CROSSING OVER BETWEEN GENES IN THE IMMUNOGLOBULIN HEAVY CHAIN LINKAGE GROUP OF THE MOUSE

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The study of genetic loci containing immunoglobulin genes is of considerable interest because the genetic basis of immunoglobulin diversity is not yet understood. Some major questions that arise concern the number of immunoglobulin genes involved and their organization on the chromosome. The mouse is particularly advantageous in studies of immunoglobulin genetics because much is known about the linkage groups (1) and immunoglobulin structure in this species (2–6). One linkage group in the mouse has been shown to contain the heavy chain genes for four immunoglobulin classes, γA, γF (γ_1), γG (γ_{2a}), and γH (γ_{2b}) (5, 6). A heavy chain locus homologous to that in the mouse has been found in man which contains three γG genes (γG_1, γG_2, and γG_3) (7).

Structural studies on heavy chains derived from a single inbred strain of mouse have shown that for each class there are multiple structural variants. These have been demonstrated by reproducible differences in tryptic peptide maps (3, 5, 6, 8). The structural basis for these variants of the same heavy chain class may be that the carboxyl-terminal half (or more) of each chain has a constant amino acid sequence while the amino-terminal half contains variable amino acid sequences. The same general structural plan also holds for the light chains, which contain common carboxyl-terminal halves and variable amino-terminal halves (9). This leads to the question of how the genes that control immunoglobulin heavy chains are organized in the heavy chain linkage group. Is there a linear repetitive stretch of genes for each class, each carrying the same "common component" but differing by a variable sequence, or are there two different types of genes, one for the common region and a second set for each of the variable segments, as has been postulated for light chains (4, 10, 11)?

An experimental approach to this problem is to study crossing over in the heavy chain linkage groups and compare the gene products of the recombined heavy chain linkage groups with the original linkage groups from which they were derived. This is experimentally feasible in the mouse and should reveal whether the genes or gene segments controlling common and variable polypeptide segments are dissociable. Furthermore, specific immunoglobulin gene products from genetically different sources can be obtained in the mouse by recovery from plasma cell tumors induced in backcross progeny (5, 6). Crossing over in the heavy chain linkage group in the inbred mouse has not been observed thus far, although large numbers of progeny (over 3000) have been examined for this purpose (12–14). An alternative to this approach is to establish

whether crossing over has occurred in wild mouse populations, and therefore we have sought to find wild mice that have recombinant genotypes based upon our characterization of those present in the inbred strains. Most of the wild mice we have examined, however, exhibited immunoglobulin phenotypes that are usually found in the inbred strains. Since many of the strains of inbred mice were originally derived from wild mice from Europe, Asia, and North America, the similarity of immunoglobulin allotypes of wild and domestic mice that were widely separated geographically would indicate that the heavy chain linkage groups are quite stable and that the mouse immunoglobulin allotypes are of ancient origin. However, in a previous study of sera from 122 wild *Mus musculus* from seven different locations in the United States, we encountered some unusual combinations of allotypes (15), which, when compared with inbred strains or hybrids of inbred strains, could only be explained as recombinants of immunoglobulin genotypes or as new allelic forms.

In this investigation, wild mice with unusual immunoglobulin phenotypes selected from a survey of wild mice from North America and Asia were characterized by genetic and immunochemical tests. The immunoglobulin allotypes of these mice could not be easily explained as new alleles. Our studies indicate that the combinations of immunoglobulin genetic determinants found in these mice could only have occurred by recombination of immunoglobulin genes. Some evidence on the ordering of genes within the complex heavy chain locus was also obtained in these studies.

Materials and Methods

Immunoglobulin Heavy Chain Allotypes in Inbred Mice.—γG, γH, γA, and unassigned allotypes: In the inbred mouse allotypic specificities (immunoglobulin determinants) have been identified on the γG, γH, and γA heavy chains, specifically in the Fc region (5, 6). These heavy chain determinants have been identified with alloantisera prepared in inbred mice by immunization either with immune agglutinates containing mixtures of normal immunoglobulins or with specific myeloma immunoglobulins from BALB/c or BALB/c-2 strains (5). Immunoglobulin determinants are assigned to a specific immunoglobulin heavy chain when myeloma proteins bearing that specificity are available. Other determinants not yet assigned to specific heavy chains are determined to be on immunoglobulin heavy chains by the characteristic position of their precipitin arcs on immunoelectrophoresis.

There are two groups of immunoglobulin determinants in inbred strains. The first group, comprising the G^1 and the unassigned 2, 3, 4, and 5 determinants, are designated the *distinct determinants*. Each distinct determinant genetically defines a group of inbred strains, and no inbred strain has more than one of these determinants. An alloantiserum that identifies a distinct determinant is specific for that determinant and does not identify any of the other distinct determinants. Two other *distinct* determinants, A^{15} and H^{16}, have recently been identified and are found in strains having the unassigned 2 determinant. Alloantisera specific for the A^{15} and H^{16} determinants were prepared by immunization of BALB/c and other strains of mice with the γA myeloma protein MOPC 320 and the γH myeloma protein MOPC 352. The tumors producing these myeloma proteins were induced in BALB/c-2 mice (6) and were originally typed as to class by heterologous antisera and tryptic peptide map studies.[1] Neither of these two myeloma proteins was precipitated by anti-2 alloantisera.

[1] Unpublished data.

The second group of determinants, 10, G^6, G^7, G^8, H^9, H^{11}, A^{12}, A^{13}, and A^{14}, differ from the *distinct determinants* inasmuch as they are shared by inbred strains carrying different *distinct determinants* (5, 6). These are designated *cross-reacting antigenic* determinants. The alloantisera that identify cross-reacting antigenic determinants are mixtures of antibodies that identify different known antigenic determinants. Selection of the appropriate alloantiserum to identify a specific cross-reacting determinant depends on the distinct determinants that are present in the serum to be tested.

γF allotypes: The γF immunoglobulins in the mouse have not been shown to have allotypic (antigenic) specificities. However, two electrophoretic allotypes of γF have been described in inbred mice: the γF fast type, which has the widest distribution among inbred strains, and the γF slow type, which is found only in inbred strains having the 2 determinant. The alleles of the γF genes may be detected by immunoelectrophoretic methods (6, 16). For this method, whole serum is digested with papain and the position of the γF-Fc fragment arc is determined in immunoelectrophoresis by means of a specific heterologous anti-γF antiserum. In this study we used a goat anti-mouse myeloma γF-Fc (MOPC 31) antiserum.

Alleles of Heavy Chain Genes in Inbred Mus musculus.—The alleles of the heavy chain genes in the inbred mouse derived from serological and immunoelectrophoretic studies are shown in Table I.

TABLE I

Alleles of Heavy Chain Genes in Inbred Mus musculus

Immunoglobulin		Genetic antigen from inbred strain prototypes					
Class	Heavy chain	BALB/c	C57BL/6	DBA/2	AL	NH	DD
γA	α	$A^{12,13,14}$	A^{15}	A^-	A^{13}	A^{14}	$A^{12,13,14}$
γF (γ1)	Φ	F^f	F^s	F^f	F^f	F^f	F^f
γG (γ2a)	γ	$G^{1,6,7,8}$	G^-	G^8	$G^{6,7,8}$	$G^{7,8}$	$G^{1,6,7,8}$
γH (γ2b)	η	$H^{9,11}$	$H^{9,16}$	$H^{9,11}$	H^-	$H^{9,11}$	$H^{9,11}$
Unassigned			2	3	4,10	5	10

Serological Methods for Identifying Immunoglobulin Determinants in Wild Mice.—Specific alloantisera that identify immunoglobulin heavy chain determinants (allotypic specificities) in inbred mice were selected to test the sera of wild mice for precipitation in double diffusion agar gels. Most of these alloantisera have been previously described (5, 6).

