10 grafts were involved.

[4]Also accepted to 330 days were 4/4 grafts on male hosts and 9/9 grafts on female hosts from donors of unknown sex.

[5]Reported by Bacon and Craig (1966).

[6]Unpublished results of Craig at this laboratory.

time the results with line C were known. As grafts were rejected in RPL-6 it became evident that here also (Table 5) fewer ♀ → ♂ grafts were retained than were grafts in other sex combinations, i.e., 29 vs. 64%, respectively (P = .017).

Further evidence that female tissue contained a histocompatibility antigen(s) foreign to males was indicated by analyzing the times of rejection for B locus compatible grafts. Since the variance of ♀ → ♂ graft rejections was less than the variance of rejection times for other sex combinations, the data were analyzed by the Kruskal-Wallis non-parametric test (see Siegel, 1956, pp. 184-194). The ♀ → ♂ grafts were rejected faster than those rejected in other sex combinations (P = .007) as is evident from Figure 1.

Evidence of W Chromosome Histocompatibility Antigens in F_1 Recipients

As B-12 and B-13 antisera had been prepared for use with RPL-6 chickens, and lines RPL-6 and R were available for experimental work, further experiments were conducted with these lines and their reciprocal F_1 crosses.

Lack of allelic differences for the W chromosome histocompatibility locus with lines R and RPL-6. Parental line wattle grafts possessing the W chromosome unlike that of F_1 recipient females were accepted at nearly the same high frequency as F_1 wattle grafts having a W chromosome of common origin with that of the recipient, i.e., 8/11 vs. 9/10 grafts accepted (Table 6). These same data show no evidence of a maternal effect on graft acceptance.

The second comparison in Table 6 shows no significant difference

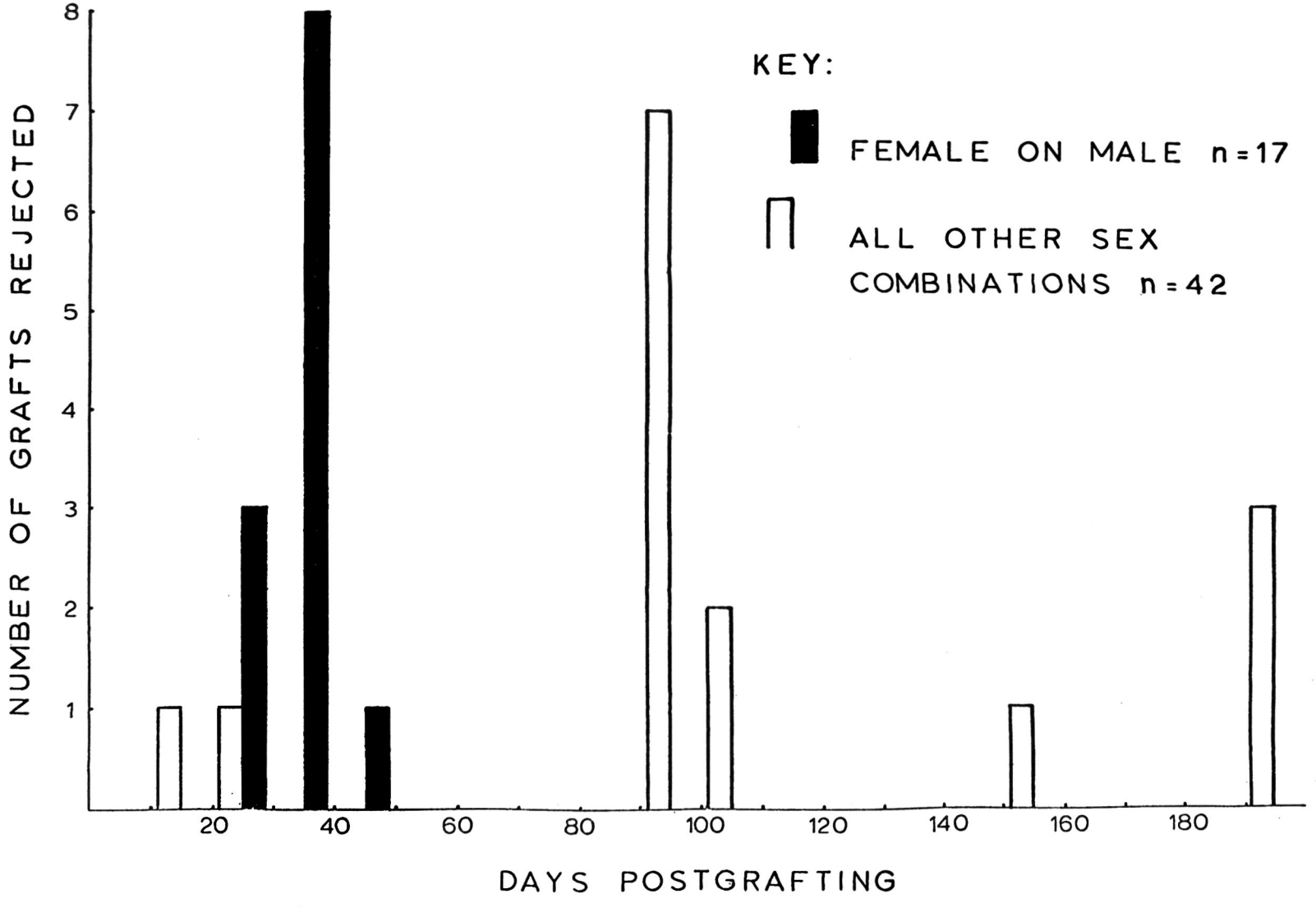

Fig. 1. Line RPL-6 adult wattle graft rejection rate as influenced by sex of donor and host.

