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GENE INTERACTIONS IN IMMUNOGLOBULINS

We are all aware, of course, that the modern interest in genetics of immunoglobulins and the subject of this symposium stems from the now classical work on the recognition of the Gm groups in man by our convener and chairman, Professor Grubb (Grubb and Laurell, 1956). The effective beginning of our understanding of genetically determined antigenic differences in the immunoglobulins of experimental animals is justly attributed to Jacques Oudin (Oudin, 1960a, b). It was Oudin who first applied the word "allotype" (Oudin, 1956) to these serologically identified differences which are controlled by alleles. Through the work of Oudin and others with rabbits (see Kelus and Gell, 1967, for review) and the work of many with human immunoglobulins (see Natvig and Kunkel, 1968, for review), it became clear that two independently segregating gene loci (or sets of gene loci, see below) controlled the allotype of group markers of the heavy (H) and light (L) chains respectively. Recognition of allotypes in the mouse followed soon thereafter (Kelus and Moor-Jankowski, 1961; Lieberman and Dray, 1964, Wunderlich and Herzenberg, 1963), and since that time the findings in the three species have complemented and confirmed each other so that progress in understanding the genetic control of immunoglobulins has occurred with gratifying speed (Herzenberg, McDevitt, and Herzenberg, 1968).

By the early 1960's several classes of immunoglobulins, defined by their H-chains as well as the kappa and lambda L-chain types, had been recognized, presaging the findings of alleles (allotypes) at several loci. In 1964 several workers studying human and mouse immunoglobulin virtually simultaneously recognized the existence of a set of linked structural genes for several immunoglobulin H-chains or classes constituting an H-chain chromosome region (Herzenberg, 1964). In man, Martenson, Kunkel and collaborators (Martensson, 1964; Natvig *et al.*, 1967) found a strict association of the three Gm factors (specificities) Gma, Gmb, and Gmf with two γG subclasses defined, at that time, by heterologous antisera. Since the Gm specificities were already known to be inherited *en bloc* in families and occurred in certain combinations within particular human populations, it was reasonable to assert that they were controlled either at one gene locus or within a chromosome region comprising several loci. The finding of Gm specificities on more than one subclass clearly suggested a chromosome region.

At the same time, as the Gm results were found, we reported that allotypic or isoantigenic specificities in the mouse associated with one or the other of two distinct immunoglobulin classes, γG_{2a} and γA, were determined at closely linked gene loci (Herzenberg, 1964). No

recombinants were found in that study comprising 149 backcross progeny. A more extensive search by Lieberman and Potter for a recombinant revealed none in 1054 back-cross progeny (Lieberman and Potter, 1966).

Interpreting the human and mouse findings together, we formulated the hypothesis in 1964 that the γG and γA H-chains or, more precisely, at least the Fc portions of these chains, were determined by linked gene loci in the H-chain (Ig) chromosome region (Herzenberg, 1964).

Subsequent finding of allotypic specificities on γG_{2b} globulins and allelic electrophoretic mobility differences for Fc fragments of γG_1 globulins defined two more loci Ig-3 and Ig-4 (Herzenberg, Minna and Herzenberg, 1967). These two Ig loci also turned out to be closely linked to each other and to Ig-1 and Ig-2. To date, no crossovers in the Ig region have been detected in some 2000 progeny tested by Potter's group and our own (Potter and Lieberman, 1967; Herzenberg and Warner, 1967). Thus within this species we can conclude that all the known γG and γA H-chains (strictly speaking, the Fc moiety) are controlled by genes in a single chromosome region. From this point, I will stay with my assigned topic, the mouse and rabbit immunoglobulins, and leave discussion of the quite parallel findings in human Gm groups to others in this symposium.

Since we could not, until recently, make antisera which could detect allotypic differences in the γG_1 class, we used, as mentioned above, genetically determined electrophoretic mobility differences to define alleles in this class. A simple method of immunoelectrophoretic analysis of papain digest of the globulins in whole, unfractionated sera was used. After electrophoretic migration of digests of individual mouse serum samples, the γG_1 Fc lines were developed with rabbit antisera specific for these fragments. The positions of the arcs gave three readily distinguished phenotypes, corresponding to the two allelic homozygotes and the heterozygote (Herzenberg, Minna, and Herzenberg). Recently we have obtained potent alloantisera (the presently conventional name for sera obtained by immunizing animals within a species and thus detecting allotypes) which recognize the same two alleles by precipitation. We have only begun analyses with these antisera and do not yet know whether more than two Ig-4 alleles will be found.

