

Two Unusual Gm Alleles: Their Implications for the Genetics of the Gm Antigens

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INTRODUCTION

The Gm antigens of human IgG are inherited in different complexes in different races (reviewed by Muir and Steinberg, 1967; Steinberg, 1967). The complexes usually found in Caucasians with the reagents routinely used in this laboratory (reagents to detect Gm antigens 1, 2, 3, 5, 6, 13, and 14) are Gm^1 , $Gm^{1, 2}$, and $Gm^{3, 5, 13, 14}$. (The nomenclature is that recommended by an Expert Committee of the World Health Organization; see Table 2.) Exceptions to these usual patterns have been reported by many investigators. Those of most interest for the present report concern the Gm^- and Gm^3 alleles.

Nielsen and Henningsen (1961) presented evidence for a Gm allele which produced neither Gm(1), Gm(2), nor Gm(5). They called this a silent allele. Steinberg (1962) published similar evidence for such an allele in three other families belonging to a religious isolate. Similar findings were subsequently reported by other investigators (Ropartz *et al.* 1962, 1963; Deicher *et al.*, 1963; Bütler and Greuter, 1964). All these analyses were based on tests for the Gm(1), Gm(2), and Gm(5) antigens. The study of the subsequently discovered antigens Gm(3) (Steinberg and Wilson, 1963) and Gm(4) (Gold *et al.*, 1965), which is similar to if not identical with Gm(3) (Steinberg, 1965a), revealed that the assumed silent allele in two of the three families reported by Steinberg (1962) produced Gm(3) (Steinberg and Goldblum, 1965). The phenotypes and presumed genotypes of these three families are shown in Table 1. The allele in the families reported by Ropartz *et al.* (1963) and by Nielsen and Henningsen (1961) also produced Gm(3) (Ropartz *et al.*, 1965; Mårtensson *et al.*, 1966). Ropartz, Rivat, and Rousseau (1967) reported a family with an allele that produces Gm(21) and possibly Gm(22) in addition to Gm(3); that is, they identified a $Gm^{3, 21}$ allele and perhaps a $Gm^{3, 21, 22}$ allele.

The allele in the third family (68.08) originally reported by Steinberg (1962) does not produce Gm(3) (see phenotypes in Table 1); that is, it is silent for Gm(1), Gm(2), Gm(3), and Gm(5). Further evidence for such a silent allele was found in a Brazilian family (no. 227) reported by Steinberg and Goldblum (1965).

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All of the individuals carrying the silent allele and the $Gm^{3, 21}$ allele were heterozygotes. Ropartz *et al.* (1962) reported an individual homozygous for an allele producing neither Gm(1), Gm(2), nor Gm(5). Unfortunately, it was not possible to undertake family studies, nor was sufficient serum available to permit storage and subsequent testing for newly discovered Gm antigens.

We now report a more extensive study of the members and relatives of the three families previously reported from this laboratory (Steinberg, 1962; Steinberg and Goldblum, 1965). During these studies, an individual homozygous for the silent allele and 70 heterozygotes were found. In addition, it was possible to establish the presence of a $Gm^{1, 3, 17, 21}$ allele.

TABLE 1
SUMMARY OF THE Gm DATA FOR FAMILIES
68.08, 61.10, AND 151.07

Family and Individual*	Gm Phenotype†
68.08:	
Father.....	3, 5, 13, 14
Mother.....	1, 3, 5, 13, 14, 21
3‡.....	1, 21
5.....	3, 5, 13, 14
61.10:§	
Father.....	Dead
Mother.....	1, 3, 17, 21
3.....	1, 3, 5, 13, 14, 21
4‡.....	3, 5, 13, 14, —17
151.07:	
Father.....	3, 5, 13, 14
Mother.....	1, 3, 17, 21
4.....	1, 3, 5, 13, 14, 21
4‡.....	3, 5, 13, 14, —17

NOTE.—In this and all other tables all samples were tested for Gm(1), Gm(2), Gm(3), Gm(5), Gm(6), Gm(13), Gm(14), and Gm(21) unless otherwise indicated.

* The digits indicate the number of children with the indicated phenotype.

† The generic term Gm is omitted.

‡ Indicates children with unexpected phenotypes. The digit indicates the number of children with indicated phenotype.

§ To conserve reagent, only certain individuals (as indicated in the table) were tested for Gm(17).

Preliminary reports of these data were presented at the Ninth Annual Meeting of the American Society of Hematology and at the 1967 meetings of the American Society of Human Genetics (Steinberg, Muir, and McIntire, 1966, 1967).

MATERIALS AND METHODS

The serum samples were those collected during the course of a medicogenetic study of a large religious isolate, the H-leut (Steinberg, 1965*b*; Steinberg, Bleibtreu *et al.*, 1967). Samples were collected from all those 5 years of age or older. Pedigree data for the members of the isolate are essentially complete back to 1800 and extensive but not complete to 1700. Several of the red-blood-cell antigens and various serum factors other than Gm have been determined for these samples. These data will be reported elsewhere (however, see Steinberg, Bleibtreu, *et al.*, 1967).

All samples shown in the tables were tested for Gm(1), Gm(2), Gm(3), Gm(5), Gm(6), Gm(13), Gm(14), Gm(21), and Inv(1). Some samples were also tested for some or all of the following: Gm(4), Gm(8), Gm(10), Gm(11), Gm(12), Gm(15), Gm(16), Gm(17), Gm(19), Gm(20), Gm(22), and Gm(23). We are indebted to Mrs. Holbrook and to Drs. Litwin, Van Loghem, Mårtensson, Natvig, and Ropartz for reagents to test some of the antigens listed above and to Drs. Kunkel, Litwin, and Ropartz for some of the determinations and for confirming some of our Gm tests.

TABLE 2
REAGENTS USED TO DETECT THE GM AND INV ANTIGENS

ANTIGEN		ANTI- γ -GLOBULIN		ANTI-D	
Current*	Previous	Antibody	Dilution	Name	Dilution
Gm(1)	Gm(a)	Wils	1/4	251	1/3
Gm(2)	Gm(x)	Taylor	1/16	Ham	1/3
Gm(3)	Gm(b ²)	Ewe	1/4	Jack	1/3
Gm(4)	Gm(f)	Lu	1/16	Tö	1/5
Gm(5)	Gm(b ¹)	Dr	1/8	21,369	1/32
Gm(6)	Gm(c)	Debose	1/4	War	1/3
Gm(8)	Gm(e)	P.C.E. 64	†	535	1/2
Gm(10)	Gm(b ^a)	Let	1/8	29	8/9
Gm(11)	Gm(b ^{β})	{ Caz	1/4	29	8/9
		{ 2,357	1/5	21,369	1/32
Gm(12)	Gm(b ^{γ})	All	1/8	29	8/9
Gm(13)	Gm(b ³)	Th	1/4	21,369	1/32
Gm(14)	Gm(b ⁴)	Bu	1/16	21,369	1/32
Gm(15)	Gm(s)	Van Dijk	1/4	Vai	1/3
Gm(16)	Gm(t)	2,639	1/4	Vai	1/3
Gm(17)	Gm(z)	Anti-Gm(17)	1/8	{ AD ₃	2/5
				{ O'Brian	1/3
Gm(20)	Gm(20)	Gar	1/16-1/32-1/64	Nis	1/3
Gm(21)	Gm(g)	{ 3,978	1/64	4,752	1/5
		{ Monkey F	1/8	Ham	1/3
Gm(22)	Gm(y)	Anti-Gm(22)	1/2	AD ₃	2/5
Inv(1)	Inv(1)	Math	1/4	Roehm	9/10
Inv(3)	Inv(b)	Lucas	1/8	Ham	1/5
ISf(1)	ISf(1)	03/227	1/32	Roehm	1/2

NOTE.—Gm(19) (Rouen(3)) was tested by Dr. Ropartz and Gm(23) (Gm(n)) was tested by Dr. Kunkel.