Table 6. Survival of integumental grafts on F_1 hosts as associated with line of origin of the W chromosome.

Kind of exchange	Kind of graft	Age of host	Donor		Host		Survival to 200 days		
			Sex	Chrom.[1]	Sex	Chrom.[1]	No.		%
$P_1 \rightarrow F_1$	Adult wattle	3 weeks	♀	Z^6W^6	♀	Z^6W^R	3/5	8/11	73
			♀	Z^RW^R	♀	Z^RW^6	5/6		
$F_1 \rightarrow F_1$	Adult wattle	3 weeks	♀	Z^RW^6	♀	Z^RW^6	5/5	9/10	90
			♀	Z^6W^R	♀	Z^6W^R	4/5		
$F_1 \rightarrow F_1$	3 week skin	3 weeks	♀	Z^RW^6	♂	Z^6Z^R	1/6		
	Adult wattle	3 weeks	♀	Z^RW^6	♂	Z^6Z^R	2/8	5/22	23
$P_1 \rightarrow F_1$	Adult wattle	3 weeks	♀	Z^6W^6	♂	Z^6Z^R	2/8		
$F_1 \rightarrow F_1$	3 week skin	3 weeks	♀	Z^6W^R	♂	Z^6Z^R	2/11		
	Adult wattle	3 weeks	♀	Z^6W^R	♂	Z^6Z^R	4/7	9/24	38
$P_1 \rightarrow F_1$	Adult wattle	3 weeks	♀	Z^RW^R	♂	Z^6Z^R	3/6		

[1] Superscript denotes parental line contributing the Z or W chromosome.

($P = .29$) in degree of rejection of female tissue by F_1 male hosts due to origin of the W chromosome in F_1 or parental line female tissue. When the graft contained the W chromosome originating from RPL-6 the acceptance rate was 5/22 as compared with 9/24 if from R females.

The evidence for a W-linked histocompatibility antigen obtained from experiments with F_1 recipients. Table 7 presents comparisons of chickens which are B locus and Z chromosome compatible. Line of origin of W chromosomes in female donor-host combinations is ignored as results cited above fail to yield convincing evidence of allelic differences in the W-linked antigen.

When adult wattle (Experiment 5) or skin of 3-week-old chicks (Experiment 3) was grafted on the backs of 3-week-old F_1 hosts significantly more grafts were accepted if there was no W chromosome incompatibility. The W incompatible grafts in Experiments 3, 4 and 5 were rejected at a significantly faster rate than rejected grafts where no W incompatibility existed when compared by the Kruskal-Wallis test ($P < .005$).

Results from adult wattle-on-shank grafts (Experiment 4) with 4-month-old F_1 hosts did not give so clear an indication of this effect. This was apparently due to lower survival of like F_1 ♀ → ♀ grafts and was especially noted with $6RF_1$ female hosts. If the data of these females are excluded, the comparison of ♀ → ♂ vs. ♂ → ♂ grafts in the same experiment showed a difference approaching significance (26/42 accepted vs. 34/43, $P = 0.086$).

Table 7. Survival of integumental grafts on F_1 hosts as associated with compatibility or incompatibility of the W chromosome.[1/]

Kind of exchange	Kind of graft	Age of host	Donor		Host		Survival to 200 days		Chi-square prob.
			Sex	Chrom.[1/]	Sex	Chrom.[1/]	No.	%	
$P_1 \rightarrow F_1$	Adult wattle	3 weeks	♀	Z^6W^6	♂	Z^6Z^R	2/8		
	Adult wattle	3 weeks	♀	Z^RW^R	♂	Z^6Z^R	3/6		
$F_1 \rightarrow F_1$	Adult wattle	3 weeks	♀	Z^RW^6	♂	Z^6Z^R	2/8	11/29 38	
	Adult wattle	3 weeks	♀	Z^6W^R	♂	Z^6Z^R	4/7		
									< .005
$P_1 \rightarrow F_1$	Adult wattle	3 weeks	♀	Z^6W^6	♀	Z^6W^R	3/5		
	Adult wattle	3 weeks	♀	Z^RW^R	♀	Z^RW^6	5/6		
$F_1 \rightarrow F_1$	Adult wattle	3 weeks	♀	Z^RW^6	♀	Z^RW^6	5/5	17/21 81	
	Adult wattle	3 weeks	♀	Z^6W^R	♀	Z^6W^R	4/5		
$F_1 \rightarrow F_1$	3 week skin	3 weeks	♀	Z^RW^6	♂	Z^6Z^R	1/6		
	3 week skin	3 weeks	♀	Z^6W^R	♂	Z^6Z^R	2/11	3/17 18	
									.047
$F_1 \rightarrow F_1$	3 week skin	3 weeks	♂	Z^6Z^R	♂	Z^6Z^R	6/11	55	

Table 7. (Continued)