Since details of mouse allotypic specificities have been amply covered in a recent review and elsewheres (Herzenberg, McDevitt, and Herzenberg, 1968; Herzenberg, Warner, and Herzenberg, 1965), I would rather not dwell on this subject here. It is worth mentioning, however, that a considerable number of allotypic specificities have now been found in the mouse immunoglobulins. Most of these are restricted to one or another of the Ig classses, i.e. determined by one or another of the Ig loci. Eleven Ig-1 specificities, distributed in various combinations among eight alleles and representing the cross reactions between these alleles have been defined. This number is certainly not an upper limit but reflects the degree of effort devoted to the ever-decreasing reward of simply defining new specificites. Four Ig-2, seven Ig-3, and two Ig-4 specificities have been described and, again, further efforts would certainly reveal more. In addition to these class-restricted specificities, several specificities shared between two or more classes have been found.

The meaning of allotypic specificities common to two or more classes is uncertain. The suggestion has been made (see, e.g., Warner and Herzenberg, 1967) that the various H-chain genes arose from a common ancestor by successive gene duplication, perhaps due to unequal

crossing over among tandem duplicated genes. The common allotypic specificities must then either have arisen prior to the final gene duplications or else occurred independently in each gene which carries the specificity. Neither explanation is particularly attractive but either could account for the findings.

Since the subclasses γG_{2a} and γG_{2b} are rather similar to one another serologically and together differ considerably from γG_1, we have initiated structural studies of the Fc fragments from myeloma proteins of these two subclasses. These studies will make it possible to define the amino acid sequence differences which differentiate these classes and will also provide the basis of comparison needed to define the amino acid differences underlying allotypy in these classes. Figure 1 shows acrylamide gel electrophoresis patterns of several γG_{2a} and γG_{2b} myeloma protein papain digests. Fc fragments from γG_{2a} give a multibanded pattern whereas those from γG_{2b} give one major and one minor band. These different banding pat-

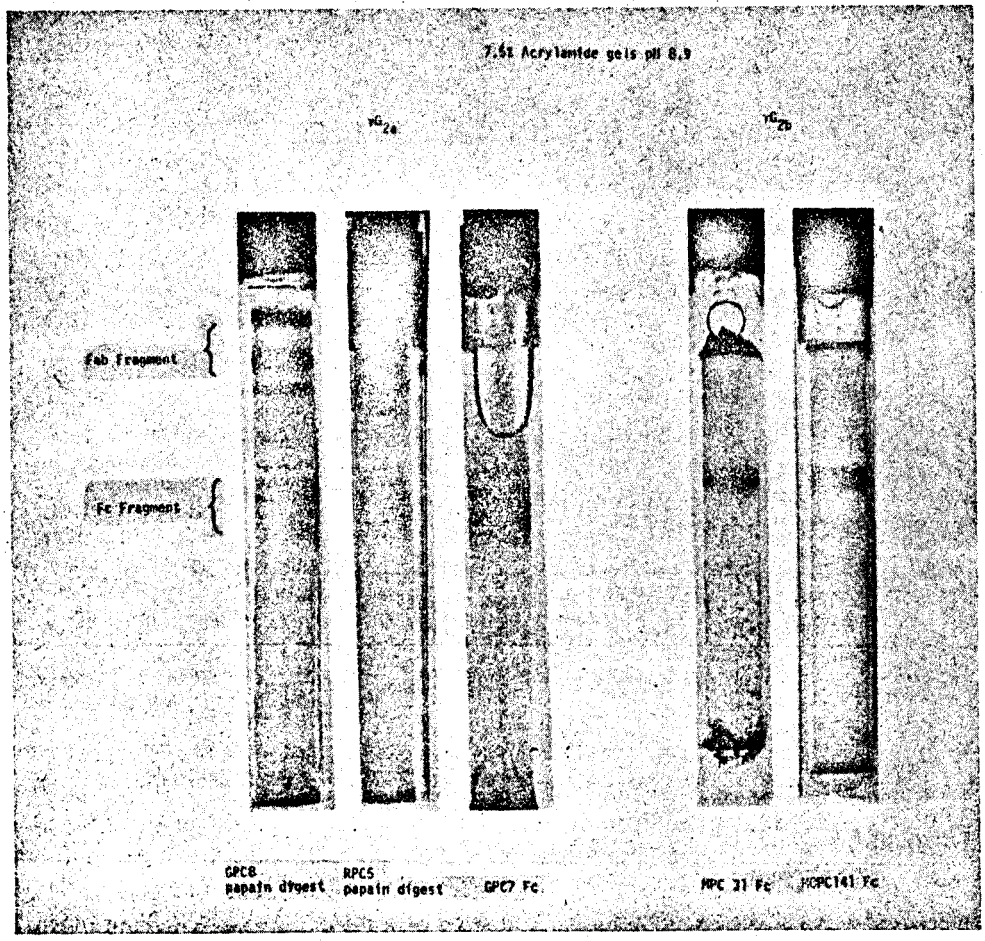

FIG. 1. Acrylamide gel patterns of papain digest and Fc fragments of γG_{2a} and γG_{2b} myeloma proteins.