* World Health Organization (1965).

† See Table 6.

The reagents used to determine the Gm and Inv factors are presented in Table 2. The Gm and Inv tests were done on microflocculation slides by a method previously described (Steinberg, 1962).

Tests for IgG and for the heavy-chain subclasses were done by double immunodiffusion. Commercial anti-IgG (Hyland Lot GP-8-65) was used for the former tests, and reagents generously supplied by Dr. W. Terry were used for the latter. We are indebted to Drs. Kunkel and Terry for confirming our determinations of the heavy-chain subclasses.

Isolation of the heavy and light chains and preparation of the Fab and Fc fragments were done by methods previously described (Polmar and Steinberg, 1967).

For immunization of rabbits and rhesus monkeys, 1 mg of protein emulsified in complete Freund's adjuvant was given as the first injection. One mg of protein in incomplete adjuvant was given at 3-week intervals for the subsequent subcutaneous or intramuscular injections.

A Spinco model E centrifuge equipped with Schlieren optics was used for ultracentrifugation of IgG molecules (in phosphate buffered saline, pH 7.2) and of isolated heavy chains (in 1 N acetic acid). The S_{20} rates reported in this paper are the observed rates uncorrected for dilution or solvent.

THE DATA

The Gm⁻ Allele

1. *Family studies.* During routine testing of specimens from the H-leut for Gm(1) and Gm(5), a serum (2,904) that was negative for both of these antigens was found.

TABLE 3
GM PHENOTYPES AND PROBABLE GENOTYPES
OF FAMILY 712.06*

Individual	Gm Phenotype†	Probable Genotype‡
Father	1, 21	1, 21/-
Mother	3, 5, 13, 14	3, 5, 13, 14/-
3‡	3, 5, 13, 14	3, 5, 13, 14/-
1‡	1, 21	1, 21/-
1‡	-	-/-

* See NOTE to Table 1. All members of this family were Inv(-1). The serum of the child (2,904) with no detected Gm antigens was tested for Inv(3) and found to be positive.

† The generic term Gm is omitted.

‡ Indicates children with unexpected phenotypes. The digits indicate the number of children with the indicated phenotype.

Subsequent testing for Gm(2), Gm(3), Gm(4), Gm(6), Gm(8), Gm(10), Gm(11), Gm(12), Gm(13), Gm(14), Gm(15), Gm(16), Gm(17), Gm(19), Gm(20), Gm(21), Gm(22), Gm(23), Inv(1), and Inv(3) showed that the serum was negative for all of these antigens *except* Inv(3) and Gm(8). (However, see below with reference to Gm(8)). The donor of serum 2,904, a child in the family 712.06 (Steinberg, Bleibtreu, *et al.*, 1967), is a healthy woman who was 28 years old when the sample was drawn. She is the mother of three children from whom no samples were drawn, because they were less than 5 years of age when we visited the colony.

The sera of the other members of family 712.06 were tested for Gm(1), Gm(2), Gm(3), Gm(5), Gm(6), Gm(13), Gm(14), Gm(21), and Inv(1). The results are shown in Table 3. All members of the family were Inv(-1). Because of a great shortage of anti-Inv(3), only the serum of the Gm(-) donor (serum 2,904) and known controls were tested for Inv(3), and, as noted above, it was positive. Each of the parents appears, on the basis of phenotype, to be homozygous for the Gm alleles. However, if the father were indeed $Gm^{1, 21}/Gm^{1, 21}$ and the mother $Gm^{3, 5, 13, 14}/Gm^{3, 5, 13, 14}$, the Gm phenotypes listed in the table would indicate nonpaternity of the first three children, nonmaternity of the next child, and "nonparentage" of the last child. We

therefore assume that each parent is heterozygous for a chromosome that does not specify any of the eight Gm antigens (1, 2, 3, 5, 6, 13, 14, 21) determined in this family. Inasmuch as none of the Gm antigens listed in Table 2 (except Gm(8), but see later) was detected in the serum of the proband, it is reasonable to assume that one of the two alleles in each parent does not produce any of these antigens, permitting the gene designation of Gm^- . This conclusion was supported by subsequent tests on the families of the parental siblings.

The results of testing five of the father's sibs, their spouses, and their children and six of the mother's sibs, their spouses, and their children are presented in Table 4.

The data in Table 4A show that of the two paternal sibs who carry the Gm^- allele, one (mother of 720.01) has the genotype $Gm^{3, 5, 13, 14}/Gm^-$ and the other (mother of 725.04) has $Gm^{1, 21}/Gm^-$, which is the same as her brother, the father of 712.06. Two (father of 712.08 and mother of 712.12) are heterozygous for known Gm alleles ($Gm^{1, 21}/Gm^{3, 5, 13, 14}$). The genotype of the fifth sib (mother of 725.01) cannot be determined from the data. It is thus evident that the genotype of one of the parents was $Gm^{1, 21}/Gm^{3, 5, 13, 14}$ and the genotype of the other was either $Gm^{1, 21}/Gm^-$ or $Gm^{3, 5, 13, 14}/Gm^-$. If the mother of family 725.01 has the genotype $Gm^{3, 5, 13, 14}/Gm^{3, 5, 13, 14}$, the latter alternative would be the correct one.

The data in Tables 3 and 4B show that the known genotypes of the tested siblings in the mother's family are $Gm^{3, 5, 13, 14}/Gm^-$ (mothers of 710.16 and 712.06) and $Gm^{1, 21}/Gm^-$ (father of 712.09 and mother of 721.12). The genotype of two sibs (the mothers of 712.08 and 771.03) may be either $Gm^{1, 21}/Gm^-$ or $Gm^{1, 21}/Gm^{1, 21}$. The data are inconclusive for the mother of 712.08, because only five offspring were tested, but there is good evidence that the mother of 771.03 is $Gm^{1, 21}/Gm^{1, 21}$, since she transmitted a $Gm^{1, 21}$ allele to all nine of her tested offspring. The mother of 712.03 may have genotype $Gm^{3, 5, 13, 14}/Gm^{3, 5, 13, 14}$ or $Gm^{3, 5, 13, 14}/Gm^-$. Her spouse is probably $Gm^{3, 5, 13, 14}/Gm^{3, 5, 13, 14}$, so no further information can be obtained directly. However, some indirect evidence is available, as follows: Since both the $Gm^{3, 5, 13, 14}$ and the $Gm^{1, 21}$ alleles occur with the Gm^- allele, one parent of this set of siblings was probably $Gm^{1, 21}/Gm^{3, 5, 13, 14}$. The mother of family 771.03 is probably $Gm^{1, 21}/Gm^{1, 21}$; hence, the second parent of these sibs was probably $Gm^{1, 21}/Gm^-$. If this assumption is correct, the mother of family 712.03 carries the Gm^- allele.