Kind of exchange	Kind of graft	Age of host	Donor Sex	Donor Chrom.[1]	Host Sex	Host Chrom.[1]	Survival to 200 days No.		Survival to 200 days %	Chi-square prob.
$P_1 \rightarrow F_1$	Adult wattle	4 months	♀	Z^6W^6	♂	Z^6Z^R	8/11			
	Adult wattle	4 months	♀	Z^RW^R	♂	Z^6Z^R	6/11			
$F_1 \rightarrow F_1$	Adult wattle	4 months	♀	Z^RW^6	♂	Z^6Z^R	6/10	26/42	62	
	Adult wattle	4 months	♀	Z^6W^R	♂	Z^6Z^R	6/10			
$P_1 \rightarrow F_1$	Adult wattle	4 months	♂	Z^6Z^6	♂	Z^6Z^R	9/11			
	Adult wattle	4 months	♂	Z^RZ^R	♂	Z^6Z^R	9/11			
$F_1 \rightarrow F_1$	Adult wattle	4 months	♂	Z^6Z^R	♂	Z^6Z^R	16/21	41/59	69	
$F_1 \rightarrow F_1$	Adult wattle	4 months	♀	Z^RW^6	♀	Z^RW^6	5/8			
	Adult wattle	4 months	♀	Z^6W^R	♀	Z^6W^R	2/8			

[1] It is assumed that allelic differences associated with the W-linked antigen are absent or of minor importance and are therefore ignored in these comparisons.

Attempts to Demonstrate a Second-set Immune Response Against W-Linked Antigens

Experiment 6 was conducted to determine whether second-set responses could be shown for rejections believed due to W-linked histoincompatibility. One $R6F_1$ and one $6RF_1$ male that had each rejected 5 first-set female grafts by 100 days postgrafting in Experiment 5 were regrafted with 4 female grafts each, one from a female of each parental strain and one from a female of each reciprocal F_1 cross. Controls were two previously ungrafted $R6F_1$ males. Contrary to expectation the second grafts appeared healthy over a 200-day observation period with more abundance of transplanted tissue growth than normally observed with primary grafts. One control F_1 male rejected 3/4 female grafts, and the other accepted all 4 female grafts over the same observation period.

RPL-6 males that had accepted primary female grafts in Experiment 2 were classified female compatible (FC) and those that rejected first female grafts as female incompatible (FIn). Second grafts on these males in Experiment 7 were applied 347 days after primary grafting when the hosts were 430-470 days old. Controls were previously ungrafted RPL-6 males of the same age that received primary grafts. They were classified 200 days after grafting as FIn if they rejected both female grafts, or FC if they accepted one or both female grafts. Results are shown in Table 8.

Only 1 of 6 second ♀ → FIn ♂ grafts was still surviving after 200 days. Only two small remnants of this wattle graft persisted, the rest having been rejected. The other 5 grafts underwent an accelerated rejection in contrast to the 4 first ♀ → FIn ♂ graft rejections. The two FIn males

Table 8. Comparison of first and second female graft rejection phenomena on RPL-6 males.

First or second graft	Class of male	No. of males	Number of survivals to 200 days/total	Survival time of grafts rejected, days
1st[1]	FIn	2	0/4	80, 80, 80, 134
2nd[2]	FIn	3	1*/6	14, 29, 32, 32, 39
1st[1]	FC	10	19/20	35**
2nd[2]	FC	2	3/4	193**

[1] Males grafted for the 1st time at > 400 days of age received 2 grafts and were classified as FC if one or both survived > 200 days or FIn if both were rejected.

[2] Males grafted for the 1st time at 75-120 days of age were classified as FC or FIn on the same basis. These were regrafted at ≥ 400 days of age.

* Partially rejected, see text.

**Rejections assumed due to other than W-linked incompatibility as the same recipient male accepted one other ♀ graft for > 200 days.

that rejected both second female grafts had each rejected two female grafts in Experiment 2. The FIn male that accepted portions of one second female graft had rejected only one primary female graft, as well as a B locus compatible male graft and a B locus incompatible male graft.

The rejection of one primary ♀ graft and one secondary ♀ graft by FC males is presumably a discrepancy associated with the lack of complete isohistogenic status in RPL-6. This could lead to misclassification of some FC males as FIn, especially if only one primary ♀ graft was used.

The Acceptance of Tissue Between RPL-6 Female Compatible (FC) and Female Incompatible (FIn) Males

Experiment 7 was also designed to see if FIn males would reject FC male tissue, which might be expected if one postulated a translocation of W-chromosomal material in the genome of FC males. There was some evidence from the primary grafts in Experiment 2 with RPL-6 males which had been classified as FC or FIn at 200 days after grafting that this might be true. The one FC ♂ → FIn ♂ graft in that experiment was rejected, and 4/4 FIn ♂ → Fin ♂ grafts were accepted. No grafts were classified as FC ♂ → FC ♂ or FIn ♂ → FC ♂ in that experiment. Alternatively, the design was set up to see if differences might exist in the response of FC and FIn ♂♂ as hosts to FC and FIn ♂ tissue. The results are presented in Table 9. Males receiving first grafts were judged FC or FIn on the basis of two ♀ grafts, those receiving second grafts had been judged FC or FIn on the basis of results from Experiment 2.

It is noted in Table 9 that 2/4 first-set and 4/6 second-set FC and FIn ♂ grafts on FIn ♂ hosts were accepted to 200 days after grafting. In

Table 9. Comparison of RPL-6 female compatible (FC) and female incompatible (FIn) males as hosts of male wattle grafts.