The alloantisera selected to identify the *distinct immunoglobulin* determinants in wild mice are listed in Table II. The alloantisera selected to identify the *cross-reacting determinants* in wild mice are listed in Table III.

Determination of Phenotypes and Genotypes of Wild Mice.—The phenotypes of wild mice are based on the immunoglobulin determinants that were identified. Genotypes were then established by appropriate matings with inbred mice and were derived from the allotypes or combinations of immunoglobulin determinants found in their progeny.

Origin of Wild Mice.—

Kitty Hawk mice: Four wild mice were trapped by us in Kitty Hawk, N.C., under a shed on a sandy terrain 300 yards from the ocean. These mice were successfully bred to laboratory inbred mice, and complete genetic studies on two of the mice were done.

Kyushu mice: 10 wild mice were sent to us by Professor Fusanori Hamajima of Kyushu

TABLE II

*Alloantisera Used to Identify Distinct Immunoglobulin Determinants in Wild Mice**

Antiserum	Determinant	Reference strain
C57BL anti-BALB/c	G^1	BALB/c
BALB/c anti-C57BL	2	C57BL
BALB/c anti-DBA/2	3	DBA/-2
DD anti-AL	4	AL
BALB/c anti-NH	5	NH
YBR anti-MOPC 352 (BALB/c-2)‡	H^{16}	C57BL
AL anti-MOPC 320 (BALB/c-2)	A^{15}	C57BL

* Alloantisera that identify distinct determinants are specific for that determinant and will not identify any other immunoglobulin determinants. Determinants (e.g. G^1, A^{15}, and H^{16}) were assigned to a specific class of heavy chain when myeloma protein heavy chains having that determinant were available. Determinants (e.g. 2, 3, 4, and 5) not yet assigned to a specific immunoglobulin heavy chain class have been determined to be on immunoglobulin heavy chains by the characteristic position of their precipitin arcs in immunoelectrophoresis.

‡ BALB/c-2 are progeny of backcross (BALB/c × C57BL) × BALB/c in which the specific plasma cell tumor was induced.

TABLE III

*Alloantisera Used to Identify Cross-reacting Immunoglobulin Determinants in Wild Mice**

Antiserum	Determinant	Reference strain
Kitty Hawk, (KH) mice		
C57BL anti-BALB/c	G^6	AL
C57BL anti-BALB/c	G^7	NH
C57BL anti-BALB/c	G^8	DBA/2
AL anti-C58	H^9	C57BL
AL anti-C58	H^{11}	NH
A/He anti-PC-6A (BALB/c)	A^{14}	NH
Kyushu (Ky) mice		
C57BL anti-AL	G^6	BALB/c
DBA/2 anti-NH	G^7	BALB/c
C57BL anti-DBA/2	G^8	BALB/c
AL anti-MOPC 195 (BALB/c)	H^9	C57BL/6
A/He anti-PC6A (BALB/c)	$A^{12,14}$	BALB/c, NH
DE anti-MOPC 153 (BALB/c)	$A^{12,13}$	BALB/c, AL

* Alloantisera used to identify the cross-reacting determinants contain mixtures of antibodies, each of which identifies a known immunoglobulin determinant. Thus the C57BL anti-BALB/c antiserum may identify the G^6 determinant alone, or the G^6 and G^7 determinants, or the G^6, G^7, and G^8 determinants. The selection of an antiserum to identify specific determinants depended on the time during immunization when the antiserum was collected as well as on the inherent ability of some individual mice to make antibodies to determinants of a limited number. To confirm the determinant being identified, a serum from an inbred reference strain carrying that determinant must show complete coalescence of precipitin lines with the precipitin lines produced with the serum of the wild mouse to be tested.

University, Fukuoka, Japan, who trapped them in a field. A complete genetic profile of one of these mice will be presented.

RESULTS

Linkage of Genes Controlling the 3 and 5 Distinct Determinants in Wild Mice.—

In a previous study we identified the G^1, 3 and 5 immunoglobulin heavy chain determinants in the sera of some individual wild mice. These determinants, in addition to the 2 and 4 determinants, are always separated in inbred strains, and each determinant is directed by a different heavy chain linkage group (5, 6). In hybrids of inbred strains, two, but never three, of these distinct determinants are found. The heavy chain gene complexes for prototype inbred strains are shown in Table I. The previous findings of three determinants (G^1, 3, and 5) in one mouse (15), together with our present ones, made us suspect that a new haplo type expressing two of these determinants might be found. We had our first opportunity to determine whether this was true with four wild mice from Kitty Hawk (KH), N. C. The four KH mice probably represented a small inbreeding population, since all were trapped under the same shed. The phenotypes of the four KH mice are given in Table IV.

TABLE IV

Phenotypes of Wild Mice From Kitty Hawk, N. C.

Mouse No.	Phenotype
38	^{3,5,} $G^8H^9A^-$
39	^{3,5,} $G^{7,8}H^9A^-$
40	^{3,5,} $G^{7,8}H^9A^-$
41	^{3,4,5} $G^{6,7,8}H^9A^{13}$

The first unusual observation was that one wild mouse (KH41) carried the unassigned 3, 4, and 5 determinants. Precipitation of serum from this mouse with anti-3, anti-4, and anti-5 homologous antisera is shown in Fig. 1. The 3, 4 and 5 determinants of KH41 were each identical with those present in the respective prototype inbred strains, DBA/2, AL, and NH. This is shown in Ouchterlony plates by complete coalescence of the precipitin lines produced with the 3 and 5 determinants (4 determinant not shown) in sera of the wild KH41 mouse and the respective inbred strains with specific anti-3 and anti-5 alloantisera (Fig. 2-top). In addition, a further experiment was done in which the anti-3 and anti-5 antisera were reacted with a mixture of sera from DBA/2 and NH mice and also with serum from the 3,5 (KH41) wild mouse. A reaction of nonidentity was obtained, with the former indicating that the 3 and 5 determinants were on separate molecules. With the latter (KH41) serum, a reaction of partial identity was obtained. We interpreted this to mean that the

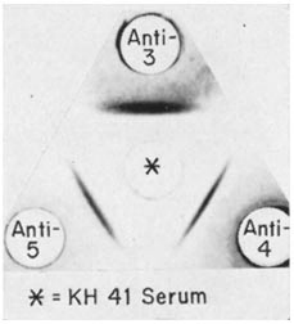


FIG. 1. Precipitation of the 3, 4, and 5 immunoglobulin heavy chain determinants in serum of the wild KH41 mouse with specific homologous anti-3, anti-4, and anti-5 antisera (Table II).

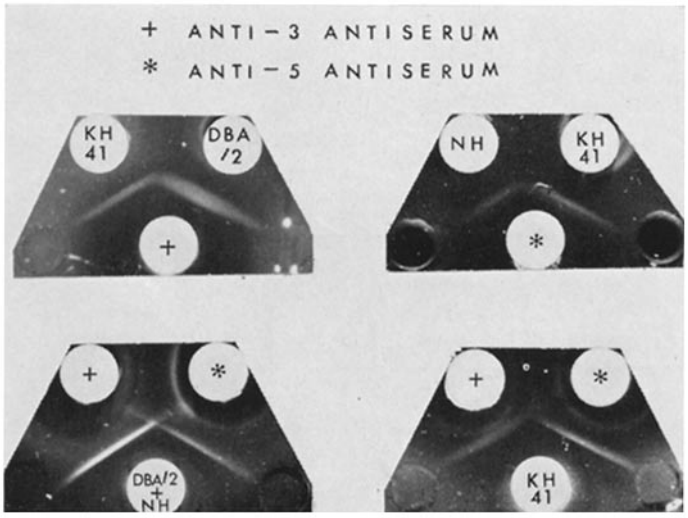


FIG. 2. (Top). Coalescence of precipitin lines showing complete identity of the 3 determinant present in the sera of an inbred (DBA/2) and a wild (KH41) mouse after reaction with a specific homologous anti-3 antiserum (Table II). Similarly, complete identity of the 5 determinant present in the sera of inbred (NH) and wild (KH 41) mice reacted with a specific homologous anti-5 antisera is shown.