Serum from over 3,300 members of the H-leut was tested to locate other individuals with the Gm^- allele or another rare allele producing Gm(3) (see Table 2) but not Gm(5), Gm(13), or Gm(14). The former, but not the latter, requires studies of families for its detection. Nine additional families with the Gm^- allele were found (Table 5) and 13 others with the "Gm(3)-producing" allele were identified (family 720.01 in Table 4A and 12 families in Table 9). The latter families will be discussed in a later section of this paper.

More extensive testing of the serum samples from families 68.08, 61.10, and 151.07 (Table 1) confirmed that the father of family 68.08 is heterozygous for the Gm^- allele and led to a reinterpretation of the genotypes in families 61.10 and 151.07. The mothers of these families are sisters. Although their phenotype is Gm(1, 3, 17, 21), neither transmitted Gm(1), Gm(17), and Gm(21) to several of their children. The possible transmission of Gm(3) cannot be determined, because the children inherited

TABLE 4—GM PHENOTYPES AND PROBABLE GENOTYPES OF THE SIBS OF THE PARENTS OF FAMILY 712.06 AND OF THE SIBS' SPOUSES AND CHILDREN*

Family and Individual	Gm Phenotype†	Probable Genotype‡
A. Families of the Father's Sibs		
712.08:		
Father‡	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
Mother§	1, 21	1, 21/1, 21 or 1, 21/-
4	1, 17, 21	1, 17, 21/1, 17, 21 or 1, 17, 21/-
1	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
712.12:		
Father	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
Mother‡	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
3	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
4	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
720.01:		
Father#	1, 3, 17, 21	1, 3, 17, 21/1, 17, 21
Mother‡	3, 5, 13, 14, -17	3, 5, 13, 14/-
2**	1, 17, 21	1, 17, 21/-
3**	1, 3, 17, 21	1, 3, 17, 21/-
2	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21 or 3, 5, 13, 14/1, 3, 21
725.01:		
Father	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
Mother‡	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14 or 3, 5, 13, 14/-
5	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14 or 3, 5, 13, 14/-
725.04:		
Father	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
Mother‡	1, 17, 21	1, 17, 21/-
3**	3, 5, 13, 14	3, 5, 13, 14/-
9	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
B. Families of the Mother's Sibs		
710.16:		
Father	1, 21	1, 21/1, 21
Mother§	3, 5, 13, 14	3, 5, 13, 14/-
5**	1, 17, 21	1, 17, 21/-
712.03:		
Father	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
Mother§	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14 or 3, 5, 13, 14/-
2	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14 or 3, 5, 13, 14/-
712.09:		
Father§	1, 17, 21	1, 17, 21/-
Mother	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
2**	3, 5, 13, 14	3, 5, 13, 14/-
5	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
721.12:		
Father	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
Mother§	1, 17, 21	1, 17, 21/-
1**	3, 5, 13, 14, -17	3, 5, 13, 14/-
3	1, 17, 21	1, 17, 21/1, 17, 21 or 1, 17, 21/-
4	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
771.03:		
Father	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
Mother§	1, 17, 21	1, 17, 21/1, 17, 21
9	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21

* See NOTE to Table 1.

‡ Sib of father of family 712.06.

† The generic term Gm is omitted.

§ Sib of mother of family 712.06.

|| To conserve reagent, only certain individuals (as indicated in the table) were tested for Gm(17).

See text section on the Gm^{1, 3, 17, 21} allele for justification of this genotype.

** Indicates children with unexpected phenotypes. The digit indicates the number of children with the indicated phenotype.

TABLE 5
GM PHENOTYPES AND PROBABLE GENOTYPES OF
NINE FAMILIES WITH THE *Gm*⁻ ALLELE*

Family and Individual	Gm Phenotype †	Probable Genotype ‡
511.06:		
Father	Dead
Mother	1, 21	1, 21/-
4 ‡	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
3 §	3, 5, 13, 14	3, 5, 13, 14/-
513.07:		
Father	1, 21	1, 21/-
Mother	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
4	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
3	1, 21	1, 21/1, 21 or 1, 21/-
1 §	3, 5, 13, 14	3, 5, 13, 14/-
515.02:		
Father	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
Mother	1, 21	1, 21/-
3	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
3	1, 21	1, 21/1, 21 or 1, 21/-
2 §	3, 5, 13, 14	3, 5, 13, 14/-
517.01:		
Father	Dead
Mother	1, 21	1, 21/-
2	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
3	1, 21	1, 21/1, 21 or 1, 21/-
1	1, 21	1, 21/- (mother of family 515.02)
3 §	3, 5, 13, 14	3, 5, 13, 14/-
539.09:		
Father	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
Mother	3, 5, 13, 14	3, 5, 13, 14/-
2	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
4	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14 or 3, 5, 13, 14/-
3 §	1, 21	1, 21/-
545.08c:		
Father	1, 21	1, 21/-
Mother	Dead
2	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
6	1, 21	1, 21/1, 21 or 1, 21/-
1 §	3, 5, 13, 14	3, 5, 13, 14/-
546.01:		
Father	1, 21	1, 21/-
Mother	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
6	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
3 §	3, 5, 13, 14	3, 5, 13, 14/-
558.13:		
Father	1, 21	1, 21/-
Mother	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
5	1, 21	1, 21/1, 21 or 1, 21/-
3 §	3, 5, 13, 14	3, 5, 13, 14/-
562.07:		
Father	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
Mother	1, 21	1, 21/-
1	1, 3, 5, 13, 14, 21	3, 5, 13, 14/1, 21
3	1, 21	1, 21/1, 21 or 1, 21/-
1 §	3, 5, 13, 14	3, 5, 13, 14/-

* See NOTE to Table 1.

† The generic term Gm is omitted.

‡ The digit indicates the number of children with the indicated phenotype.

§ Indicates children with unexpected phenotypes.

their father's $Gm^{3, 5, 13, 14}$ allele. Therefore, the genotypes of these women could be either $Gm^{1, 17, 21}/Gm^3$ or $Gm^{1, 3, 17, 21}/Gm^-$. It will be shown in a later section that the latter genotype is probably the correct one.

Of the 71 individuals found to carry the Gm^- allele (Tables 1, 3, 4, and 5), all except the members of one family (545.08c) can be traced to a common ancestral family (48). Condensed pedigrees of these two kindreds are presented in Figures 1 and 2. All individuals in families numbered 200, 400, 401, 500, 501, 700, and 701 and in those with only two- or three-digit numbers are dead. We have less pedigree data for the father of family 545.08c (Fig. 2) than for any of the other individuals who carry the Gm^- allele. We know that his paternal grandmother was born in 1831 and his maternal grandmother in 1825, but we do not know who their parents were. This leaves a gap of almost 100 years between their birth dates and those of the parents of family