First or second graft	Type of male graft exchange[1/] Donor	Host	Number of recipient males	Number of survivals to 200 days/ total	Survival time of grafts rejected, days
1st	FC	FIn	2	1/2	60
1st	FIn	FIn	2	1/2	148
	(FC + FIn)	FIn	4	2/4	60, 148
1st	FC	FC	10	10/10	0
1st	FIn	FC	10	10/10	0
	(FC + FIn)	FC	20	20/20	0
2nd	FC	FIn	4	2/3	32
	FIn	FIn	4	2/3	20
	(FC + FIn)	FIn	8	4/6	20, 32
2nd	FC	FC	2	2/2	0
2nd	FIn	FC	2	2/2	0
	(FC + FIn)	FC	4	4/4	0

[1/] Males were classified as FC or FIn as previously indicated in text and Table 8.

contrast 20/20 first-set and 4/4 second-set FC and FIn ♂ grafts on FC ♂ hosts were accepted. There was a faster rejection in the second-set as compared to first-set rejections, which might have been associated with minor histocompatibility differences. There was no evidence for FC ♂ donor tissue being different antigenically from FIn ♂ tissue.

Evidence of Z Chromosome Histocompatibility Antigens

As indicated earlier, allelic differences between W-linked antigens of lines R and RPL-6 were not found. The data were therefore examined to determine whether a Z-linked histocompatibility antigen(s) was present on the assumption (where necessary) that differences in W-linked antigens were not involved. Results are shown in Table 10.

No significant difference in acceptance of grafts due to Z chromosome incompatibility was indicated in Experiment 3 using 3-week-old F_1 chicks (14/32 for Z incompatible vs. 6/11 for Z compatible, $P = .55$). However the lower percentage acceptance of Z incompatible grafts (44 vs. 55) was in the direction expected if a Z-linked histocompatibility antigen(s) was present. Therefore wattle grafts were exchanged between 4-month-old females of each F_1 type (Experiment 4). A significant difference ($P < .01$) was observed between the acceptance of 5/16 Z incompatible grafts vs. 41/59 for Z compatible grafts.

Experiment 5 was designed to determine specifically whether there were differences in the W-linked antigens of RPL-6 and R and/or to show evidence for a Z-linked antigen. The results obtained by grafting adult wattle on these 3-week-old chicks strongly suggest that grafts containing Z chromosomes foreign to the host were accepted to a significantly lesser degree

Table 10. Survival of integumental grafts on F_1 hosts as associated with presence or absence of a "foreign" Z chromosome.

Kind of exchange	Kind of graft	Age of host	Donor		Host		Survival to 200 days			Chi-square prob.
			Sex	Chrom.[1]	Sex	Chrom.[1]	No.		%	
$F_1 \rightarrow F_1$	3 week skin	3 weeks	♀	Z^RW^6	♀	Z^6W^R	2/6			
	3 week skin	3 weeks	♀	Z^6W^R	♀	Z^RW^6	2/5			
	3 week skin	3 weeks	♂	Z^6Z^R	♀	Z^6W^R	7/14	14/32	44	
	3 week skin	3 weeks	♂	Z^6Z^R	♀	Z^RW^6	3/7			.55
$F_1 \rightarrow F_1$	3 week skin	3 weeks	♂	Z^6Z^R	♂	Z^6Z^R	6/11		55	
$F_1 \rightarrow F_1$	Adult wattle	4 months	♀	Z^RW^6	♀	Z^6W^R	0/8			
	Adult wattle	4 months	♀	Z^6W^R	♀	Z^RW^6	5/8	5/16	31	
$F_1 \rightarrow F_1$	Adult wattle	4 months	♀	Z^RW^6	♀	Z^RW^6	5/8			< .01
	Adult wattle	4 months	♀	Z^6W^R	♀	Z^6W^R	2/8			
	Adult wattle	4 months	♂	Z^6Z^R	♂	Z^6Z^R	16/21	41/59	69	
$P_1 \rightarrow F_1$	Adult wattle	4 months	♂	Z^6Z^6	♂	Z^6Z^R	9/11			
	Adult wattle	4 months	♂	Z^RZ^R	♂	Z^6Z^R	9/11			

Table 10. (Continued)

Kind of exchange	Kind of graft	Age of host	Donor		Host		Survival to 200 days		Chi-square prob.
			Sex	Chrom.[1]	Sex	Chrom.[1]	No.	%	
$P_1 \rightarrow F_1$	Adult wattle	3 weeks	♀	Z^6W^6	♀	Z^RW^6	2/5		
	Adult wattle	3 weeks	♀	Z^RW^R	♀	Z^6W^R	1/4		
$F_1 \rightarrow F_1$	Adult wattle	3 weeks	♀	Z^RW^6	♀	Z^6W^R	0/5	4/19 21	
	Adult wattle	3 weeks	♀	Z^6W^R	♀	Z^RW^6	1/5		
$P_1 \rightarrow F_1$	Adult wattle	3 weeks	♀	Z^6W^6	♀	Z^6W^R	3/5		< .005
	Adult wattle	3 weeks	♀	Z^RW^R	♀	Z^RW^6	5/6		
$F_1 \rightarrow F_1$	Adult wattle	3 weeks	♀	Z^RW^6	♀	Z^RW^6	5/5	17/21 81	
	Adult wattle	3 weeks	♀	Z^6W^R	♀	Z^6W^R	4/5		

[1] It is assumed that allelic differences associated with the W-linked antigen are absent or of minor importance and are therefore ignored in these comparisons.

than grafts made in Z compatible combinations (4/19 vs. 17/21, $P < .005$).