FIG. 2 (Bottom). Complete crossing of precipitin lines showing nonidentity and indicating that the 3 and 5 determinants are on different molecules in the DBA/2 (3 determinant) and NH (5 determinant) mice.

Partial identity of precipitin lines indicating that the 3,5 of the wild mouse (KH41) are on the same molecular species. In addition, spurring suggests that more 3 than 5 determinants are on these molecules.

3 and 5 determinants were a single molecular species and that this molecular species contained more 3 than 5 determinants in the wild mouse (Fig. 2-bottom).

Our previous findings of G^1, 3, and 5 determinants in one mouse suggested that two of the distinct determinants were linked (15). The presence also of the 3 and 5 determinants in the three other KH wild mice suggested that there were wild mice homozygous for a heavy chain linkage group that directed both the 3 and 5 determinants. All these possibilities could be established by genetic tests. For convenience, genotypes will sometimes be abbreviated and will express only those genes pertinent to the data being presented. Two of the KH wild mice (KH40 and KH41) were successfully mated to inbred strains or hybrids of inbred strains, and further matings of these F_1 progeny were made. KH41, which carried the 3, 4, and 5 unassigned determinants, was mated to a $G^{1/5}$ heterozygote and three types of progeny were produced: $G^1/^4$, $G^1/^{3,5}$, and $^5/^{3,5}$ (Table V). One of the progeny (mouse 214) with a $G^1/^4$ genotype was mated to a BALB/c (G^1/G^1 genotype) mouse, and two types of progeny, G^1/G^1 and $G^1/^4$, were obtained, showing that the 4 became segregated from the 3 and 5 of the wild mouse (Table V). Another progeny (mouse 217) of the same cross, having the 5/3,5 genotype was also mated to a BALB/c mouse and progeny with $G^1/^5$ and $G^1/^{3,5}$ genotypes were obtained, which indicated that 3,5 and 5 were segregated and that the determinants 3 and 5 from the wild mouse were linked. This was further reinforced by the genetic study of another mouse, KH40, whose phenotype was $^{3,5}G^{7,8}A^-$ (Table VI). KH40 (similar to KH41)

TABLE V

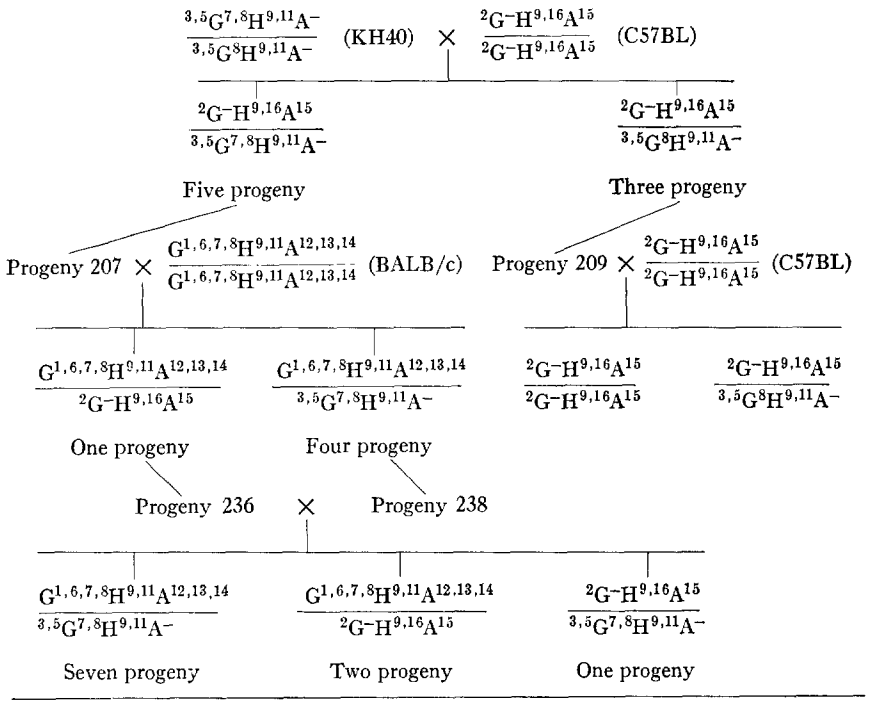
Progeny Tests Showing a New 3,5 Heavy Chain Linkage Group in Wild Mice

$\frac{^{3,5}G^{7,8}H^{9,11}A^-}{^4G^{6,7,8}H^-A^{13}}$ (KH41) ×		$\frac{G^{1,6,7,8}H^{9,11}A^{12,13,14}}{^5G^{7,8}H^{9,11}A^{14}}$ (C × NH)F_1		
$\frac{G^{1,6,7,8}H^{9,11}A^{12,13,14}}{^4G^{6,7,8}H^-A^{13}}$		$\frac{G^{1,6,7,8}H^{9,11}A^{12,13,14}}{^{3,5}G^{7,8}H^{9,11}A^-}$		$\frac{^{3,5}G^{7,8}H^{9,11}A^-}{^5G^{7,8}H^{9,11}A^{14}}$
One progeny		Three progeny		One progeny
Progeny 214 × BALB/c				Progeny 217 × BALB/c
$\frac{G^{1,6,7,8}H^{9,11}A^{12,13,14}}{G^{1,6,7,8}H^{9,11}A^{12,13,14}}$	$\frac{G^{1,6,7,8}H^{9,11}A^{12,13,14}}{^4G^{6,7,8}H^-A^{13}}$	$\frac{G^{1,6,7,8}H^{9,11}A^{12,13,14}}{^5G^{7,8}H^{9,11}A^{14}}$		$\frac{G^{1,6,7,8}H^{9,11}A^{12,13,14}}{^{3,5}G^{7,8}H^{9,11}A^-}$
Eight progeny	Five progeny	One progeny		Three progeny

did not have the A^{14} determinant, which is found in inbred strains having the 5 determinant (Table I). KH40 was mated to a C57BL ($^2G^-/^2G^-$ genotype) mouse and all eight progeny carried the 2, 3, and 5 determinants but none showed the A^{14}. Another unusual finding was that three of the eight progeny did not have the G^7 determinant while five did. In inbred mice the 5, G^7, and G^8

TABLE VI

Progeny Tests Showing Two Alleles in the Linked 3,5 Heavy Chain Locus



determinants are linked. The finding of two genotypes, $^{3,5}G^{7,8}A^-/^2G^-A^{15}$ and $^{3,5}G^8A^-/^2G^-A^{15}$ among the progeny of the KH40 cross suggested two different allotypes; $^{3,5}G^{7,8}$ and $^{3,5}G^8$. Thus the genotype of KH40 is established as $^{3,5}G^{7,8}A^-/^{3,5}G^8A^-$. It lacked the A^{14} gene and was homozygous for the linkage groups that directed the 3 and 5 determinants. Some further matings of selected progeny of the same cross (KH40 × C57BL) were done to confirm these findings (Table VI). Mouse 209 ($^{3,5}G^8A^-/^2G^-A^{15}$), which did not have the G^7 determinant, was backcrossed to a C57BL mouse ($^2G^-A^{15}/^2G^-A^{15}$). Some progeny from this cross were genetically $^{3,5}G^8A^-/^2G^-A^{15}$ and did not have the G^7

or A^{14} determinants associated with the 5 determinant in inbred strains, while the remaining progeny resembled the C57BL in their genotypes. Another progeny, mouse 207 of this same cross (KH40 × C57BL), which had the G^7 determinant ($^2G^-A^{15}/^{3,5}G^{7,8}A^-$), was crossed to a BALB/c mouse to obtain two types of progeny, $^2G^-A^{15}/G^1$ and $^{3,5}G^{7,8}A^-/G^1$. These two types of progeny were then crossed to each other to obtain mice with three different genotypes, including $^{3,5}G^{7,8}A^-/^2G^-A^{15}$. Thus the two alleles were recovered, one with the G^7 determinant and the other without it (Table VI). The original KH mice having the 3 and 5 distinct determinants were mated many times, and in over 70 progeny studied segregation of the 3 and 5 genes was never encountered.