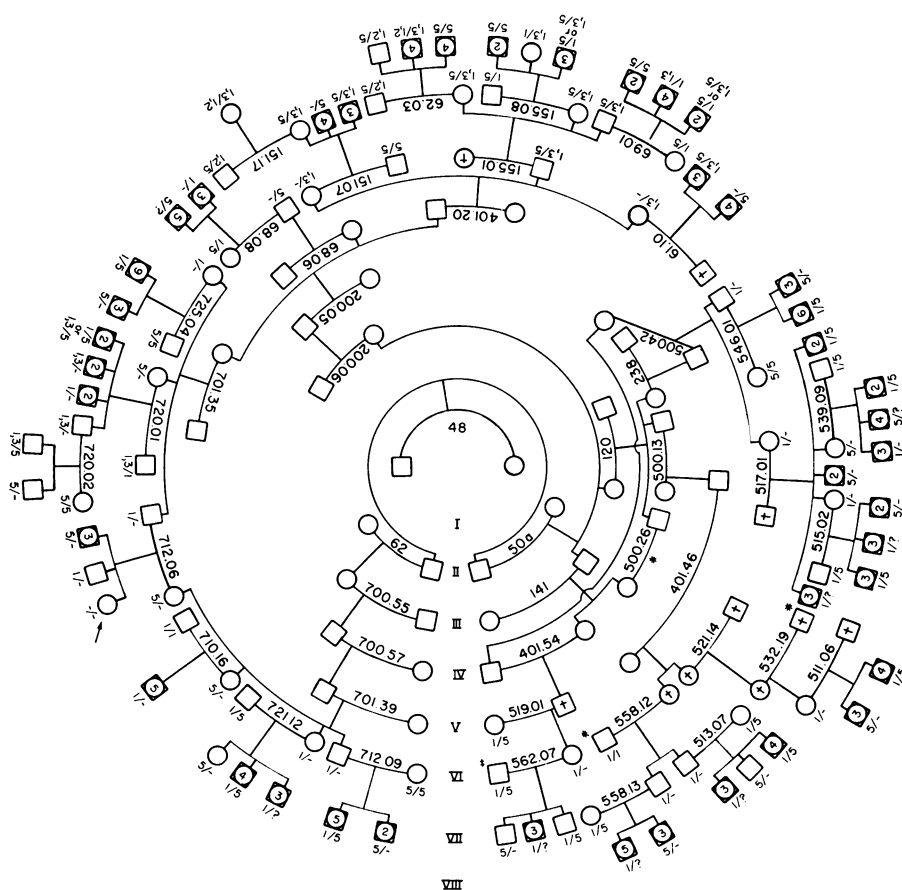


FIG. 1.—Kindred of all families (except family 545.08c) with the Gm^- allele. 5 = $Gm^{3, 5, 13, 14}$; 1 = $Gm^{1, 17, 21}$; 1, 3 = $Gm^{1, 3, 17, 21}$ (this allele also produces $Gm(20)$ and $Gm(22)$, but these are omitted here because of the unavailability of antisera to detect them); - = Gm^- ; ? = uncertainty concerning allele; * indicates that the family (or individual) occurs in Fig. 2 also; † indicates that the family (or individual) occurs in Fig. 3 also.

48. The ancestral data for the father of 545.08c are more extensive via his grandfathers, but, even so, his paternal grandfather can be traced for only two more generations, to people born between 1771 and 1777. In fact, we can trace only one of his four grandparents, his maternal grandfather, to ancestors born prior to 1740.

2. *The Gm⁻ homozygote.* As stated earlier, the serum (sample 2,904) of the woman with the *Gm⁻/Gm⁻* genotype was tested for all the antigens listed in Table 2. No tests were done for Gm(7), Gm(9), and Gm(18), because reagents were not available for these. Serum 2,904 was negative for all Gm factors except Gm(8) and possibly ISf(1) (Ropartz, Fudenberg, *et al.*, 1967). Work in progress in collaboration with Ropartz indicates that Gm(8) is present on some γ G2 myeloma proteins (as well as on γ G1 myeloma proteins which are also Gm(3) but not on those which are Gm(1)). Hence, the Gm(8) factor in serum 2,904 may be on the γ 2 heavy chain rather than the γ 1

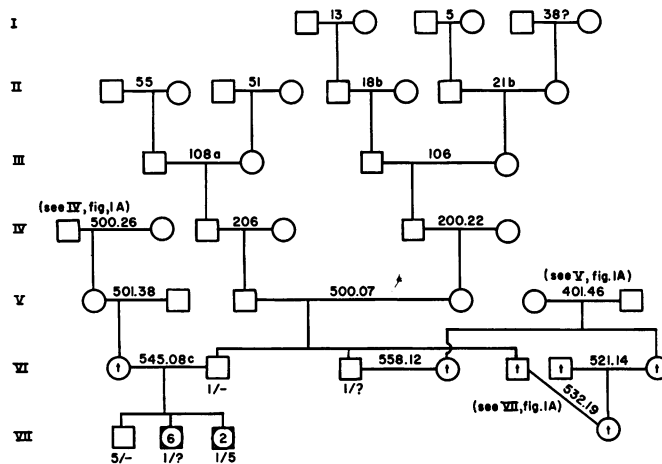


FIG. 2.—Kindred of family 545.08c, the only family with the *Gm⁻* allele that cannot be shown to have descended from family 48. See Fig. 1 for an explanation of the abbreviations of the alleles.

heavy chain. This surmise is supported by the observation that only 7 of 20 serum samples from *Gm^{1,21}/Gm⁻* individuals were Gm(8), while 3 were clearly Gm(-8) and 10 gave equivocal tests. The data are presented in Table 6. If the *Gm⁻* allele determined Gm(8), all the *Gm^{1,21}/Gm⁻* individuals should have been Gm(8). Since some are Gm(-8), we must assume that the *Gm⁻* allele does not determine this reaction. Incidentally, the reader will note that the scores (including the controls and sample 2,904) range from 1 to 25, representing continuous variation with no indication of a dichotomy.

Dr. L. Rivat in Dr. Ropartz' laboratory twice found serum 2,904 positive for ISf(1), an antigen thought by Ropartz, Fudenberg, *et al.* (1967) to be determined by a gene independent of the *Gm* locus. We have not been able to confirm her findings. The tests in this laboratory were done with anti-ISf(1) SNagg 03/227 and red blood cells from donor Talbot, both of which were generously sent to us by Dr. Ropartz. In both laboratories, the test is done with anti-D Roehm used routinely in this

laboratory. The results of titrations of the sera from 2,904, her father, her mother, and of control sera sent by Dr. Ropartz are shown in Table 7. We are at a loss to explain the discrepancy, unless it can be related to the observation that red blood cells sensitized with anti-D Roehm are more often agglutinated by sera from nonrheumatoid adult donors than cells coated with any other anti-D and that the vast majority of the agglutinating sera have antibodies that cannot be characterized (Wilson and Steinberg, 1965). At any rate, we may conclude that serum 2,904 is negative for all

TABLE 6
SUMMARY OF Gm(8) TYPING OF Gm^{1+21}/Gm^- SAMPLES

Family	Individual	Score*	Conclusion †
712.06.	2,070	16	-?
725.04.	2,727	8	+
720.01.	{2,389	19	-?
	{2,351	6	+
710.16.	{2,800	25	-
	{1,848	16	-?
	{1,773	9	+
	{1,850	14	-?
	{1,840	23	-
712.09.	2,069	17	-?
558.13.	5,303	17	-?
513.07.	4,025	6	+
539.09.	{5,234	12	+?
	{5,177	2	+
546.01.	3,921	3	+
517.07.	4,437	1	+
545.08.	5,354	14	-?
562.07.	5,083	16	-?
511.06.	4,304	9	+
515.02.	3,314	23	-

* Scores based on results of two-way titrations: sera: 1/8-1/32; Ragg: 1/256-1/4,096. Reagents: P.C.E. 64/anti-D 535-1/10. Note: 2,904 (-/-) done at the same time: score = 4.