The Relationship of Age of Donor and Host on the Rejection of Grafts

An experiment was not designed explicitly to show the effect of age on tissue rejection. However, there appear to be more rejections associated with weak antigenic differences if hosts are 3 weeks of age than when adult. In Experiment 2, only one of four RPL-6 male hosts that were 123 days old at grafting rejected B-locus compatible female grafts consistently, and the other three accepted female grafts. The other nine hosts were 83 or 90 days old at grafting and rejected their female grafts. This could indicate that at 4 months of age the capacity to reject was weakening. The results with control RPL-6 chickens in Experiment 7 also suggest that older hosts may be less reactive to the weak W histocompatibility antigen. Nine previously ungrafted males over 400 days old accepted two female grafts as well as two male grafts. One male rejected one female graft and accepted the other three grafts. Only two males rejected both female grafts. One of these accepted two male grafts and the other rejected both male grafts.

Lack of a Local Graft vs. Host Reaction from Adult Wattle Donor Tissue

Results of Experiment 4 $\sigma \rightarrow \sigma$ grafts give no evidence that adult wattle tissue can elicit a local immune response against B-locus incompatible hosts. If this were true, parental adult tissue on F_1 hosts would show more rejections than would F_1 tissue. In this experiment, line R and RPL-6 parental male grafts on $6RF_1$ males were rejected at the same level

(4/22 rejections) as were $6RF_1$ and $R6F_1$ adult male grafts on the same hosts (5/21 rejections, see Table 7). F_1 ♀ → ♂ data from Experiments 4 and 5 likewise gave no indication of a graft vs. host reaction. These results fail to support the hypothesis that adult chicken wattle contains enough immunologically competent cells to cause tissue rejection from local graft vs. host reactions as suggested by Billingham and Silvers (1957). These authors suggested that adult grafts having long outlived the tolerance responsive phase should react against their hosts and reject themselves. The results reported here are in agreement with those of Schierman and Nordskog (1964).

Evidence for Differences in Immune Responsiveness of Reciprocal F_1 Hybrid Females

Table 11 presents data from Experiments 4 and 5 where $R6F_1$ ♀♀ and $6RF_1$ ♀♀ hosts received identical grafts. In Experiment 4 each 4-month-old F_1 ♀ host received two like and two reciprocal F_1 ♀ grafts. In Experiment 5 each 3-week-old F_1 host received a parental RPL-6 and line R ♀ wattle graft and a like and reciprocal F_1 ♀ wattle graft. The results indicate that $R6F_1$ females accepted significantly more grafts than did $6RF_1$ females (23/37 vs. 10/35, $P < .005$). These results included Z chromosome incompatible as well as Z chromosome compatible graft rejections. An analysis of only Z chromosome compatible graft rejections was made to exclude the hypothesis that the greater rate of rejection was due only to a stronger Z^R than Z^6 histocompatibility antigen. The limited data indicate that $R6F_1$ ♀♀ accepted more Z chromosome compatible grafts than did $6RF_1$ ♀♀ (9/18 vs. 15/19, $P = .069$). Those grafts rejected in the latter comparisons

Table 11. Immune responsiveness of $R6F_1$ and $6RF_1$ female chickens to identical wattle tissue grafts transplanted from $R6F_1$ and $6RF_1$ and parental line R and RPL-6 females.

Kind of graft	Age of host	Donor Sex	Donor Chrom.[1]	Host Sex	Host Chrom.[1]	Survival to 200 days No.			Chi-square prob.
Adult wattle	3 weeks	♀	Z^6W^6	♀	Z^6W^R	3/5			
Adult wattle	3 weeks	♀	Z^6W^R	♀	Z^6W^R	4/5	9/18		
Adult wattle	4 months	♀	Z^6W^R	♀	Z^6W^R	2/8			
								10/35	
Adult wattle	3 weeks	♀	Z^RW^R	♀	Z^6W^R	1/4			
Adult wattle	3 weeks	♀	Z^RW^6	♀	Z^6W^R	0/5	1/17[2]		
Adult wattle	4 months	♀	Z^RW^6	♀	Z^6W^R	0/8			
									< .005
Adult wattle	3 weeks	♀	Z^RW^R	♀	Z^RW^6	5/6			
Adult wattle	3 weeks	♀	Z^RW^6	♀	Z^RW^6	5/5	15/19		
Adult wattle	4 months	♀	Z^RW^6	♀	Z^RW^6	5/8			
								23/37	
Adult wattle	3 weeks	♀	Z^6W^6	♀	Z^RW^6	2/5			
Adult wattle	3 weeks	♀	Z^6W^R	♀	Z^RW^6	1/5	8/18[2]		
Adult wattle	4 months	♀	Z^6W^R	♀	Z^RW^6	5/8			

[1] It is assumed that allelic differences associated with the W-linked antigen are absent or of minor importance and are therefore ignored in these comparisons.

[2] Z chromosome incompatible grafts.

are assumed due to minor histocompatibility antigens other than Z or W. Although these results cannot argue against different strengths in the Z-linked histocompatibility antigen, they strongly indicate $6RF_1$ ♀♀ are more responsive immunologically than are $R6F_1$ ♀♀.

