Table 1. Distribution of chromosome numbers in analyzed metaphases for two Przewalski's horses (karyotypes in parentheses).

Chromosome numbers				
63	64	65	66	67
		Animal No. 253		
0	1	3	30(6)	1
		Animal No. 171		
1	3	6	35(4)	1

evidence from skeletal studies of museum specimens which suggests that some of these animals may have had domestic horse blood introduced in their ancestors. Indeed, there is some concern over our inability to decide now whether the animals originally imported were not already partially hybridized with wild-running domesticated Mongolian horses. These aspects are fully discussed by Mohr (*4*). Inasmuch as these animals may proceed toward extinction, it would appear urgent to investigate the chromosome complement of the remaining specimens. Moreover, since the pedigree of most animals is not well known it may be possible to reconstruct, after such studies, whether indeed the original Przewalski's horse had the suggested

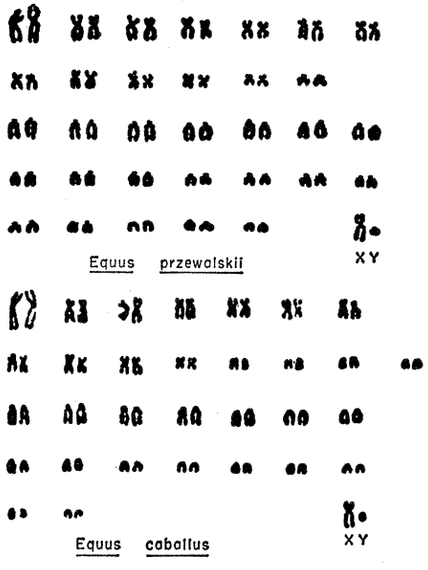

Fig. 2. (Top) Karyotype of metaphase from Romeo (No. 253). There are 66 chromosomes. The chromosomes in the first two rows are considered to be metacentric in this context although, clearly, the last pair could also be considered acrocentric. (Bottom) Karyotype of metaphase from a culture of testis cells from a domestic horse ($2n = 64$). The chromosomes in the first two rows are considered metacentric; however, the last pair may validly be construed as acrocentric.

karyotype or whether it was even more "primitive" and became what has been presented here by a process of hybridization. The pedigree of the animals described here is well substantiated (*4, 5*); they are direct descendants of the original imports and have not been hybridized with domestic horses after capture.

Another interesting aspect of our finding is that hybrids between the Przewalski's horse and various domestic horses have been reported by Iwanoff (*8*) and Lotsy (*9*); others have been referred to by Mohr (*4*). These hybrids are said to be fully fertile (*8, 9*). This is in contrast to the infertility of mules in whom meiotic pairing is impossible because of the extensive rearrangement of chromosomes of the parental species. The reported fertility of the horse hybrids suggests a much simpler type of chromosome rearrangement which is capable of allowing synapsis. Bender and Chu (*10*) have discussed the implications of such potential hybrids for primates (in which they are not known to have occurred) and have drawn attention to the desirability of studying meiotic figures in such instances. By such analysis, possible homologs could be identified, and most autosomes of these two species would have to be homologous to enable meiosis to proceed. Also, this might allow identification of those elements whose robertsonian centric fusion presumably led to the creation of new metacentric autosomes during the reduction of chromosome number in the possible evolutionary step from *Equus przewalskii* to *Equus caballus*. This mechanism of evolution in certain families has been investigated by Bender and Chu (*10*) and is also discussed by Hamerton *et al.* (*11*). In the equines discussed here it may be possible to study this process in greater detail.

Note added in proof: While this manuscript was in press we were able to obtain satisfactory preparations by short-term lymphocyte cultures on Belle (No. 163) and Marcia (No. 263). Both have a complement of $2n = 66$.

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Hybrid Antibody Molecules with Allotypically Different L-Polypeptide Chains

Abstract. *Isolated L-chains with either the A4 or the A5 allotypic markers from rabbit antibodies to 2,4-dinitrophenyl determinant recombine at random with antibody H-chains in the presence or abscence of the hapten to produce hybrid precipitating antibody molecules.*