† + control 5; - control 21.

TABLE 7
SUMMARY OF THE RESULTS OF THE TESTS OF THE SERUM OF DONOR 2,904, OF HER PARENTS (FAMILY 712.06), AND OF CONTROLS FOR ISf(1)

DONOR	DILUTION OF DONOR'S SERUM			
	1/2	1/4	1/8	1/16
Father	3*	4	4	4
Mother	±	2	3	4
2,094	3	4	4	S
02/14	0	±	2	3
03/258	3	4	4	4

* The digits indicate the degree of agglutination: 0 = no agglutination; 1-4 = increasing degrees of agglutination; S = solid agglutination, i.e., no free cells.

known genetically determined antigens on the $\gamma 1$ and $\gamma 3$ heavy-chain subclasses of IgG.

One possible explanation for the absence of the Gm antigens in serum 2,904 is that there is a deficiency of $\gamma 1$ and $\gamma 3$ heavy chains. (Absence of IgG is excluded on a priori grounds, because the donor is a healthy adult and because direct tests indicate a normal level of IgG.) Tests for the heavy-chain subclasses $\gamma 1$, $\gamma 2$, and $\gamma 3$ showed that all are present and that the concentration of $\gamma 3$ is approximately twice that in sera from donors without the Gm^- allele. Drs. Terry and Kunkel have confirmed these findings. Dr. Terry has found a considerable reduction of $\gamma 1$ and a marked increase of $\gamma 2$ as well as of $\gamma 3$ in the serum of 2,904. No test for $\gamma 4$ ($\gamma 2d$) has been done.

Six serum samples, four from heterozygotes for the Gm^- allele and two from individuals not carrying the allele, were sent to Dr. Terry, who tested them without knowing which, if any, carried the Gm^- allele. The data, presented in Table 8, strongly

TABLE 8
RESULTS OF DETERMINATIONS OF RELATIVE CONCENTRATIONS OF $\gamma G1$ AND $\gamma G3$ IN SERUM SAMPLES FROM HETEROZYGOTES FOR Gm^- AND FROM DONORS NOT CARRYING THE Gm^- ALLELE*

SPECIMEN NUMBER	FROM FAMILY	GENOTYPE	RELATIVE CONCENTRATION OF	
			$\gamma G1$	$\gamma G3$
2,389.....	720.01	$Gm^{1, 17, 21}/Gm^-$	Normal	Elevated
2,351.....	720.01	$Gm^{1, 17, 21}/Gm^-$	Slightly reduced	Elevated
2,717.....	725.04	$Gm^{3, 5, 13, 14}/Gm^-$	Slightly reduced	Elevated
2,720.....	725.04	$Gm^{2, 5, 13, 14}/Gm^-$	Normal	Elevated
2,770.....	725.04	$Gm^{1, 21}/Gm^{3, 5, 13, 14}$	Normal	Normal
2,716.....	725.01	$Gm^{1, 21}/Gm^{3, 5, 13, 14}$	Normal	Normal

* See NOTE to Table 1. Tests done "blind" by Dr. Terry of the National Institutes of Health.

indicate that the Gm^- allele in the heterozygous as well as the homozygous condition is associated with an increased production of the $\gamma 3$ heavy-chain subtype. If sufficient anti- $\gamma 3$ antiserum becomes available, this observation may be used to determine whether the Gm^- allele is present when family data are not instructive. It is possible, but unlikely, that the Gm^- allele in the heterozygous state also has an effect on the $\gamma 1$ heavy chain, because in each of the two families one heterozygous sib showed a "normal" level of $\gamma 1$ while the other showed a slight reduction.

The IgG was isolated from the serum of 2,904 by column chromatography. The heavy and light chains were isolated by reduction and alkylation. The isolated heavy chain was negative for the Gm antigens 1, 2, 3, 5, 6, 13, and 14. The isolated light chain was Inv(3), as was the whole serum (Table 3). The sedimentation coefficients of the isolated IgG (5 mg/ml) and the isolated heavy chain (3 mg/ml) were determined by ultracentrifugation in a Spinco model E, with the control and test samples run simultaneously in phosphate buffered saline, pH 7.2. The sedimentation coefficient (S_{20}) was 6.7 for IgG from 2,904 and from the control. S_{20} was 1.9 for the heavy chain

from 2,904 and 2.0 for the heavy chain from the control. It appears that there is no difference in molecular weight between the IgG molecules or the heavy chains of donor 2,904 and those of the control serum.

Additional evidence for the presence of $\gamma 1$ and $\gamma 3$ (as well as $\gamma 2$ and $\gamma 4$) in the serum of 2,904 was obtained from further studies, in which two rabbits and two rhesus monkeys were immunized with IgG isolated from the serum of 2,904. Both of the rabbits and one of the monkeys made precipitating antibody against IgG. Furthermore, the three antisera contained antibodies that both detected and differentiated heavy chains of all four subclasses. Details of the immunological studies and of fingerprint studies will be presented in another paper.

The Gm^{1, 3, 17, 21} Allele

The first evidence that an allele which produces Gm(3) and not Gm(5) is present in the H-leut was found when families 61.10 and 151.07 (Table 1 and Steinberg and Goldblum, 1965) were tested for Gm(3). These data were interpreted as indicating the presence of a Gm^3 allele, and it was thought that the genotype of the mothers of these families was Gm^1/Gm^3 . Further family studies and tests for Gm(21) and subsequently Gm(17) in some samples show that this interpretation is probably incorrect.

The most direct evidence for a $Gm^{1, 3, 17, 21}$ allele comes from family 720.01 (Table 4A). The two Gm(1, 17, 21) children and the three Gm(1, 3, 17, 21) children indicate that the mother, whose phenotype is Gm(3, 5, 13, 14), has the Gm^- allele known to be present in her family. The Gm(1, 17, 21) children must have obtained these antigens via an allele inherited from the father, because they are not present in the mother; similarly, the Gm(1, 3, 17, 21) children must have obtained these antigens via an allele from the father. A reasonable hypothesis is that the father's genotype is $Gm^{1, 3, 17, 21}/Gm^{1, 17, 21}$. The father of family 720.02 (Table 9) is a son in family 720.01. His probable genotype, therefore, is $Gm^{1, 3, 17, 21}/Gm^-$. The phenotypes of his two children are in agreement with this assumption.

Further support for the existence of a $Gm^{1, 3, 17, 21}$ allele comes from family 520.21 (Table 9). (Only the father was tested for Gm(17).) The mother is probably homozygous for $Gm^{1, 21}$. The seven Gm(1, 3, 21) children must have obtained the Gm(3) antigen from an allele transmitted by the father. If Gm(3) were due to a Gm^3 or $Gm^{3, 21}$ allele, the father's second allele would have to be $Gm^{1, 3, 5, 13, 14, 21}$ or $Gm^{1, 3, 5, 13, 14}$ to explain his Gm(1, 3, 5, 13, 14, 21) phenotype. Such alleles have not been detected among the H-leut. Therefore, we conclude that the father's genotype is $Gm^{1, 3, 21}/Gm^{3, 5, 13, 14}$ (and, if Gm(17) is considered, $Gm^{1, 3, 17, 21}/Gm^{3, 5, 13, 14}$).