The findings in the KH mice provided genetic proof of two new allotypes wherein the genes controlling the 3 and 5 determinants are linked on the same heavy chain linkage groups. These two new allotypes are $^{3,5}G^{7,8}A^-$ and $^{3,5}G^8A^-$.

Further characterization of the two new heavy chain linkage groups in respect to the three other linked heavy chain genes, H, F, and A, was needed. Both $^{3,5}G^{7,8}$ and $^{3,5}G^8$ homozygous progeny of the KH mice carried the $H^{9,11}$ determinants. The $H^{9,11}$ determinants are found in inbred strains having either the 3 or 5 determinants, and we have no homologous alloantiserum that will distinguish γH differences among these two strains. Thus γH differences could not be determined in the mice having the linked 3,5 genes. The γF alleles were also alike in the 3/3, 5/5, and 3,5/3,5 mice. This was established in immunoelectrophoretic studies using papain-digested serum and a goat antiserum that was specific for mouse γF-Fc fragments. All three types of γF-Fc fragments were of the fast type (not shown).

Inbred strains having the distinct 5 determinant also have the A^{14} determinant. No γA determinant has been identified thus far in our laboratory in strains having the 3 distinct determinant (Herzenberg has identified a γA determinant in mice having the 3 distinct determinant) (14). In the original KH mice having the linked 3,5 determinants, as well as in their progeny (e.g. 3,5/2 or 3,5/3,5), the A^{14} determinant was not present. This finding suggested that the A gene in the linkage groups controlling $^{3,5}G^{7,8}$ and $^{3,5}G^8$ resembled that seen in inbred strains (e.g. DBA/2, carrying the $^3G^8H^{9,11}F^fA^-$ heavy chain linkage group) and did not resemble the A gene in inbred strains carrying the distinct 5 determinant (e.g. NH, which has the $^5G^{7,8}H^{9,11}F^fA^{14}$ genotype).

The possibility that the papain Fc fragments of immunoglobulins carrying either the $^{3,5}G^8$ or $^{3,5}G^{7,8}$ determinants might differ in immunoelectrophoretic mobility was explored. This was determined on papain-digested serum from $^{3,5}G^8$ (KH209) and $^{3,5}G^{7,8}$ (KH207) wild mice (Table VI) and compared with that from inbred strains $^3G^8$ (DBA/2) and $^5G^{7,8}$ (NH). Homologous anti-3 and anti-$5G^7$ antisera were used in this system (Fig. 3). It was found that Fc fragments carrying the 3 and the 3,5 determinants had similar fast electrophoretic

mobilities, while molecules carrying the $^{5}G^{7,8}$ and $^{3,5}G^{7,8}$ determinants showed slower electrophoretic mobility. This latter difference in mobility was probably due to the change in net charge caused by the presence of the G^{7} determinant. Thus the genetically linked 3 and 5 determinants in the wild mouse are on molecules having the same electrophoretic mobility as molecules carrying the 3 and is not a composite of the 3 plus the 5 in the inbred strains. In addition, the $^{5}G^{7,8}$ determinants in inbred strains (NH) and the $^{3,5}G^{7,8}$ determinants in the wild mouse (KH207) appear to be on molecules with similar electrophoretic mobilities. These findings suggest that $^{3,5}G^{7,8}$ and $^{5}G^{7,8}$ are alleles and the $^{3}G^{8}$ may be considered another allele. Thus the previously unassigned 3 and 5

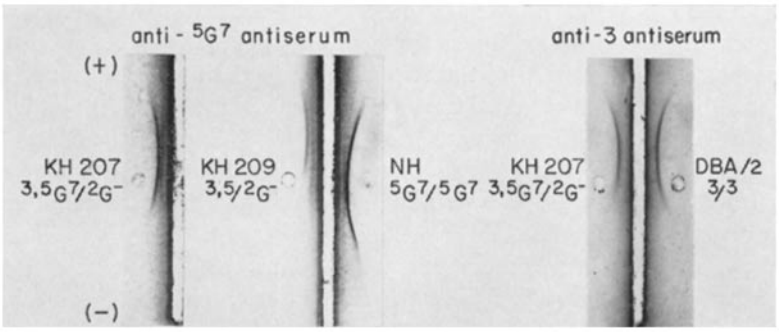


FIG. 3. Comparison of immunoelectrophoretic mobility of papain-digested serum (Fc fragments) carrying the $^{3,5}G^{7}$ and ^{3,5} determinants from KH wild mice and the $^{5}G^{7}$ determinants from an inbred strain (NH), using a specific homologous anti-$^{5}G^{7}$ antiserum. Similarly, a comparison of the electrophoretic mobility of Fc fragments carrying the $^{3,5}G^{7}$ determinants from a KH wild mouse and the 3 determinant from an inbred strain (DBA/2), using a specific homologous anti-3 antiserum, is presented.

determinants of inbred strains are now assigned to the γG heavy chains and the four tentative γG alleles are $G^{3,8}$, $G^{5,7,8}$, $G^{3,5,7,8}$, and $G^{3,5,8}$. It may now be assumed that the 3,5 alleles could have evolved by an intragenic crossing over involving the $G^{3,8}$ and $G^{5,7,8}$ alleles.

In summary, we have shown that in KH wild mice there are two new heavy chain linkage groups, each of which controls both the 3 and 5 determinants. Two alleles, $G^{3,5,7,8}H^{9,11}F^{f}A^{-}$ and $G^{3,5,8}H^{9,11}F^{f}A^{-}$ have been found, and it is suggested these new linked 3,5 heavy chain alleles probably evolved from a homologous cross-over of two prototype heavy chain linkage groups, one controlling the $G^{5,7,8}$ and the other the $G^{3,8}$ determinants.

Genetic Linkage of $G^{1,6,7,8}$ and 2 Determinants in Kyushu (Ky) Wild Mice.— The unassigned 2 immunoglobulin determinant found in many inbred strains was not found in any North American mice nor in the limited number of wild mice from South America that were tested.

We suspected that the 2 determinant might be endemic to Asia, since some ancestors of laboratory mice were of Asian origin. We found the 2 determinant in 9 of 10 wild mice sent to us from Kyushu, Fukuoka, Japan. Five of these mice had phenotypes $^{2}G^{1,6,7,8}H^{9,16}F^{s}A^{15}$ as determined by serological and immunoelectrophoretic methods. Initially the Ky mice appeared to be hybrids similar to the ones produced by crossing BALB/c and C57BL inbred mice to produce $G^{1,6,7,8}H^{9,11}F^{f}A^{12,13,14}/^{2}G^{-}H^{9,16}F^{s}A^{15}$ progeny. However, it immediately became apparent that the phenotypes of the Ky wild mice were unusual, since the $H^{11}F^{f}$ and $A^{12,13,14}$ determinants, which are always present in inbred