terns are absolutely characteristic of the subclass (see Fig. 2). Whether the multiplicity of bands is due to amide content heterogeneity or some other cause, we do not know. The Fab fragments show no clear correlation with the subclass from which they come.

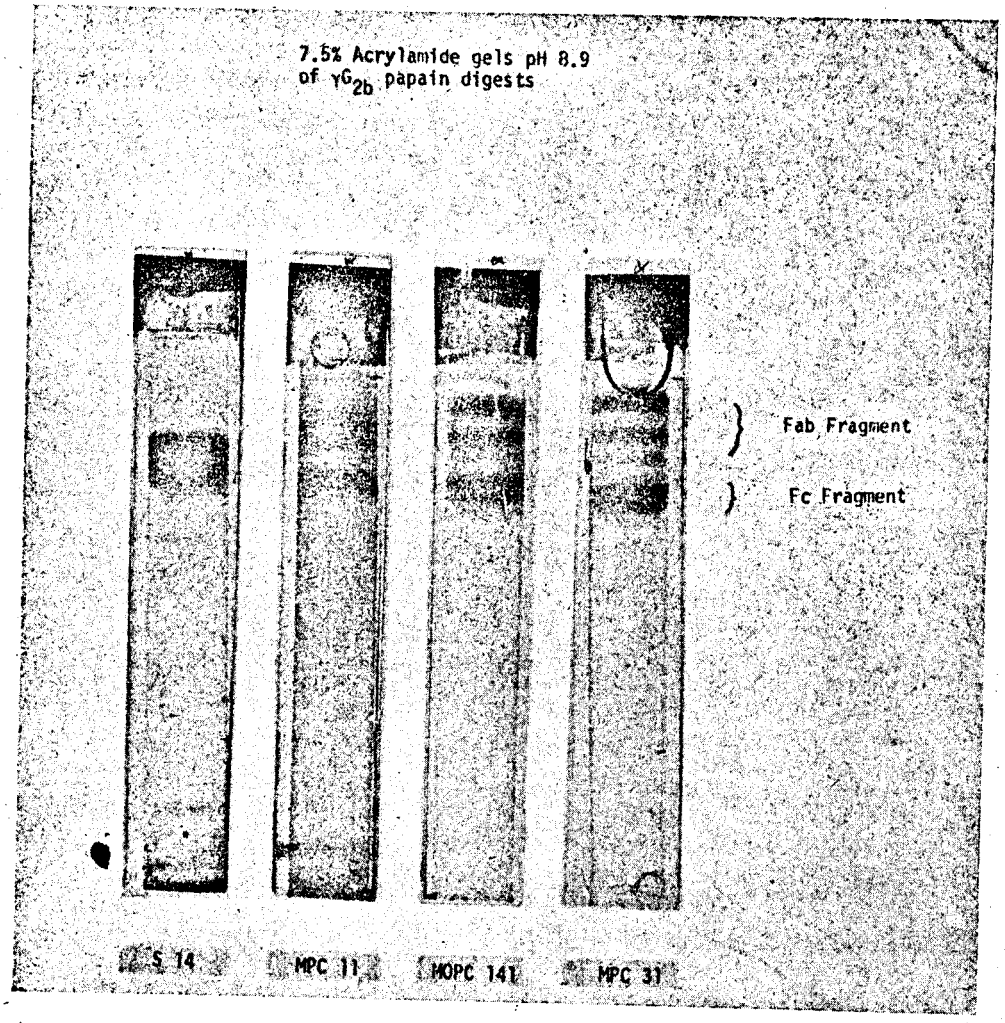

FIG. 2. Acrylamide gel patterns of papain digest of γG_{2b} myeloma proteins.