DISCUSSION

The present study indicates that the W and possibly the Z chromosome of the chicken contain one or more histocompatibility factors. Why such effects were not previously detected by others is of interest. Several possible reasons are as follows: (a) Lines from unrelated sources were used. (b) Lines differ in responsiveness to such weak antigens, e.g., lines C and RPL-6 are reactive to the weak W-linked antigen as expressed by skin graft rejection whereas line R, and perhaps other inbred lines, are not. (c) The graft size was apparently not so large as to overwhelm completely the immune response mechanism in most cases. (d) Observations of grafts were made for at least a 200-day period.

Larger numbers of ♀ → ♂ grafts were rejected in all experiments using lines C and RPL-6 chickens than grafts between other sex combinations. W-linkage of a histocompatibility gene is proposed as an explanation. These results with chickens are in agreement with those of Kozelka (1932) but later workers were not able to show such an effect: Polley (1960), Cock (1962b), Solomon (1962). The analogous Y-linked histocompatibility antigen in mice has been demonstrated by many workers: Eichwald and Silmser (1955), Hauschka (1955) (see Review of Literature section). Likewise these authors have reported acceptance of ♂ → ♀ grafts in some strains of mice, a result similar to acceptance of ♀ → ♂ grafts in line R.

The W-linked antigen is present in line R female skin although such grafts are not rejected by line R males. This is evident since line R ♀ and $6RF_1$ ♀ grafts were rejected by F_1 males in excess of grafts made in other sex combinations. These results are analogous to those reported by

Zaalberg (1959) for the Y-linked antigen in mice.

The W-linked antigens of lines R and RPL-6 are considered to be similar or identical as results showed that (a) parental line wattle grafts possessing the W chromosome unlike that of F_1 female recipients were accepted at nearly the same high frequency as F_1 wattle grafts having a W chromosome of common origin with that of the recipient, and (b) there was no significant difference in degree of rejection of female tissue by F_1 male hosts due to origin of the W chromosome in F_1 or parental line female tissue. Many workers have also failed in attempts to show differences in Y-linked antigens of different strains of mice (Bailey, 1963; Bernstein et al., 1958; Billingham and Silvers, 1960; Eichwald et al., 1958; Zaalberg, 1959).

It was not possible to demonstrate differences of female tissues of different origin in capacity to survive the immune response. Thus R ♀ → F_1 ♂ and RPL-6 ♀ → F_1 ♂ exchanges did not differ in survival rate (10/19 vs. 9/17, Table 7). This test is similar to one suggested by Michie and McLaren (1958) for the Y-linked antigen in mice. They pointed out the possible androgen-dependence of male skin would constitute such a case if it varied from strain to strain.

The capacity to accept (as in line R) or reject (as in RPL-6) skin grafts carrying the female specific antigen was presumably due to a genetic (or line) difference in respect of male recipient's capacity to respond. The absence of Y-linked histocompatibility rejections in certain lines of mice has been similarly attributed to the incapacity of the female recipient to respond to the Y-linked antigen (Bernstein et al., 1958; Hauschka et al., 1959; Zaalberg, 1959; Billingham and Silvers, 1960; Klein and

Linder, 1961; Eichwald and Wetzel, 1965). Applying the hypothesis of Michie and McLaren (1958) to chickens, the strain differences in respect of the male recipients' capacity to respond to the W-linked antigen might be either specific, e.g., the males of some strains such as line R could possess an autosomally controlled replica of a W-controlled antigen, or nonspecific.

Hauschka and Holdridge (1963) presented evidence for a Y-linked autosomal translocation in a strain of DBA/2 mice (see Review of Literature). However, Hauschka and Holdridge (1963), Billingham and Silvers (1960), and Eichwald and Wetzel (1965) using other strains of mice failed to find evidence for this. Lack of rejection of FC ♂ grafts by FIn ♂♂ in RPL-6 chickens argue against a W-chromosome translocation in that line. Although this would not necessarily be true for line R, there is evidence from Experiment 4 that line R males do not contain a translocation of W chromosome. Line R FC ♂ → F_1 ♂ grafts were accepted the same as RPL-6 ♂ → F_1 ♂ grafts (9/11 vs. 9/11, Table 7). The RPL-6 male donors were previously ungrafted. In contrast 26/42 ♀ → ♂ grafts were accepted. If the 5 FC line R donor males contained W chromosomal translocations, tissue from them grafted onto F_1 males would presumably have been accepted similarly to ♀ → ♂ grafts (62%) and not as RPL-6 male grafts (82%).

Since F_1 males were responsive to female grafts carrying the W chromosome of either parental line, it is hypothesized that the rejection capability is determined by the presence of one or more dominant genes which allow the males to react against the weak W-linked antigen. Klein and Linder (1961) and Eichwald and Wetzel (1965) have suggested from their experimental results that either of two dominant autosomal genes at

separate loci produce the capability in female mice of rejecting grafts carrying the Y-linked antigen.

Data from these studies suggest that males incapable of rejecting grafts having the W-linked antigen are also less capable of rejecting skin grafts with other weak histocompatibility antigens. Thus RPL-6 FIn males which rejected female tissue accepted only 2/4 first-set and 4/6 second-set FC and FIn male grafts while RPL-6 FC males accepting female tissue also accepted 20/20 first-set and 4/4 second-set FC and FIn male grafts. If this hypothesis is correct, line R could still be covertly segregating at some minor histocompatibility loci. This could explain the rejection of 2/11 line R ♂ → $R6F_1$ ♂ grafts in Experiment 4 (Table 7).