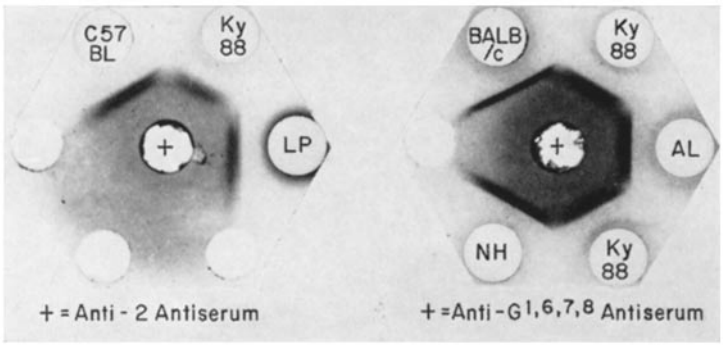


FIG. 4. Ouchterlony plates showing coalescence of precipitin lines produced with specific homologous anti-2 antiserum reacted with serum of the (Ky88) wild mouse (genotype $^{2}G^{1,6,7,8}/^{2}G^{1,6,7,8}$) and the sera of inbred mice (C57BL and LP) (genotypes $^{2}G^{-}/^{2}G^{-}$). Similarly, coalescence of precipitin lines produced with a specific homologous anti-$G^{1,6,7,8}$ antiserum reacted with the serum of the Ky88 and the sera of inbred strains BALB/c ($G^{1,6,7,8}$), AL ($G^{6,7,8}$), and NH ($G^{7,8}$) is shown.

strains or hybrids of inbred strains having the $G^{1,6,7,8}$ determinants, were absent, whereas all of the determinants $^{2}G^{-}H^{9,16}F^{s}A^{15}$, characteristic of the heavy chain linkage group in inbred mice having the distinct 2 determinant, were present. The $G^{1,6,7,8}$ determinants in the sera of a Ky mouse (Ky88) and the inbred BALB/c appeared to be identical when precipitated with a homologous anti-$G^{1,6,7,8}$ antiserum in Ouchterlony plates. Similarly, the 2 determinants of the Ky88 and of the inbred C57BL appear to be the same (Fig. 4). Papain digests of serum from Ky88, BALB/c, and C57BL were each tested with a goat anti-γF-Fc antiserum by immunoelectrophoresis (Fig. 5). Both the Ky88 and C57BL mice had γF^{s} determinants, while the BALB/c had γF^{f} determinants.

Some Ky mice were successfully mated to various inbred mice. Detailed immunogenetic and immunochemical studies of one of these mice, Ky88, are presented here. Ky88 (phenotype $^{2}G^{1,6,7,8}H^{9,16}F^{s}A^{15}$) was mated to one of an inbred BALB/c strain (genotype $G^{1,6,7,8}H^{9,11}F^{f}A^{12,13,14}/G^{1,6,7,8}H^{9,11}F^{f}A^{12,13,14}$),

and 23 progeny were obtained, all of the same phenotype ($^{2}G^{1,6,7,8}H^{9,11,16}F^{s,f}A^{12,13,14,15}$). This suggested that the $G^{1,6,7,8}$ determinants were linked to the 2 determinant in the Ky88 mouse, since the $G^{1,6,7,8}$ did not segregate. The F_1 hybrids were mated to inbred YBR ($^{3}G^{8}H^{9,11}F^{f}A^{-}/^{3}G^{8}H^{9,11}F^{f}A^{-}$ genotype) mice to determine the genotype of the Ky88 parental strain. Progeny with genotypes of $^{2}G^{1,6,7,8}H^{9,16}F^{s}A^{15}/^{3}G^{8}H^{9,11}F^{f}A^{-}$ were obtained (Table VII).

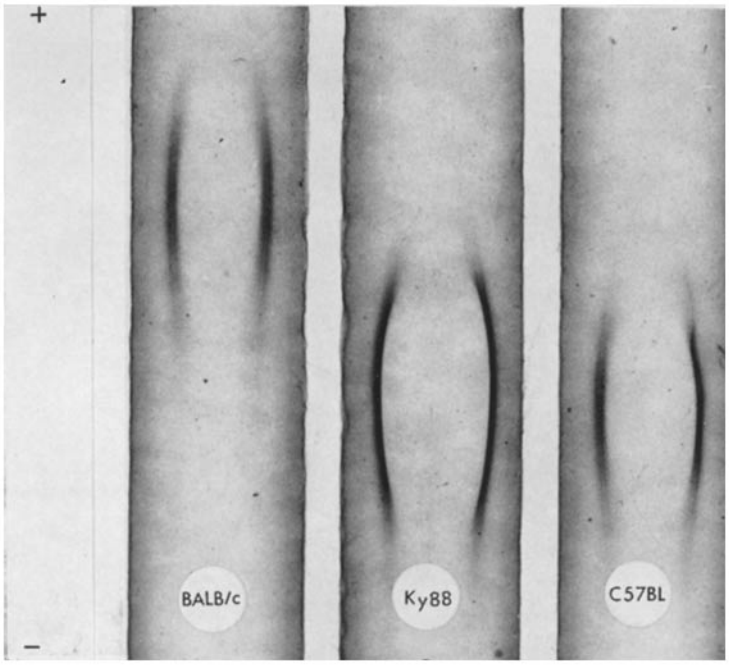


FIG. 5. γF-Fc (MOPC 31). Comparison of immunoelectrophoretic mobility of papain-digested serum (Fc fragments) of Ky88 wild mouse and inbred BALB/c and C57BL strains, using a heterologous goat anti-mouse myeloma antiserum. The γF-Fc mobility of Ky88 and C57BL is slow, while that of BALB/c is fast.

Precipitin reactions of serum from one of these progeny having the 2, and 3, and the G^{1} antigens with the respective alloantisera are shown in Fig. 6. The genotypes of these F_2 hybrids ($F_1 \times$ YBR) were thus assumed to be $^{3}G^{8}H^{9}F^{f}A^{-}/^{2}G^{1,6,7,8}H^{9,16}F^{s}A^{15}$, and the genotype of the Ky88 parental strain was proved to be $^{2}G^{1,6,7,8}H^{9,16}F^{s}A^{15}/^{2}G^{1,6,7,8}H^{9,16}F^{s}A^{15}$. In further studies of (Ky88 $\times$ BALB/c) F_2 progeny for the F^{f} and F^{s} immunoglobulins, three types of progeny were found: F^{f} in $G^{1,6,7,8}H^{9,11}A^{12,13,14}$ homozygotes, $F^{f,s}$ in $^{2}G^{1,6,7,8}H^{9,16}A^{15}/G^{1,6,7,8}H^{9,11}A^{12,13,14}$ heterozygotes, and F^{s} in $^{2}G^{1,6,7,8}H^{9,16}A^{15}$ homozygotes (not shown).

Thus the findings in the Ky mouse provided genetic proof of a new heavy chain linkage group controlling the $G^{1,6,7,8}$ and 2 determinants.

It was of interest to determine how the new heavy chain linkage group $^{2}G^{1,6,7,8}$ evolved. However, it was first necessary to characterize further the

TABLE VII

Progeny Tests Showing New $^{2}G^{1,6,7,8}$ Heavy Chain Linkage Group in Wild Mice

$$\text{Ky88}\ \frac{^{2}G^{1,6,7,8}H^{9,16}F^{s}A^{15}}{^{2}G^{1,6,7,8}H^{9,16}F^{s}A^{15}} \times \text{BALB/c}\ \frac{G^{1,6,7,8}H^{9,11}F^{f}A^{12,13,14}}{G^{1,6,7,8}H^{9,11}F^{f}A^{12,13,14}}$$

$$\text{YBR}\ \frac{^{3}G^{8}H^{9,11}F^{f}A^{-}}{^{3}G^{8}H^{9,11}F^{f}A^{-}} \times \frac{^{2}G^{1,6,7,8}H^{9,16}F^{s}A^{15}}{G^{1,6,7,8}H^{9,11}F^{f}A^{12,13,14}}$$

23 progeny

$\frac{^{3}G^{8}H^{9,11}F^{f}A^{-}}{G^{1,6,7,8}H^{9,11}F^{f}A^{12,13,14}}$	$\frac{^{3}G^{8}H^{9,11}F^{f}A^{-}}{^{2}G^{1,6,7,8}H^{9,16}F^{s}A^{15}}$

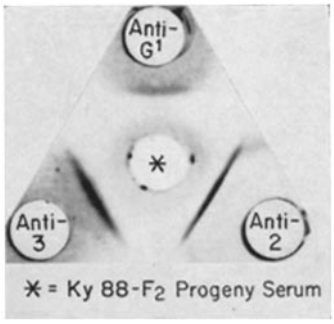


FIG. 6. Precipitation of the $G^{1,2}$ and 3 heavy chain immunoglobulin determinants in the serum of an F_2 progeny of a cross of (Ky88 × BALB/c)F_1 × YBR, having a $^{2}G^{1,6,7,8}/^{3}G^{8}$ genotype, with specific homologous anti-G^{1}, anti-2, and anti-3 antisera.

distinct 2 determinant, which has not yet been assigned to a specific immunoglobulin heavy chain in inbred strains. We tested three different myeloma heavy chain proteins, γF (MOPC 300), γH (MOPC 352), and γA (MOPC 320), induced in BALB/c-2 variants for the 2 determinant, and none of these was positive. A γG myeloma tumor has not yet been induced in BALB/c-2 variants.