Tryptic peptide maps of Fc fragments are, again, characteristic of the subclass (see also Potter and Lieberman, 1967). However, comparison of such maps for Fc fragments of different allotypes within a class do show differences. We are attempting to determine which portions of the sequence are different between classes and allotypes and what amino acid changes have occurred. We hope thereby to come closer to understanding the evolution of allotypes and subclasses. The data in Table 1 show that the gross amino acid compositions of several Fc fragments of each class are constant, within experimental errors, but there is a considerable difference noted between classes. There are differences for nearly every amino

TABLE 1. AMINO ACID COMPOSITIONS OF γG_{2a} AND γG_{2b} Fc FRAGMENTS OF Ig^a TYPES

	γG_{2b}	γG_{2a}		γG_{2b}	γG_{2a}
Lys	17.3*(1.0)	19.2 (1.0)	Gly	13.4 (1.1)	9.5 (0.4)
His	3.6 (0.4)	7.7 (0.6)	Ala	7.0 (0.9)	6.3 (0.4)
Arg	8.8 (1.4)	8.5 (0.2)	Val	24.4 (1.2)	28.6 (0.6)
Asp	32.8 (1.0)	32.5 (0.9)	Ilu	15.1 (0.9)	9.0 (0.9)
Thr	18.3 (0.4)	19.3 (0.1)	Leu	17.3 (0.4)	15.3 (0.5)
Ser	22.9 (0.5)	19.5 (0.5)	Tyr	8.9 (0.7)	9.8 (0.5)
Glu	23.0 (1.0)	29.5 (0.9)	Phe	8.6 (0.1)	8.4 (0.3)
Pro	20.4 (0.8)	17.9 (0.9)			

* Number of residues, based on assumption of a unit of 250 residues. Standard deviation given in parenthesis.

acid, but the largest relative differences are for histidine, glycine, and isoleucine. We present these early results only because they may be of some use to others.

All the allotypic specificities so far detected and studied in the mouse are on the Fc region of the H-chains. We are on the lookout for Fab allotypes, but so far none has been found among the inbred strains.

NEGATIVE EVIDENCE FOR L-CHAIN ALLOTYPES IN MICE

Mr. Facon, a graduate student in my lab, has gone to considerable effort to find any difference between L-chains of several mouse strains which could be revealed by amino caid composition analyses. The bulk of γG globulins from AKR/J, BALB/CJ, B10.D2/Sn and C57BL/10 Sn were individually purified by ammonium sulfate precipitation, DEAE chromatography and Sephadex G-200 filtration. The L-chains were obtained, after reduction and alkylation in 8 M urea, by two or three fractionations on G-100 Sephadex in 8 M urea and 1 M proprieonic acid. In Table 2 the results of multiple analyses of two completely independent L-chain isolations, are presented. Line 1 gives the average and standard deviations of three amino acid analyzer runs on the first batch of L-chains isolated. Lines two and three are the data from, respectively, three analyzer runs of one hydrolysate and a single run of a second hydrolysate. As can be seen, no consistent or significant difference in the gross amino acid compositions of these L-chains was found.

Our negative results can be contrasted with the large and readily detected amino acid compositional differences between allotypically different L-chains or H-chains in the rabbit (Reisfeld, Dray, and Nisonoff, 1965; Koshland, 1967). Of course, though disappointing, such results do not prove that there is no L-chain allotypy in these strains. However, they make it more likely that if L-chain allotypes are to be found, sequence studies will have to be done.

The mouse strains we used for this work were chosen from among the common laboratory strains on the basis of known independent histories (Staats, 1966). It is possible that wild mice, or strains more recently derived from wild mice, may reveal L-chain allotypy.