There was evidence for a shorter average time of rejection of ♀ → ♂ grafts if the host was of the reactive RPL-6 parental line as compared with the $R6F_1$ hybrid, i.e., 32.43 $\pm$ s.d. 7.04 days vs. 69.38 $\pm$ s.d. 28.15 days, respectively. Klein and Linder (1961) failed to find evidence for a slower rejection of ♂ → ♀ grafts by F_1 females as compared to responsive parental line females (100% rejected male grafts) for the Y-linked antigen in mice. A slower rejection of female grafts by the F_1 male chickens in this study could be expected as not all RPL-6 males are capable of responding to the W-linked antigen and the postulated immune response genes would presumably be segregating in this line. If there were partially dominant genes at more than one locus with a cumulative effect between loci the presence of only one would bring about a slower rejection than if two or more were present as suggested by Klein and Linder. Thus some F_1 males would have received less of the dominant response genes than others from RPL-6 and none from the nonresponsive line R parent.

Results from Experiment 4 in which $R6F_1$ and $6RF_1$ females each received the same donor type tissue and from Experiment 5 in which $R6F_1$ and $6RF_1$ females and males each received the same donor type tissue suggest that at least one of the postulated genes determining immunological response to weak antigens is located on the Z chromosome. In these experiments significantly more grafts were accepted by $R6F_1$ female hosts than by $6RF_1$ female hosts (23/37 vs. 10/35, $P < .005$). It is hypothesized that this is due to a dominant gene on some Z^6 chromosomes which helps to bring about a significantly larger number of rejections in $6RF_1$ females than in $R6F_1$ females which lack a Z^6 chromosome. This result should not be due to maternal effects since in Experiment 5, 5/14 grafts were accepted by $6RF_1$ males and 6/15 grafts were accepted by $R6F_1$ male hosts.

Evidence from RPL-6 chickens over 400 days of age indicates that second ♀ → FIn ♂ grafts will undergo an accelerated rejection. This is further immunological evidence for a histocompatibility gene located on the W chromosome. Such a response was also demonstrated against the Y-linked antigen in mice by Eichwald et al., (1958), Billingham et al., (1965). The acceptance of 4 second female grafts by two F_1 males following their rejection of 5 first female grafts (one of which was B-locus incompatible) was contrary to expectation. In mice, Zaalberg (1959) noted F_1 females gave an accelerated rejection to second male grafts from either parental line. There was no evidence for a W-chromosomal translocation into the male genome of the $R6F_1$ ♂ or $6RF_1$ ♂ as skin grafts from line R and RPL-6 males that accepted female tissue (FC males) was accepted by these F_1 males as well as grafts from males that rejected female tissue (FIn males).

A very tentative hypothesis to account for the acceptance of the

second-set female grafts by the F_1 males is that the additional four grafts on each male of approximately 1 cm^2 was sufficient to overwhelm the immunological response mechanism of the host, thereby producing tolerance of the new grafts. Martinez et al., (1961) have shown that very large grafts can produce tolerance of Y-linked histocompatibility antigen in mice. Prehn (1961) reported that repeated grafting of adult C57BL/An females for seven times at 14-day intervals with adult male skin isografts led to loss of their ability to respond to the Y antigen. They concluded that prior exposure to numerous male grafts in strain C57BL/An females appeared to have produced a distinct degree of tolerance to the male antigen. There are several differences in the second-set grafting experiments with RPL-6 and the F_1 chickens. First, the FIn RPL-6 ♂♂ had each rejected only one or two ♀ grafts previous to receiving second female grafts. Secondly, it appears, as stated above, that RPL-6 chickens are more capable of rejecting skin due to a weak antigenic difference than is line R or the F_1's of the two lines. If F_1 males are less responsive to histocompatibility antigens then the capacity for their immune mechanism to become overwhelmed would be easier than if it were stronger as in RPL-6. Therefore the greater amount of antigen on the weaker responding F_1 males may have induced tolerance, allowing the grafts to be accepted. Subbarayudu (1966) found that line R chickens were more susceptible to tolerance induction as induced by $6RF_1$ or $R6F_1$ whole blood at hatching than were RPL-6 chickens. This is evidence to support the hypothesis that chickens weak in immunological responsiveness (line R) are more susceptible to tolerance induction than are chickens strong in immunological responsiveness (RPL-6).

Evidence for the existence of a Z-linked histocompatibility antigen

arises from results of two experiments (4 and 5) with reciprocal F_1 females (Table 10). A significantly higher rejection frequency of Z incompatible grafts as compared with Z compatible grafts was found. The postulated Z-linked antigen would necessarily be different in RPL-6 and line R chickens. In Experiment 3 (Table 10) Z-linked effects were not significant. Theoretically a Z histoincompatibility effect would be exhibited in ♂ → ♀ exchanges between F_1 chickens if allelic differences were present in the parental lines. Tests similar to those of Experiment 5 using maternal line ♂ and F_1 ♂ → F_1 ♀ grafts would be of interest in further establishment of a Z-linked antigen(s). The hypothesis of a Z-linked histocompatibility antigen in chickens is in agreement with the results of Polley (1960). Bailey (1963) reported an X-linked antigen in mice in an analogous experiment utilizing reciprocal F_1 hybrid males. He made no F_1 ♀ → F_1 ♂ exchanges but reported that paternal ♀ grafts onto F_1 ♂♂ which had rejected a reciprocal F_1 male graft were rejected as in a second-set immune response.