The Ky88 mouse presented us with an opportunity to characterize the immunoglobulin carrying the 2 determinant, because it was linked to the well characterized $G^{1,6,7,8}$ determinants. Papain-digested serum (Fc fragment) from Ky88 ($^{2}G^{1,6,7,8}/^{2}G^{1,6,7,8}$) was tested with two homologous antisera, one

identifying the 2 and the other the $G^{1,6,7,8}$ determinants in immunoelectrophoresis. Papain-digested sera from C57BL ($^2G^-/^2G^-$) and BALB/c ($G^{1,6,7,8}/G^{1,6,7,8}$) inbred strains were run for comparison (Fig. 7). In Ky88 the electrophoretic mobilities of the Fc fragments carrying the 2 and the $G^{1,6,7,8}$ determinants were different (fast and slow) and indicated that these determinants were on different molecules. It appears that the heavy chain linkage group of the Ky88 mouse carries independent genes that separately control the 2 and $G^{1,6,7,8}$ determinants. The 2 determinant in the C57BL inbred mouse and the

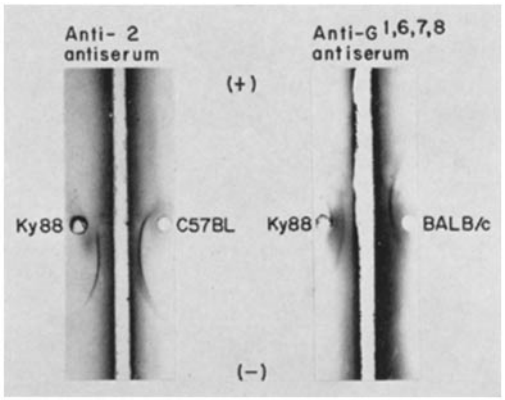


FIG. 7. Comparison of immunoelectrophoretic mobility of papain-digested serum (Fc fragments) of the Ky88 wild mouse ($^2G^{1,6,7,8}/^2G^{1,6,7,8}$) and the inbred strains BALB/c $G^{1,6,7,8}/G^{1,6,7,8}$) and C57BL ($^2G^-/^2G^-$), using specific homologous anti-2 and anti-$G^{1,6,7,8}$ antisera. Electrophoretic mobility of the Fc fragments carrying the 2 and $G^{1,6,7,8}$ determinants is different in the Ky88 homozygote. Fc fragments carrying the 2 determinant in Ky88 and C57BL have the same mobility, whereas some difference in electrophoretic mobility is observed for the $G^{1,6,7,8}$ determinants in Ky88 and BALB/c.

Ky88 wild mouse were on molecules of the same electrophoretic mobility, whereas there was a difference in the mobilities of the molecules carrying the $G^{1,6,7,8}$ determinants in the inbred BALB/c and the wild Ky88.

In summary, in the Ky88 mouse a new heavy chain linkage group has been found that has two genes, one controlling the 2 and the other the $G^{1,6,7,8}$ determinants. There are at least six heavy chain classes, γM, γA^{15}, γF^s, $\gamma G^{1,6,7,8}$, $\gamma H^{9,16}$, and γ molecules, carrying the 2 determinant in the wild Ky mouse, whereas only five heavy chain classes are present in the inbred C57BL strain, which lacks the heavy chain having the $G^{1,6,7,8}$ determinants.

DISCUSSION

The two new heavy chain linkage groups, $G^{3,5,7,8}H^{9,11}F^fA^-$ and $^2G^{1,6,7,8}$-$H^{9,16}F^sA^{15}$, found in homozygous wild mice described here probably arose from crossing over.

In the new heavy chain linkage group, $G^{3,5,7,8}H^{9,11}F^fA^-$, the G^3 and G^5 linked determinants were found to be identical with the G^3 in the DBA/2 and the G^5 in the NH strains. The $G^{3,5,7,8}H^{9,11}F^fA^-$ heavy chain linkage group was found in several different, widely separated geographic locations, including Kitty Hawk, N. C.; Calgary, Alberta; Parma, Italy; and Frederick, Md., and also in a laboratory strain bred from wild stocks trapped on the island of Skockholm. The wide distribution and prevalence of this heavy chain linkage group suggests that its origin must be quite ancient, since its spread most likely was from a single focal point. The G^3 and G^5 determinants probably evolved independently, and the finding of 3-like and 5-like determinants in other species of *Mus* (to be described in a separate publication) supports this. Furthermore, the identity of G^3 from the homozygous $G^{3,5}/G^{3,5}$ wild mouse with G^3 from the G^3/G^3 inbred strains and of G^5 from the $G^{3,5}/G^{3,5}$ wild mice with G^5 of the G^5/G^5 inbred strains indicates that the $G^{3,5}$ gene of wild mice is a fusion of heavy chain linkage groups carrying the G^3 and G^5 genes in inbred strains.

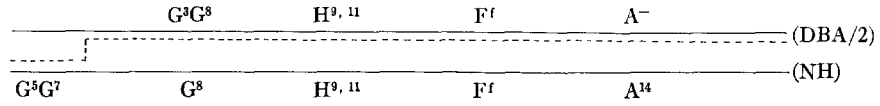
There are at least two genetic mechanisms that could account for the origin of the $G^{3,5,7,8}H^{9,11}F^fA^-$ heavy chain linkage group in wild mice. The first is based on the assumption that the new heavy chain linkage group arose from a series of sequential mutations of the same gene. Each successive mutation would involve at least a single amino acid substitution, with each mutation recurring and establishing that mutational type before the next mutation was superimposed; for example,

$$O \rightarrow G^3 \rightarrow G^{3,5} \rightarrow G^{3,5,8} \rightarrow G^{3,5,7,8}.$$

To evolve the heavy chain linkage groups found in the KH mice would require at least four recurring superimposed successive mutations. This is unlikely, since G^3, G^5, $G^{7,8}$, and G^8 genes all occur independently of each other in inbred strains.

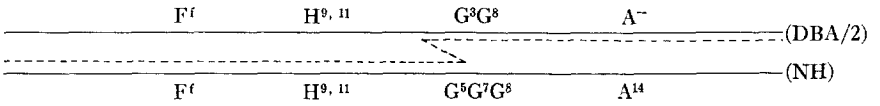
A more plausible genetic mechanism for evolving the heavy chain linkage groups in the KH wild mice would be accomplished from a cross-over in heavy chain linkage groups typical of those found in the inbred strains such as DBA/2 (having the G^3 determinant) and NH (having the G^5 determinant). Although previously the 3 and 5 determinants were not assigned to specific immunoglobulin heavy chains in inbred strains, the data presented in this paper suggest that the 3 and 5 determinants are on γG heavy chains or at least on molecules having the same electrophoretic mobility, and we have tentatively assigned them to the γG. The possibility must then be considered that a homologous cross-over occurred whereby part of the $G^{5,7,8}$ gene was fused to the $G^{3,8}$ gene to produce the new $G^{3,5,7,8}$ type, and that the rest of the heavy chain linkage group found in mice having the G^3 determinant remained the same. Thus there are several possible single cross-over events that would explain the

origin of the $G^{3,5,7,8}H^{9,11}F^fA^-$ wild mice heavy chain linkage group. We will consider four of these (Schemes 1–4).