TABLE 2. AMINO ACID COMPOSITION OF POOLED LIGHT CHAINS OF INBRED MOUSE STRAINS

	AKR		BALB/C		B10.D2		C57BL/10	
Lys	12.3	(1.3)	12.5	(1.0)	12.2	(0.6)		
	12.5	(0.3)	12.5	(0.6)	12.6	(1.6)	12.3	(0.2)
	9.3		10.0		10.8		11.6	
His	3.2	(0.2)	3.2	(0.2)	3.4	(0.1)		
	3.2	(0.2)	3.2	(0.3)	3.8	(0.5)	3.6	(0.1)
	2.8		3.2		3.3		3.7	
Arg	7.0	(0.3)	7.4	(0.2)	7.4	(0.5)		
	7.3	(0.5)	7.4	(1.2)	6.9	(0.7)	7.8	(0.3) — line up
	7.2		7.5		7.9		7.1	
Asp	23.1	(0.2)	22.5	(0.1)	23.7	(0.2)		
	26.1	(0.6)	24.7	(0.06)	23.6	(0.4)	23.2	(0.2)
	24.1		23.7		22.0		23.9	
Thr	21.0	(0.7)	20.2	(0.4)	20.1	(0.3)		
	19.9	(0.2)	20.1	(0.2)	20.0	(0.2)	20.3	(0.2)
	20.0		19.9		20.2		20.4	
Ser	29.2	(0.4)	29.8	(0.8)	28.7	(1.8)		
	27.9	(0.3)	27.8	(0.5)	27.2	(0.1)	27.2	(0.3)
	27.8		29.4		27.7		28.4	
Glu	21.1	(0.2)	20.6	(0.1)	20.3	(0.3)		
	23.2	(0.2)	22.8	(0.4)	21.7	(0.1)	20.8	(0.05)
	22.5		23.1		22.7		23.3	
Pro	11.1	(0.4)	11.3	(0.9)	11.5	(0.5)		
	12.0	(0.7)	10.8	(0.7)	11.1	(0.3)	10.8	(0.6)
	12.4		11.6		11.5		11.6	
Gly	14.4	(0.5)	15.1	(0.8)	14.8	(0.5)		
	14.4	(0.1)	14.5	(0.1)	14.4	(0.1)	14.4	(0.3)
	15.6		16.3		15.2		15.1	
Ala	12.0	(0.2)	11.4	(0.3)	10.6	(0.3)		
	11.1	(0.3)	11.4	(0.3)	11.3	(0.3)	10.9	(0.2)
	11.7		12.0		11.7		11.3	
Val	11.0	(1.0)	10.8	(0.1)	12.1	(0.3)		
	12.6	(0.4)	11.9	(0.1)	12.2	(0.1)	11.5	(0.3)
	13.7		12.5		12.7		12.0	
Ilu	9.0	(0.5)	9.0	(0.2)	9.2	(0.3)		
	8.7	(1.2)	9.5	(0.4)	9.7	(0.2)	9.2	(0.2)
	9.7		9.2		9.8		9.6	
Leu	13.9	(0.3)	13.5	(0.2)	14.3	(0.3)		
	14.8	(0.2)	14.6	(0.4)	14.7	(0.1)	14.4	(0.3)
	15.7		13.3		15.0		14.0	
Tyr	9.9	(0.05)	9.4	(0.2)	8.6	(0.3)		
	9.7	(0.4)	9.5	(0.1)	10.1	(0.2)	10.1	(0.06)
	9.8		9.6		10.4		8.9	
Phe	8.3	(0.3)	8.4	(0.3)	8.8	(0.2)		
	8.6	(0.8)	8.4	(0.3)	8.9	(0.3)	8.9	(0.4)
	9.3		8.3		9.1		8.7	
Carboxy-methyl Cysteine	5.7	(0.3)	5.8	(0.2)	6.1	(0.2)	6.1	(0.1)
Met	2.1	(0.1)	2.6	(0.1)	2.5	(0.05)	2.2	(0.1)

For a given residue, except CMC and methionine, the first line shows the average and the standard deviation, in parentheses, of three analyses of one hydrolysate of the first batch of light chains prepared.

The second line shows the average and the standard deviation of three to four analyses of the hydrolysate of a second batch of light chains.

The third line shows the result of one analysis of a second hydrolysate of this second batch of light chains.

Because of great variations in the analyses for CMC and Met between hydrolysates due to oxidation, the figures shown for these residues are the averages and standard deviation of three determinations on the hydrolysate with the highest values for these residues.

SYNTHESIS OF THE H-CHAIN

In the discussion of H-chain allotypes, I have thus far concerned myself only with the allotypes in man and mouse, all of which are located in the "constant" region of their respective H-chains, and, in general, are each found on the H-chain of only one of the immunoglobulin classes. Rabbit H-chain allotypes, however, being found in both the constant and variable regions of the chain, present a more complex picture, which I would like to discuss in detail here since it perhaps gives specific insights into the mechanism of genetic control and synthesis of the H-chain.

The A 8, 11, 12, 14, 15, and 16 allotype antigens in rabbits (Hamers and Hamers-Casterman, 1967; Mandy and Todd, 1969; Dubiski, 1969; Kelus *et al.*, unpub.) are analogous to the human Gm or the mouse Ig antigens, i.e. they are found in the constant region of H-chains of only one class. The above series of rabbit allotypes are on all γ chains while various of the Gm antigens are on different γ subclasses. Ig antigens are on one or another of the mouse subclasses or on α chains. There are also analogous allotypes unique to γA or γM immunoglobulins which may be marker specific for the constant regions of α and μ chains in man (Fudenberg and Kunkel, this symposium) and rabbit (Kindt *et al.*, 1968; Masuda *et al.*, 1969; Conway *et al.*, 1969).