The phenotype of the father of family 520.19 and of the mother of 720.18 is Gm(1, 3, 21). Their genotype could be $Gm^3/Gm^{1, 21}$ or $Gm^{3, 21}/Gm^{1, 21}$ or $Gm^{1, 3, 21}/Gm^{1, 21}$. Each is mated to a Gm(3, 5, 13, 14) spouse. If $Gm^3/Gm^{1, 21}$ or $Gm^{3, 21}/Gm^{1, 21}$ is the correct genotype, half the offspring should be Gm(-1). There were 5 offspring in family 520.19 and 7 in family 720.18. All 12 were Gm(1, 3, 5, 13, 14, 21). The probability of this occurring by chance if a Gm^3 or a $Gm^{3, 21}$ allele is present is $(\frac{1}{2})^{12}$ or $\frac{1}{4,096}$. The assumption of a $Gm^{1, 3, 21}$ allele requires that all 12 offspring be Gm(1, 3, 5, 13, 14, 21), as indeed they are.

TABLE 9
GM PHENOTYPES AND PROBABLE GENOTYPES OF THIRTEEN
FAMILIES WITH THE $Gm^{1, 3, 17, 21}$ ALLELE*

Family and Individual†	Gm Phenotype‡	Probable Genotype‡
62.03:		
Father§	1, 2, 3, 5, 13, 14, 17, 21	1, 2, 17, 21/3, 5, 13, 14
Mother	1, 3, 5, 13, 14, 17, 21	1, 3, 17, 21/3, 5, 13, 14
4 	1, 2, 3, 21	1, 3, 21/1, 2, 21
1	1, 2, 3, 5, 13, 14, 21	1, 2, 21/3, 5, 13, 14
4	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
69.01:		
Father	1, 3, 5, 13, 14, 21	1, 3, 21/3, 5, 13, 14
Mother	1, 3, 5, 13, 14, 21	1, 21/3, 5, 13, 14
4 	1, 3, 21	1, 3, 21/1, 21
2	1, 3, 5, 15, 14, 21	1, 3, 21/3, 5, 13, 14 or 1, 21/3, 5, 13, 14
2	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
151.17:		
Father	1, 2, 3, 5, 13, 14, 21	1, 2, 21/3, 5, 13, 14
Mother#	1, 3, 5, 13, 14, 21	1, 3, 21/3, 5, 13, 14
1 	1, 2, 3, 21	1, 3, 21/1, 2, 21
155.08:		
Father	1, 3, 5, 13, 14, 21	1, 21/3, 5, 13, 14
Mother	1, 3, 5, 13, 14, 21	1, 3, 21/3, 5, 13, 14
1 	1, 3, 21	1, 3, 21/1, 21
3	1, 3, 5, 13, 14, 21	1, 3, 21/3, 5, 13, 14 or 1, 21/3, 5, 13, 14
2	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
513.10:		
Father	No sample	
Mother 	1, 3, 21	1, 3, 21/1, 21
1	1, 3, 5, 13, 14, 21	1, 3, 21/3, 5, 13, 14 or 1, 21/3, 5, 13, 14
517.07:		
Father 	1, 3, 21	1, 3, 21/1, 21
Mother	No sample	
6	1, 3, 5, 13, 14, 21	1, 3, 21/3, 5, 13, 14 or 1, 21/3, 5, 13, 14
520.19:		
Father 	1, 3, 21	1, 3, 21/1, 21
Mother	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
5	1, 3, 5, 13, 14, 21	1, 3, 21/3, 5, 13, 14 or 1, 21/3, 5, 13, 14
520.21:		
Father	1, 3, 5, 13, 14, 17, 21	1, 3, 17, 21/3, 5, 13, 14
Mother	1, 21	1, 21/1, 21
7 	1, 3, 21	1, 3, 21/1, 21
2	1, 3, 5, 13, 14, 21	1, 21/3, 5, 13, 14
541.10:		
Father 	1, 3, 21	1, 3, 21/1, 21
Mother	1, 3, 5, 13, 14, 21	1, 21/3, 5, 13, 14
3 	1, 3, 21	1, 3, 21/1, 21
3	1, 21	1, 21/1, 21
5	1, 3, 5, 13, 14, 21	1, 3, 21/3, 5, 13, 14 or 1, 21/3, 5, 13, 14

* See NOTE to Table 1. See also families 61.10 and 151.07 in Table 1 and family 720.01 in Table 4A.

† The numbers in this column refer to the number of children with the indicated phenotype.

‡ The generic term Gm is omitted. See text for further details.

§ To conserve reagent, only certain individuals (as indicated in the table) were tested for Gm(17).

|| Indicates individuals with unusual phenotypes.

She is a child in family 151.07 (see Table 1).

TABLE 9—Continued

Family and Individual†	Gm Phenotype‡	Probable Genotype‡
562.11:		
Father	1, 3, 5, 13, 14, 21	1, 3, 21/3, 5, 13, 14
Mother	No sample	
2 	1, 3, 21	1, 3, 21/1, 21
2	1, 3, 5, 13, 14, 21	1, 3, 21/3, 5, 13, 14 or 1, 21/3, 5, 13, 14
1	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
711.16:		
Father	No sample	
Mother 	1, 3, 21	1, 3, 21/1, 21
3	1, 3, 5, 13, 14, 21	1, 3, 21/3, 5, 13, 14 or 1, 21/3, 5, 13, 14
720.02:		
Father	1, 3, 17, 21	1, 3, 17, 21/—
Mother	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
1 	3, 5, 13, 14	3, 5, 13, 14/—
1	1, 3, 5, 13, 14, 21	1, 3, 21/3, 5, 13, 14
720.18:		
Father	3, 5, 13, 14	3, 5, 13, 14/3, 5, 13, 14
Mother 	1, 3, 21	1, 3, 21/1, 21
7	1, 3, 5, 13, 14, 21	1, 3, 21/3, 5, 13, 14 or 1, 21/3, 5, 13, 14

We believe we have established the presence of a $Gm^{1, 3, 17, 21}$ allele and will not pursue the argument further except to state that the data for all the families listed in Table 9 plus those for families 61.10 and 151.07 in Table 1 and family 720.01 in Table 4A are consistent with this interpretation. Dr. Litwin kindly tested "blind" three $Gm^{1, 3, 17, 21}/Gm^-$ and three $Cm^{3, 5, 13, 14}/Gm^-$ samples for Gm(17) and Gm(22). His Gm(17) tests confirmed our previous findings. He wrote concerning Gm(22), "Gm(y) [Gm(22)] recently has become most difficult to type with and use." He was able, however, to ascertain the presence of Gm(22) in one $Gm^{1, 3, 17, 21}/Gm^-$ sample and in all three of the $Cm^{3, 5, 13, 14}/Gm^-$ samples. Hence, it seems likely that the $Cm^{1, 3, 17, 21}$ allele leads to the production of Gm(22) also. Gm(22) is transmitted with Gm(3) in Caucasoids (Litwin and Kunkel, 1967), that is, with the $Cm^{3, 5, 13, 14}$ allele. The $Gm^{1, 3, 17, 21}/Gm^-$ samples were also tested for Gm(20) (Klemperer *et al.*, 1966). Since all were Gm(20), the allele is $Gm^{1, 3, 17, 20, 21, 22}$. We shall continue, however, to refer to the allele as $Gm^{1, 3, 17, 21}$, because reagents for Gm(22) are not readily available and because Gm(20), which usually occurs with Gm(1), is carried on the Fc portion of the $\gamma 1$ heavy chain (Klemperer *et al.*, 1966, and unpublished data from this laboratory), on which Gm(1) and (22) are also located.