SCHEME 1

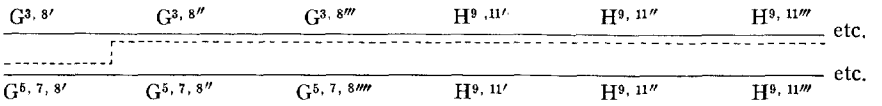
The first scheme involves an intragenic cross-over of the gene controlling the G^5 and G^7 heavy chain determinants into the γG gene in the linkage group of the mouse having the G^3 determinant. In this scheme the H and F genes are between the G and A genes.



SCHEME 2

The second scheme similarly involves intragenic cross-over in the γG gene of the mouse, but in this case the gene order is different and the G and A genes are adjacent. The difference between the two schemes becomes apparent when the origin of the $^2G^1$ linkage group is considered.

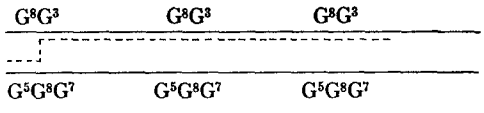
The third and fourth possibilities consider that two different molecules might exist in the $G^{3,5,7,8}$ mice. Thus the origin of the $G^{3,5,7,8}$ linkage group could have evolved still another way (Scheme 3).



SCHEME 3

Here duplications of genes controlling γG heavy chains are postulated. Cross-over would occur between two different γG subgroup genes. We can consider this scheme unlikely, however, for the following reasons. If the electrophoretic mobilities of $G^{3,8}$ and $G^{5,7,8}$ differ, it would be expected that in such a cross-over type, molecules with different electrophoretic mobilities would be found. Immunoelectrophoretic studies did not, however, reveal two electrophoretic types in $G^{3,5,7,8}$ homozygotes. The G^7 determinant is particularly helpful in this respect, since it is associated with a change in net charge; e.g. $G^{3,5,8}$ and $G^{3,5,7,8}$ differ in electrophoretic mobility.

A fourth scheme can be proposed and ruled out on the same basis as the third.



SCHEME 4

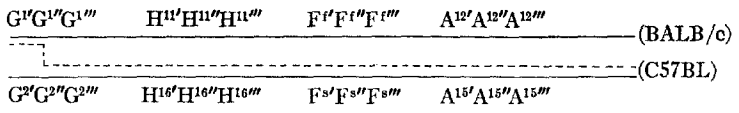
In this scheme the cross-over would give rise to $G^{3,5,8}$ and $G^{3,8}$ cistrons. If this mechanism represents the original cross-over, then the origin of the G^7 must be explained. This could most easily arise as a single base-substitution mutation affecting a charged amino acid. However, it would not be expected that such a mutation would involve all of the multiple $G^{3,5,8}$ cistrons, as would be necessary to explain the $G^{3,5,7,8}$ allele.

Before concluding which is the more likely, scheme 1 or 2, we shall consider the other new heavy chain $^2G^{1,6,7,8}H^{9,16}F^aA^{15}$ linkage group.

The origin of the $^2G^{1,6,7,8}H^{9,16}F^aA^{15}$ heavy chain linkage group in the homozygous Kyushu mouse would be difficult to explain in any way other than by crossing over. If it did occur as a result of recurring successive mutations, it would require at least six sequential mutations, one superimposed upon another. A more direct means to obtain the Ky heavy chain linkage group would be a cross-over of the $\gamma G^{1,6,7,8}$ genes from the prototype BALB/c mouse onto the heavy chain 2 gene of the prototype C57BL. This approach is more valid, as the 2 and the $G^{1,6,7,8}$ determinants found in the homozygous Kyushu wild mouse appear to be identical with the 2 determinant in the C57BL and the $G^{1,6,7,8}$ determinants in BALB/c inbred strains when examined in Ouchterlony plates. The problem is to determine the site of the cross-over, which is complicated by the fact that the immunoglobulin heavy chain carrying the 2 determinant has not yet been identified. So far we do not have a γG myeloma protein from a mouse having the 2 determinant, but we know that the 2 determinant is not on the γF, γH, or γA immunoglobulins since myeloma proteins of these classes have been produced in mice having the 2 determinant. None of these myeloma protein heavy chains precipitates with the homologous anti-2 antisera. From these findings we may indirectly infer that the determinant 2 is therefore on the γG immunoglobulins or on a γG-like (subclass) immunoglobulin. The immunoelectrophoretic distribution of the 2 determinant is clearly that of a slow ($\gamma 2$) immunoglobulin (2). However, papain digestion of whole serum from C57BL ($^2G^-/^2G^-$ genotype) and BALB/c ($G^{1,6,7,8}/G^{1,6,7,8}$ genotype) showed that the electrophoretic mobility of the Fc fragments having the 2 determinant is slow, while the mobility of the Fc fragments having the $G^{1,6,7,8}$ determinants is fast. The homozygous Kyushu mouse ($^2G^1/^2G^1$ genotype) showed two separate γG electrophoretic types, the fast molecules having the $G^{1,6,7,8}$ determinants and the slow molecules having the 2 determinant. On the basis of these findings, and in order to be able to visualize mechanisms of cross-over, we have tentatively assigned the 2 determinant to a homologue of a γG

immunoglobulin. Two cross-over mechanisms thus become feasible—homologous cross-over (Scheme 5) and nonhomologous cross-over (Scheme 6)—to explain the origin of the Kyushu heavy chain type. Both types of cross-overs would involve the heavy chain genes found in the prototype inbred strains BALB/c and C57BL.

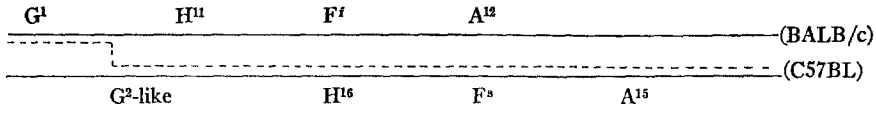
We would first assume that the 2 determinant (C57BL) is located on a homologue of the $G^{1,6,7,8}$ gene found in BALB/c. In homologous cross-over, duplications of heavy chain genes, each controlling a subgroup of γG heavy chain, would be postulated (Scheme 5). For example, if we represent the genes of BALB/c as $G^1H^{11}F^fA^{12}$ and the genes of C57BL as $G^2H^{16}F^sA^{15}$, and each gene is arbitrarily duplicated three times to become $G^{1\prime}$, $G^{1\prime\prime}$, and $G^{1\prime\prime\prime}$ or $G^{2\prime}$, $G^{2\prime\prime}$, and $G^{2\prime\prime\prime}$, then cross-over could occur as follows.



SCHEME 5

The cross-over would occur in the G″ subgroup gene carrying the 2 determinant in the C57BL, which would fuse with the gene carrying the $G^{1\prime}$ from the BALB/c to produce the homozygous $(G^{2\prime\prime}G^{1\prime})/(G^{2\prime\prime}G^{1\prime})$ Ky mouse.

Nonhomologous cross-over (Scheme 6) would be based on the fact that the G and H chains in mice have many homologies and in the BALB/c strain are known to share many tryptic peptides (17). Indeed, in the mouse, G and H are thought to have evolved by gene duplication (17). If the 2 determinant is on a G-like protein, it also would share homologies with the $G^{1,6,7,8}$ as well as with H^{11}, and G^2 would pair with H^{11} in meiosis and produce a nonhomologous crossing over type as follows.