SUMMARY

The presence of a female specific histocompatibility antigen presumably controlled by a gene(s) on the W chromosome was indicated by skin grafting results in chickens. Greater numbers of ♀ → ♂ grafts were rejected in line RPL-6 and in F_1 crosses between lines RPL-6 and R than between other sex combinations. Although line R males do not reject isogenic female grafts, parental and F_1 grafts containing the W chromosomes of line R were rejected by F_1 male recipients indicating that such grafts contain a W-linked antigen. The W-linked antigens of lines R and RPL-6 appeared to be identical as indicated by rejection frequencies of parental line and F_1 female skin grafts in tests with $R6F_1$ and $6RF_1$ male and female hosts.

The capacity to accept (as in line R) or reject (as in most RPL-6) skin grafts carrying the female specific antigen was assumed to be due to a genetic difference of male recipients' capacity to respond. Results strongly suggest that at least one gene determining immunological response to weak antigens is located on the Z chromosome.

Accelerated second-set rejections of ♀ → ♂ skin grafts were found in line RPL-6. Such a response was not demonstrated with 2 F_1 males which retained second female grafts. The possible induction of immunological tolerance is discussed as an explanation of these latter results.

Evidence for the existence of a Z-linked histocompatibility antigen was obtained. Higher proportions of parental and F_1 female grafts compatible for the Z chromosome were accepted by F_1 females than were Z

incompatible grafts. Allelic differences in the postulated Z-linked antigen(s) would necessarily differ between the parental lines used, i.e., RPL-6 and R. No evidence for maternal effects on graft acceptance was found.

ACKNOWLEDGMENTS

Gratitude is expressed to Dr. James V. Craig, Professor of Poultry Genetics and major advisor, for his guidance, technical advice and constructive criticism extended throughout the course of this study.

Thanks are extended to the Department of Dairy and Poultry Science for the chickens and facilities made available for this study.

Acknowledgment is made to Carl R. Polley, Research Assistant, and Wayman P. Justice and Patricia Gaskins, Graduate Students, for their aid and suggestions concerning immunological methods and procedures.

Grateful appreciation is expressed to the author's parents, Mr. and Mrs. C. E. Bacon, for their encouragement and for having given him an interest in poultry science.

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HISTOCOMPATIBILITY IN THE CHICKEN AS AFFECTED BY THE SEX CHROMOSOMES

by

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B.S., Kansas State University, 1961

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE
GENETICS

Department of Dairy and Poultry Science

KANSAS STATE UNIVERSITY
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1967

Two inbred lines of chickens, R and RPL-6, and their reciprocal F_1 hybrids were used in a series of skin grafting experiments to study the effects of the Z and W sex chromosomes on histocompatibility.

The grafting technique used was dependent upon age of donor and host. Whole thickness skin graft exchanges were made between 3 to 4 week-old chicks on their backs. Adult donor wattle tissue was grafted to the backs of 3 week-old hosts. Adult wattle-to-shank grafts were made in experiments with chickens over 2 1/2 months of age. Hosts received 4 or 5 grafts, each approximately 1 cm^2 in size in all experiments. They were scored macroscopically in the same way for all techniques from 6 to 200 or more days after grafting.

The presence of a female specific histocompatibility antigen presumably controlled by a gene(s) on the W chromosome was indicated from primary skin grafting results. Greater numbers of ♀ → ♂ grafts were rejected in line RPL-6 and in F_1 crosses between lines RPL-6 and R than between other sex combinations. The presence of some males that accepted female grafts was noted. Line R males did not reject isogenic female grafts. However, parental and F_1 grafts containing the W chromosome of line R were rejected by F_1 male recipients indicating that such grafts contained a W-linked antigen. The W-linked antigens of lines R and RPL-6 appeared to be identical as indicated by rejection frequencies of parental line and F_1 female skin grafts in tests with $R6F_1$ and $6RF_1$ male and female hosts.

The capacity to accept (as in line R) or reject (as in most RPL-6) skin grafts carrying the weak female specific antigen was assumed to be due to a genetic difference of male recipients' capacity to respond to weak

histocompatibility antigens. In an experiment involving wattle-to-shank graft exchanges between line RPL-6 males that accepted or rejected prior or simultaneous RPL-6 female grafts, males that accepted female grafts also accepted all male grafts, whereas those that rejected female grafts rejected one-third of their male grafts as well. The rejection of the male grafts was assumed due to other weak histocompatibility antigens segregating in the line. Results of tests with parental and F_1 female skin grafts to F_1 male and female hosts strongly suggested that at least one gene determining immunological response to weak antigens is located on the Z chromosome. There was no evidence that maternal effects influenced immunological response.

An experiment with RPL-6 chickens over 400 days of age indicated that second ♀ → ♂ grafts underwent an accelerated second-set rejection. Such a response was not demonstrated with 2 F_1 males which retained second female grafts. The possible induction of tolerance is discussed as an explanation of the latter results.

Evidence for the existence of a Z-linked histocompatibility antigen was obtained. Higher proportions of parental and F_1 female grafts compatible for the Z chromosome were accepted by F_1 females than were Z incompatible grafts. Allelic differences in the postulated Z-linked antigen(s) would therefore necessarily differ between the parental lines used, i.e., RPL-6 and R.

Results failed to indicate that adult chicken wattle contains enough immunologically competent cells to cause tissue rejection from local graft-versus-host reactions. Line R and RPL-6 parental line male grafts on $6RF_1$ males were accepted at the same level as were $6RF_1$ and $R6F_1$ adult male grafts on the same hosts.