All the individuals believed to have the $Gm^{1, 3, 17, 21}$ allele can be traced to ancestral family 30 (Fig. 3). In addition, many, but not all, had ancestors in family 48. Similarly, many but not all individuals with the Gm^- allele are traceable to family 30. (These observations are not surprising inasmuch as about 45% of the individuals in this population are descended from family 48 and about 60% from family 30 [Martin and Steinberg, unpublished observations].)

Our present conclusion is that the Gm^- allele was introduced via family 48 and

some segment subject to suppression is sometimes inherited from the father and sometimes from the mother. Such a pattern is extremely unlikely. Furthermore, the pattern of relationship (Fig. 1) among those heterozygous for the chromosome that produces no Gm antigens on the $\gamma 1$ or $\gamma 3$ heavy chains suggests dominant rather than recessive inheritance.

A dominant inhibitor, if it exists, must be closely linked to the Gm locus, because only in this way does limitation of its effect to a portion of only one chromosome of a pair seem reasonable. Crossing over between the inhibitor and the Gm locus could be detected if a child with Gm(3, 5, 13, 14) in its phenotype were recovered from a mating of $Gm^{1, 21}/Gm^- \times Gm^{1, 21}/Gm^{1, 21}$ or if a child with Gm(1, 21) in its phenotype were recovered from a mating of $Gm^{3, 5, 13, 14}/Gm^- \times Gm^{3, 5, 13, 14}/Gm^{3, 5, 13, 14}$. No such children have been observed in known matings of this type, nor in matings between assumed Gm(1, 21) homozygotes, nor in those between assumed Gm(3, 5, 13, 14) homozygotes, although we have looked at many hundreds of such matings among the H-leut. Thus, it seems unlikely that the absence of Gm antigens is due to an inhibitor, either recessive or dominant. The most reasonable assumption is an allele (Gm^-) at the Gm locus that fails to produce any of the known Gm antigens.

The presence or absence of the Gm^- allele could be determined for 100 of the 136 children in the 18 families in which the Gm^- allele was segregating in one parent. Forty-eight of the 100 had the Gm^- allele. The maximum-likelihood estimate of the proportion (p) with the allele, using truncate selection, is $p = 0.46 \pm 0.05$. The difference from 0.50 is not significant ($P \sim 0.4$).

2. *The $Gm^{1, 3, 17, 21}$ allele.* This allele, shown to be present in 16 families and 52 individuals (Tables 1, 4A, and 9), seems to be distinct from the Gm^- allele and to have been introduced via family 30 (Fig. 3). We have not been able to establish any relation between this family and family 48, to which all except one of the individuals bearing the Gm^- allele can be traced (Fig. 1).

The $Gm^{1, 3, 17, 21}$ allele was observed to be segregating in 16 families (Tables 1, 4, and 9) with a total of 102 children. The presence or absence of the $Gm^{1, 3, 17, 21}$ allele could be determined for 66 of these 102 children. Thirty-seven of the 66 had the allele and 29 did not; $p = 0.55 \pm 0.06$, using truncate selection and Haldane's maximum-likelihood method. The difference from 0.50 is not significant ($P \sim .4$).

"Molecular" Genetics

It would be of great interest to know how the Gm^- allele affects the IgG molecules so that no Gm antigens are detected on the $\gamma 1$ or $\gamma 3$ heavy chains. Our investigations are a long way from the answer to this question. Failure to produce $\gamma G1$ and $\gamma G3$ molecules is excluded, since the double-diffusion immunoprecipitation tests indicate that both molecules are present. Single-diffusion tests indicate that the Gm-negative donor (2,904) has a greatly increased proportion of $\gamma G3$ molecules and a reduced, although detectable, amount of $\gamma G1$ molecules. The ultracentrifugation analyses indicate that no major change in molecular size or shape has occurred. On the basis of the currently available evidence, we must conclude that the IgG molecules in this donor's serum are normal, albeit probably changed in relative concentrations. We

assume that her heavy chains differ from the heavy chains of other donors only in their primary sequence.

It has been postulated that a separate gene controls the synthesis of each of the heavy-chain subclasses, that the gene which controls the synthesis of the $\gamma 1$ heavy chain produces the Gm antigens found on that chain, and that the gene which controls the synthesis of the $\gamma 3$ heavy chains produces the Gm antigens found on it (Kunkel, Allen, and Grey, 1964; Kunkel, Allen, Grey, *et al.*, 1964; Mårtensson and Kunkel, 1965). These genes are postulated to be adjacent to each other.

The multilocus hypothesis has been expanded to postulate that the portion of the gene determining the Fd segment of the $\gamma 1$ heavy chain is adjacent to the portion of the gene determining the Fc segment of the $\gamma 3$ heavy chain (Mårtensson *et al.*, 1966). A summary of this viewpoint is presented in Figure 4, which is based upon the published and the unpublished reports of various investigators (Mårtensson, 1966; Litwin and Kunkel, 1967; Ropartz, Rivat, and Rousseau, 1967). According to this hypothesis, the chromosomes commonly present in Caucasoids, using as markers the Gm antigens 1, 3, 5, 13, 14, 17, 21, and 22, are as shown in Figure 5.

This attractive hypothesis is not sufficient, however, to explain various kinds of published data (Steinberg, 1965*a*, 1967; Muir and Steinberg, 1967). As indicated

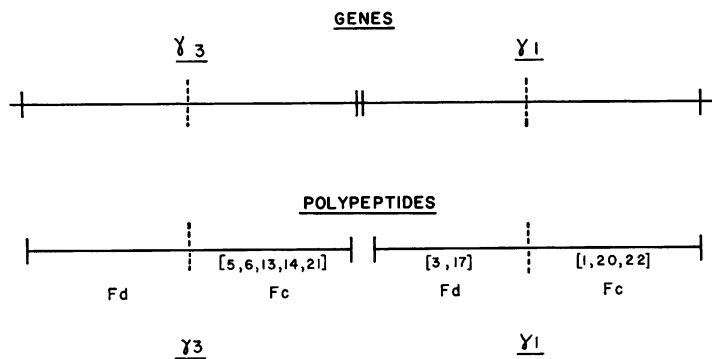


FIG. 4.—Diagram representing the multigene hypothesis. The uncertainty concerning the location of the antigens within the Fab and Fc portions of the polypeptide chains is indicated by placing them in brackets. See text for further details.