SCHEME 6

Thus the heavy chain linkage group of the homozygous wild Ky mouse could have evolved from unequal crossing over of the γG genes of BALB/c mice, for instance, and the γG-like genes of a strain such as C57BL.

In both of the latter two schemes, it is apparent that the H, F, and A genes are linked. This finding, together with the evidence presented on the new $G^{3,5,7,8}$ heavy chain linkage group, indicates that the first scheme for the origin of the $G^{3,5,7,8}H^{9,11}F^fA^-$ is most likely and that the probable order of the genes in the heavy chain linkage group is G,H,F,A. Since G and H are closely re-

lated structurally, the probable relationship G·H·F,A is based upon the assumption that G and H are duplication products that arose from a replication process of G or H that did not involve a chromosome break.

To summarize, both the tentative assignment of the 3 and 5 determinants to the γG heavy chain and the finding of new alleles of the γG ($\gamma 2_a$) heavy chain gene in KH wild mice enabled us to identify the new alleles as $G^{3,8}$, $G^{3,5,8}$, $G^{5,7,8}$, and $G^{3,5,7,8}$. Thus in the mouse we now recognize nine alleles of γG (Table VIII).

We have not assigned the 2 determinant to the G gene, although it has been established that the 2 determinant is not on the H, F, or A. The possibility still

TABLE VIII

Alleles of Heavy Chain Genes in Inbred and Wild Mice

Heavy chain linkage group	Heavy chain gene	BALB/c	C57BL	DBA/2	AL	NH	DD	KH1*	KH2‡	Ky§
	A	$A^{12,13,14}$	A^{15}	A^-	A^{13}	A^{14}	$A^{12,13,14}$	A^-	A^-	A^{15}
	F	F^f	F^s	F^f	F^f	F^f	F^f	F^f	F^f	F^s
	H	$H^{9,11}$	$H^{9,16}$	$H^{9,11}$	H^-	$H^{9,11}$	$H^{9,11}$	$H^{9,11}$	$H^{9,11}$	$H^{9,16}$
	G	$G^{1,6,7,8}$	G^-	$G^{3,8}$	$G^{6,7,8}$	$G^{5,7,8}$	$G^{1,6,7,8}$	$G^{3,5,7,8}$	$G^{3,5,8}$	$G^{1,6,7,8}$
	Unassigned		2		4,10		10			2

* KHI = Wild Kitty Hawk mouse with $G^{3,5,7,8}$ genotype.

‡ KH2 = Wild Kitty Hawk mouse with $G^{3,5,8}$ genotype.

§ Ky = Wild Kyushu mouse.

remains that the 2 gene is not an allele of G but is controlled by a γG-like gene.

The most likely tentative order of genes in the heavy chain linkage group is based on cross-over data and is G·H,F,A. However, this order is not absolutely established, because of the paucity of cross-over types.

It is of interest to compare the human heavy chain linkage group with that of the mouse. First, in rodents, the γG immunoglobulins have been divisible into two electrophoretic groups, the γ_1 or fast type and γ_2 or slow type (17–19). The principal immunoglobulin in the mouse is γ_1 (γF); these immunoglobulins appear earliest during immunization (20–21), and in greatest quantity (22). In man the electrophoretic distribution of γG immunoglobulin resembles that of γ_2. The γ_1 of the mouse has no counterpart in man and is probably not genetically comparable to the γG_1 group in man. It thus appears that the γ_1–γ_2 division may have been an evolutionary event relative to lower vertebrates. The human and mouse heavy chain genetic loci differ, then, in that the human heavy chain linkage group thus far contains γG genes, while the mouse locus contains γ_1 (γF), γ_2 (γG and γH), and γA genes.

Considerably more detailed information is available on the genes in the heavy chain linkage groups of man. Allotypes have been identified on the Fab

fragment, such as the f and z genetic antigens on the γG_1 (7, 23–24) and sequence studies on the COOH-terminal octadecapeptide have been done (25).

The presence of genetic markers on Fab and the demonstration of their close linkage to Fc determinants (26, 27) provides evidence that a segment of the heavy chain greater than that included in the Fc is encoded by the same gene. It is of interest that amino acid sequence studies on human μ and γ chains have indicated remarkable similarities in the NH_2-terminal portion (28, 29) raising the possibility that allotypes located on the Fd could be generated from segments near the amino terminus.

In caucausoids, Gm(a) and Gm(f) are found on separate γGl molecules. In mongoloids, Gm(a) and Gm(f) frequently are found on the same γGl molecule. The Gm^{af} gene may have arisen as the result of a recombinational event between Gm^a and Gm^f genes (26, 27).

In the BALB/c mouse multiple heavy chain types, each containing a single characteristic Fc polypeptide sequence plus the common peptides derived from the Fd, are linked via peptide bonds to polypeptides of a variable nature (3, 6). Present structural and amino acid sequence data cannot exclude two different hypotheses on genetic control of heavy chain (and probably light chain) sequences. The first hypothesis, based on current dogmas on protein synthesis (the one cistron, one polypeptide chain relationship) would predict multiple cistrons in a heavy chain locus, one for each of the variant types. The second hypothesis, recently propounded for light chains (4, 11, 30) would predict that a common sequence is encoded by one gene while the variable sequences are each encoded by another separate gene.

Other genetic data presented here on the new heavy chain type $^2G^{1,6,7,8}$-$H^{9,16}F^sA^{15}$ hint the possibility of multiple cistrons for each gene; e.g., G,H,F,A, etc. This, too, would be consistent with the demonstration of multiple heavy chain types for each heavy chain class in the BALB/c mouse.

Sequence studies on human heavy chain Fc fragments (31) and tryptic peptide map studies on mouse Fc fragments (3, 6) have indicated similarities in sequence within species that are difficult to explain unless the genes were duplicated subsequent to speciation. In the mouse, G and H are almost certainly murine duplication products. Amino acid sequence similarities have also been found among human γG heavy chain types. Recently evidence for duplication of genes for murine hemoglobin chains has been presented (32).

SUMMARY

Immunoglobulin heavy chain genes were found in wild mice (*Mus musculus*) that could best be explained as recombinants of immunoglobulin genotypes.

In wild mice from Kitty Hawk, N. C., two new heavy chain linkage groups, $G^{3,5,7,8}H^{9,11}F^fA^-$ and $G^{3,5,8}H^{9,11}F^fA^-$, were found, each of which genetically controls both the 3 and 5 distinct immunoglobulin determinants. In inbred

strains the 3 and 5 determinants are found independently. The new heavy chain allotype $G^{3,5,7,8}H^{9,11}F^fA^-$ probably arose from a homologous (intragenic) cross-over between $G^{3,8}H^{9,11}F^fA^-$ and $G^{5,7,8}H^{9,11}F^fA^{14}$ heavy chain linkage groups. It was suggested that genes controlling $G^{3,8}$, $G^{5,7,8}$, $G^{3,5,8}$, and $G^{3,5,7,8}$ are alleles.

Another homozygous wild mouse (Kyushu, Japan) showed a new heavy chain allotype, $^2G^{1,6,7,8}H^{9,16}F^sA^{15}$. The 2 and $G^{1,6,7,8}$ determinants are also separated in inbred strains. The 2 determinant in inbred mice is not on the γF, γH, or γA heavy chain and is probably on a γG or γG-like immunoglobulin heavy chain. Papain digestion of serum from the Kyushu mouse showed two electrophoretically different Fc fragments, one carrying the $G^{1,6,7,8}$ and the other the 2 determinant. The new heavy chain allotype, $^2G^{1,6,7,8}H^{9,16}F^sA^{15}$, of the Kyushu wild mouse probably arose from a nonhomologous (unequal) cross-over between $^2G^-H^{9,16}F^sA^{15}$ and $G^{1,6,7,8}H^{9,11}F^fA^{12,13,14}$ heavy chain linkage groups. The linkage group of the Kyushu wild mouse has at least five heavy chain genes, while that of the inbred mice has four.

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