Aa 1, 2, 3 antigens behave quite differently. These antigens are found in the Fd region of the H-chain of all major classes of rabbit immunoglobulins (see Kelus and Gell, 1967; Todd and Inman, 1967). Thus a γ chain marked with A11 in the constant region may carry Aa_1 in the Fd just as a μ chain marked with Ms_1 may carry the same Aa_1 in the Fd. Recent evidence from amino acid compositional data and amino acid sequence data show that the Aa antigens are actually located in the N terminal segment of the chain, i.e. in the variable portion of the Fd fragment (Koshland, 1967; Koshland *et al.*, 1969; Wilkinson, 1969). The composition and sequence data also confirms the presence in μ chains, and α chains of the Aa_1 and Aa_3 allotypes (Koshland, personal communication).

Restating these facts in genetic terms: the γ, α, and μ loci each code for the constant region of the H-polypeptide chains which nonetheless have a common set of N terminal sequences coded for at the Aa locus genes, i.e. each immunoglobulin H-chain is coded for by two genes! Thus not only do we have to explain how the variable region is generated, but we have to discover the mechanism whereby two genes can contribute to the synthesis of a single polypeptide chain.

Some insight into the mechanism of this gene interaction comes from recent studies showing that the interacting genes must be on the same chromosome (Prahl *et al.*, 1970; Mage *et al.*, personal communication). In doubly heterozygous rabbits carrying the alleles Aa_1 and A_{11} on the same member of a chromosome pair (i.e. in coupling, or cis, position) and Aa_3 coupled with A_{12} on the homologous chromosome, the H-chains synthesized are allotypically *either* A_1, A_{11} or A_3, A_{12}. None of the chains demonstrated a trans interaction between the two gene loci. Thus the interaction is intrachromosomal.

The interactions between the constant region genes and the variable region genes could occur at any one of the steps in protein synthesis, i.e. before transcription, at transcription, between transcription, and translation by the joining of two separately transcribed mRNA molecules or after tranlsation by the joining of two separately synthesized polypeptides.

Evidence that the polypeptides are synthesized as units (Fleischman, 1967; Lennox *et al.*, 1967), if confirmed, rules out the last possibility. Of those remaining, none has been tested directly, but as there are no known examples of joining of two mRNA molecules, I think it is not too likely that this is the case for immunoglobulins.

Interaction before or at transcription could result from joining or close approximation of two non-contiguous DNA segments, as, for example, occurs in a chromosome translocation or a process quite analagous to what happens after an episome like a temporate bacteriophage is released from the chromosome. Since there is abundant precedence for both these genetic events, perhaps they are reasonable candidates for the mechanism by which the constant region genes and Aa genes interact.

A model for an intrachromosomal interaction between the Aa locus and either γ, α, or μ genes is represented in Fig. 3. The four loci are represented on the same chromosome with a loop of variable size between Aa and the constant region loci. In this model, one or another of the constant loci is pulled near the Aa locus depending on how much the loop is enlarged. There would have to be homologous recognition regions just to the right of Aa and to the left of each of the constant loci to allow the now contiguous DNA stretches to include the entire Aa locus and all of one of the constant loci. Whether an actual break and rejoining of DNA strands, i.e. a crossover occurs, or whether an RNA synthetase can transcribe a single mRNA molecule from the contiguous genes is not necessary to precise at this time.