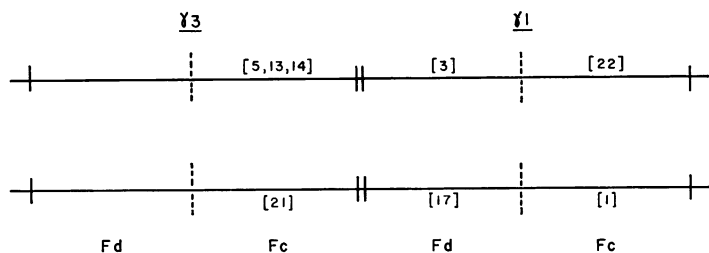


FIG. 5.—Diagram representing the chromosomes usually found in Caucasoid populations. See legend to Fig. 3 and text for further details.

earlier, no explanation has been offered for the fact that these specific complexes are common; that is, they comprise at least 99% of the chromosomes of Caucasoids. The rarer complexes are interpreted as having arisen from the two shown in Figure 5 by crossing over (Ropartz *et al.*, 1965; Mårtensson, 1966; Litwin and Kunkel, 1967; Ropartz, Rivat, and Rousseau, 1967). For example, Ropartz, Rivat, and Rousseau (1967) presented evidence for a chromosome segment which determines antigens Gm(3) and Gm(21) and possibly Gm(22), that is, a $Gm^{3, 21}$ or $Gm^{3, 21, 22}$ allele. They postulated that this allele originated in an ancestor as the result of crossing over between the genes determining $\gamma 1$ and $\gamma 3$. Although this interpretation is consistent with the data, there has been no direct demonstration of crossing over from family studies, such as that presented for the Rh locus (Steinberg, 1965*b*). Thus, mutation is an equally likely possibility.

Be that as it may, the explanation of the origin of $Gm^{1, 3, 17, 21, 22}$ within the framework of the two-locus hypothesis requires that, under the most favorable codon arrangement, a minimum of two crossovers occurred within the postulated $\gamma 1$ gene (Fig. 6). Any alternative arrangement would require at least three crossovers.

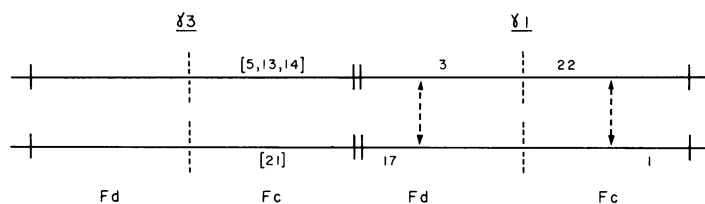


FIG. 6.—Diagram showing arrangement of “codons” determining the antigens in the postulated $\gamma 1$ locus permitting the derivation of the $Gm^{1, 3, 17, 21, 22}$ chromosome by only two crossovers (dotted arrows). Other arrangements would require more than two crossovers.

The origin of the Gm^- allele is even more challenging for the multiple-locus hypothesis. An explanation based on crossing over would require, under the most favorable arrangements of the codons for the multiple-locus hypothesis, two intragenic crossovers in the $\gamma 1$ locus and one in the $\gamma 3$ locus. An explanation based on mutation would require either that a single mutation affected the antigens of two loci or that two mutations occurred, one in each locus. Neither of these alternatives seems likely to us. Equally implausible is a deficiency or nonsense sector long enough to involve the allotypic antigens of both loci, but not the isotypic antigens.

None of the aforementioned difficulties arises if a single locus is postulated to determine the Gm antigens. The various combinations of antigens are assumed to be due to a series of alleles derived by mutation and possibly by crossing over. However, although the assumption of a single locus encounters little difficulty in explaining the antigenic combinations, it encounters considerable difficulty in explaining the genetic control of the heavy-chain subclasses and the association of groups of Gm antigens with only one heavy-chain subclass. Neither the single-locus hypothesis, the multiple-locus hypothesis, nor current genetic theory (dogma) is adequate to explain the tremendous variability of the heavy chains within a subclass. Furthermore, current genetic dogma cannot adequately explain the double amino acid substitution, requir-

ing five nucleotide changes, observed between the pentapeptide associated with Gm(1) and that associated with Gm(-1) (Frangione *et al.*, 1966; Thorpe and Deutsch, 1966; Muir and Steinberg, 1967). In brief, it is our belief that we are still far from a satisfactory explanation of the genetics of the IgG molecules. In the light of this and in the light of the available family and population data, we propose to continue to treat the pattern of inheritance as though it is due to a series of alleles (Muir and Steinberg, 1967).

SUMMARY

None of the Gm antigens carried by the $\gamma 1$ and $\gamma 3$ heavy chains of IgG was detected in the serum, the isolated IgG, or the isolated heavy chains of a healthy 28-year-old mother of three children, although both of these heavy-chain subclasses were present. Her IgG and her isolated heavy chains could not be distinguished from those of other subjects by ultracentrifugation, immunoelectrophoresis, or double immunodiffusion. However, the concentration of $\gamma G3$ in the donor's IgG was about twice that found in the IgG of "normal" donors. The donor was shown to be homozygous for an unusual Gm^- allele that was detected in 19 families of an inbred religious isolate. A second unusual allele ($Gm^{1, 3, 17, 20, 21, 22}$) was detected in 16 families of this same inbred religious isolate. We refer to the allele as $Gm^{1, 3, 17, 21}$, because reagents for Gm(20) and Gm(22) are not readily available. Some individuals were heterozygous for both alleles (i.e., $Gm^-/Gm^{1, 3, 17, 21}$).

All individuals with the Gm^- allele (except one for whom ancestral data are scanty) can be traced to one family, and all individuals with the $Gm^{1, 3, 17, 21}$ allele can be traced to a second family. These families probably introduced the alleles to the isolate.

The data are discussed in relation to the number of loci which determine the Gm antigens.

ACKNOWLEDGMENTS

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ADDENDUM

Since this paper was submitted for publication we have become aware of a report (Natvig *et al.*, 1967) of two pedigrees (I and H.W.) purporting to show crossing over between the postulated $\gamma 3$ and $\gamma 1$ loci and a third pedigree (family 777) purporting to show crossing over between the postulated $\gamma 2$ and $\gamma 3$ loci. The first two families may be explained by assuming the presence of a $Gm^{1, 3, 17, 21, 22}$ allele. This is shown in Addendum Table A. It is of interest that the phenotypes of the parents and children of these families are the same as those of some of the families in Table 9.

The exceptional child in family 777 may indeed involve a crossover, but, as the authors indicate, the child may also be the result of an extramarital mating and therefore will not be considered further here.

ADDENDUM TABLE A

INTERPRETATION OF PEDIGREES I AND H.W. FROM NATVIG ET AL. (1967),
ASSUMING THE PRESENCE OF THE $Gm^{1, 3, 17, 21, 22}$ ALLELE*

Individual	Phenotype†	Probable Genotype†
Family I		
Father	1, 2, 3, 5, 17, 21, 22	1, 2, 17, 21/3, 5, 22
Mother	1, 3, 5, 17, 21, 22, 23	1, 3, 17, 21, 22/3, 5, 22, 23
Son	1, 2, 3, 17, 21, 22	1, 2, 17, 21/1, 3, 17, 21, 22
Son's wife	1, 2, 17, 21	1, 2, 17, 21/1, 17, 21 or 1, 2, 17, 21
Grandchild	1, 2, 3, 17, 21, 22	1, 2, 17, 21/1, 3, 17, 21, 22
Family H.W.		
Father	1, 3, 5, 17, 21, 22, 23	1, 3, 17, 21, 22, 23/3, 5, 22
Mother	1, 2, 17, 21	1, 2, 17, 21/1, 17, 21 or 1, 2, 17, 21
Child 1	1, 2, 3, 5, 17, 21, 22	1, 2, 17, 21/3, 5, 22
Child 2	1, 2, 3, 17, 21, 22, 23	1, 2, 17, 21/1, 3, 17, 21, 22, 23

* All samples were tested for Gm(1), Gm(2), Gm(3), Gm(5), Gm(17), Gm(21), Gm(22), and Gm(23). Only the positive reactions are recorded.

† The generic term Gm is omitted.

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