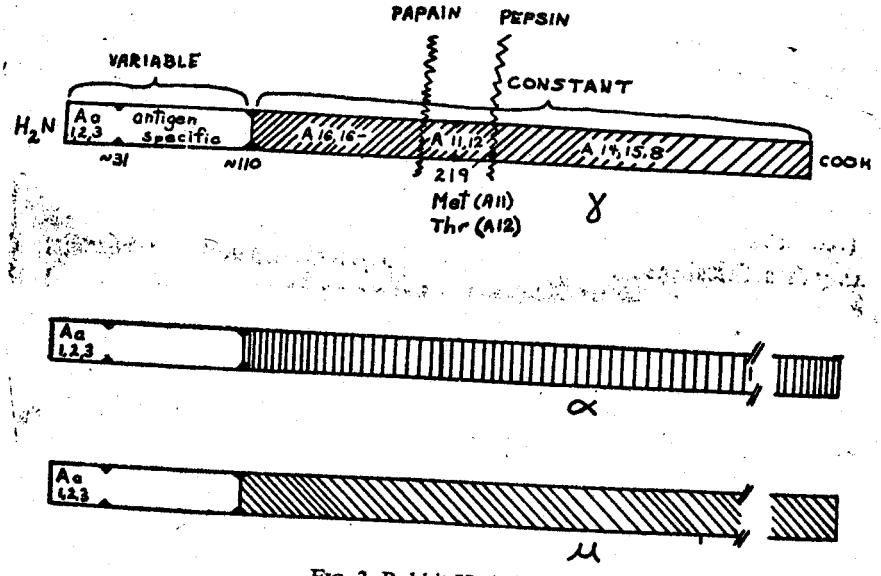

FIG. 3. Rabbit H-chains

TRANSFER OF GENETIC INFORMATION

The last part of this presentation will deal briefly with consideration of whether transfer of genetic information coding for antibody structure is a normal and obligate event in the immune response. Mr. Dale Hattis in my laboratory, in collaboration with Dr. Tom Wegmann of Harvard University, examined the correlation of Ig allotype, an antibody structure

marker, with H-2 type, a cell surface structural marker of antibody producing (and other) cells of tetraparental mice. In such mice, since homozygous cells of 2 different inbred strains exist side by side in all tissues, there is maximum possibility for intercellular information transfer under natural conditions. Early results suggest that the two genetic markers are always found paired as they are in the parental strain. No cells show recombination of the two markers.

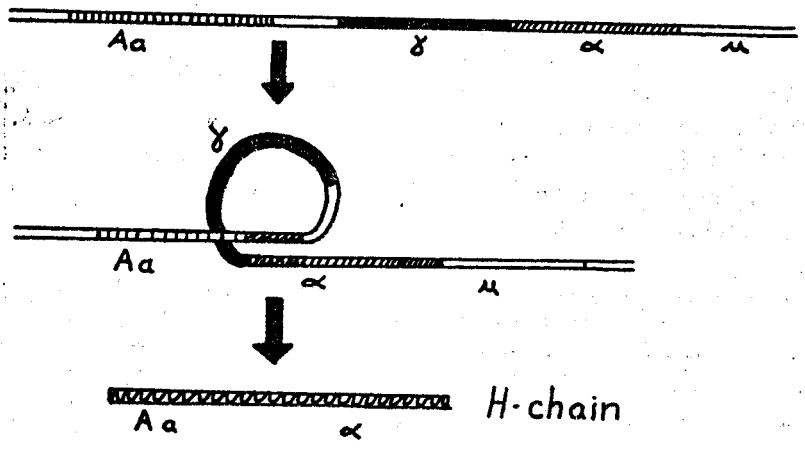

FIG. 4

Tetraparental mice are produced by combining blastomeres of two eggs from different inbred mouse strains into a single embryo (Tarkowski, 1961; Mintz, 1962). The zona pellucida of two eggs (one from each strain) is disrupted in a drop of medium at the eight-cell stage of zygote cleavage and the two zygotes allowed to fuse into a single embryo. The fused embryos are then introduced into pseudo-pregnant female mice where they develop, are born normally, and grow into adult mice which are mosaics (chimeras) of the cell types of the two original zygotic genotypes.

The two inbred strains used for this work, C3H and C57BL/6, differ at the H-2 locus for cell membrane antigens and at the Ig loci for immunoglobulin allotypes. A combination of H-2 cytotoxicity and Ig dependent, anti sheep red blood cell (SRBC) plaque development methods was used to score each antibody forming cell (AFC) for both these unrelated and unlinked loci.

In these experiments adult tetraparental mice were injected twice with 4×10^8 SRBC. There was an interval of four days between injections. Three days after the second injection, the mice were sacrificed, their spleens removed, disrupted and dispersed into suspensions. Aliquots of each suspension were treated in the presence of guinea-pig complement with either of two cytotoxic anti H-2 sera, directed against each of the two "parental" H-2 types in the chimera, and then scored for the number of remaining cells producing anti-SRBC marked with each "parental" allotype.

To score AFC for allotype, each aliquot was plated with SRBC in agarose medium onto glass slides, according to a modification of the Jerne localized-hemolysis-in-gel method for detecting individual AFC. In this method, all slides are incubated at 37°C for 60 min. With

some slides complement is added at this point and left in contact 30 min at 37°C. With other slides, a 1 hr incubation with anti-allotype antisera directed against one or the other of the two "parental" allotypes is interposed between the primary incubation and the complement treatment. The recognition of allotype marked AFC depends on an increase in the number of plaques on the slide treated with anti-allotype antiserum (developed plaques) over the number of plaques on the slide treated only with complement (direct plaques).*

The data in Table 3 show that when a cell suspension made up of a 1:1 mixture of normal C57BL/10 (H-2b, Ig-1b) and a normal C3H (H-2k, Ig-1a) spleen cells, essentially no recombinant cells appear. C57BL cells treated with C3H anti C57BL cytotoxic serum fail to make either direct plaques or plaques developed with either anti-allotype sera. Treatment of the same cells with cytotoxic anti C57BL serum, or with normal serum does not decrease their ability to form either direct plaques or developed plaques. As expected, since these are C57BL cells, only the anti b allotype serum develops any plaques.

TABLE 3. SCORING FOR RECOMBINATION OF ALLOTYPE AND CELL MEMBRANE ANTIGEN MARKERS. I. *In Vitro* MIXTURE OF TWO CELL TYPES

Cell suspension	Cytotoxic serum treatment	Direct plaques	Allotype plaques developed with	
			anti a	anti b
C57BL cells	Anti C57BL	0	5[a]	8
	Anti C3H	117	(−45)	256
	C57BL normal serum control	67	14	289
C3H cells	Anti C57BL	12	41	1
	Anti C3H	8	(−5)	(−4)
	C57BL normal serum control	15	43	0
1:1 mixture	Anti C57BL	11	48	4
	Anti C3H	57	6	268
	C57BL normal serum control	78	36	237

[a] Figures are number of plaques calculated per 10^6 cells.

The same results are obtained with C3H cells, except that it is the anti C3H cytotoxic serum which kills, and the anti a allotype serum which develops.

Finally, in the 1:1 mixture, treatment with anti C57BL cytotoxic sera kills the C57BL cells. The only plaques developed are a allotype plaques which come from the C3H cells in the mixture. Conversely, treatment of the mixture with anti C3H cytotoxic serum removes the C3H cells, and all developed plaques after this treatment are allotype b.

Testing of the tetraparental (chimeric) animals is in the preliminary stages now. Unfortunately, the response to SRBC of 6 animals available for immunization and testing so

* Antibody forming cells secrete antibodies which attach to the surrounding SRBC. Subsequent addition of complement causes lysis of those red cells combined with directly hemolytic, mostly IgM, antibody. Areas of lysis, i.e. plaques; around each AFC secreting directly hemolytic antibody can be seen with the naked eye. Interposing treatment with amplifying antisera such as anti-allotype sera between the primary incubation and the complement-lysis step allows detection of those cells secreting non-hemolytic (or weekly hemolytic) anti-sheep cell antibody. Essentially, such antisera react with the anti-sheep cell antibody found to the SRBC, providing more, and perhaps better, sites for complement attachment and action.

far was very poor in comparison with the controls presented earlier (see Table 4). This poor response may be due to the age of the animals (6–9 mos) to variations in the immunization procedure, or to something peculiar to the tetraparental animal itself. Results with a new series of animals will be available in a few months.

TABLE 4. SCORING FOR RECOMBINATION OF ALLOTYPE AND CELL MEMBRANE ANTIGEN MARKERS. II. RESULTS WITH TWO CHIMERIC ANIMALS

Chimera number	Cytotoxic serum treatment	Direct plaques	Allotype plaques developed with	
			anti a	anti b
k-26-7	Anti C57	3[a]	12	3
	Anti C3H	21	(−2)	21
	C57BL normal serum control	20	15	40
k-26-2	Anti C57	7	11	(−2)
	Anti C3H	4	(−1)	6
	C57BL normal serum control	9	24	4

[a] Figures are number of plaques calculated per 10^6 cells. In k-26-7 1.1×10^6 were plated. In k-226-2, 2.9×10^6 cells were plated in triplicate.

While the data on the two representative animals presented in the table is poor, it does indicate that there is not a massive transfer of information between the cell types. Just as in the *in vitro* mixture of C3H and C57BL cells, those cells surviving by treatment with anti C57BL cytotoxic serum, yield plaques that develop with anti a allotype, and those that survive treatment with anti C3H cytotoxic serum yield plaques that develop with anti b. Thus, within the limitation of this data, the chimeric animals have just two cell types, one carrying the H-2b and Ig-1b markers (C57BL) and the other carrying H-2k and Ig-1a (C3H).

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