Normal rabbit γ_2-globulin is composed of two H- and two L-polypeptide chains (*1*). The allelic allotypic markers of the *b*-locus, designated A4 and A5, are present only on the L-chains (*2*). Molecules of γ_2-globulin as they are produced in vivo carry either the A4 or the A5 allotypic marker on each L-chain in animals phenotypically heterozygous in respect to these markers, and no detectable molecules are synthesized in such animals that have one L-chain carrying the A4 marker and one L-chain carrying the A5 marker (*3*). The γ_2-globulin molecules can readily be reformed from the constituent H- and L-polypeptide chains (*4, 5*). Furthermore, when antigen is present during recombination of the polypeptide chains, specific antibody H- and L-chains preferentially reassociate, resulting in a higher percentage of functioning antibody molecules than in the absence of the antigen (*5*). Our experiments were designed to determine whether the presence of antigen during recombination of antibody H- and L-

Table 1. Formation of "hybrid" γ_2-globulin molecules with L-chain allotypic markers A4 and A5.

Recombination mixture	Allotypic antiserum added	Radioactivity precipitated from 7S material (%)		L5/L4 in 7S peak	Radioactivity precipited due to hybrid molecules	
		I^{131} (L4)	I^{125} (L5)		Expected*	Observed
γH4, γL4I^{131}, γL5I^{125}	anti-A5	32.0	65.7	0.92	31.6	32.0
γH5, γL4I^{131}, γL5I^{125}	anti-A4	49.3	19.4	0.92	24.8	19.4

* Sample calculation for random recombinations: If at the molar ratio of L5/L4 = 0.92 random recombination of L4- and L5- with the H4- occurs, then 22.7 percent of molecules would have two L5-chains, 50.1 percent one L5- and one L4-chain, and 27.2 percent two L4-chains. Then 48 percent of L4I^{131}-chains would be in hybrid molecules with L5I^{125} and should precipitate with antiserum to A5 marker. However, the antiserum precipitates only 65.7 percent of the L5I^{125} and thus would bring down also only 65.7 percent of expected I^{131} due to hybrid molecules. Therefore, for random recombination 31.6 percent of I^{131} would be expected to be precipitated.

chains would promote the formation of molecules with identical L-chains, and whether structural differences of the L-chains in respect to allotype dictate the formation of antibodies with two identical L-chains in heterozygous animals.

Our experiments demonstrate that antibody L-chains with differing allotypic markers recombine at random with antibody H-chains even in the presence of antigen, and form hybrid molecules with two different L-chains. Furthermore, allotypic molecular homozygosity has no functional significance with respect to antibody activity in that such hybrid antibody molecules precipitate with the specific antigen.

Experiments were carried out with rabbits homozygous at the *b* locus for either the A4 or the A5 allotypic marker (*6*). All the rabbits were homozygous at the *a*-locus for the A3 allotypic marker, localized on the H-chains (*2*). The γ_2-globulin of these animals prior to immunization was isolated from the serum; after iodination and reduction the H- and L-chains were separated (*5*). The H-chains without iodine label were prepared from isolated γ_2-globulin from both A3A4 and A3A5 rabbits (designated γH4 and γH5, respectively). The L-chains were prepared from I^{125}-labeled γ_2-globulin from A3A5 rabbits (designated γL5I^{125}) and from I^{131}-labeled γ_2-globulin from A3A4 rabbits (designated γL4I^{131}). An equimolar mixture was prepared from γH4, γL5I^{125}, and γL4I^{131}; and a similar mixture from γH5, γL5I^{125}, and γL4I^{131}. The polypeptide chains were dissolved in 1*M* propionic acid at the time of mixing. Subsequently recombination of polypeptide chains was allowed to occur during successive steps of dialysis as follows: once against 1*M* propionic acid; twice against 0.01*M* sodium acetate buffer, *p*H 5.0; and twice against 0.01*M* sodium acetate buffer, *p*H 5.0, rendered 0.15*M* in respect to sodium chloride. The re-formed 7*S* molecules were isolated by sucrose-density gradient ultracentrifugation (*5*).

A single portion of antiserum to the A4 or the A5 allotypic marker (*6*) was added to portions of the isolated recombined 7*S* material. The amounts of antiserum and antigen used were similar to those used by Dray and Nisonoff (*3*). Under these conditions about 50 to 60 percent of A4 molecules and 60 to 70 percent of A5 molecules are precipitated. Only antiserum to the A5 marker was added to the 7*S* material isolated from the γH4, γL5I^{125}, γL4I^{131} mixture. Thus only molecules that were re-formed from completely dissociated L-chains were precipitated. Antiserum to A5 would precipitate recombined γ_2-globulin molecules containing γL5I^{125}-chains, and any I^{131} radioactivity precipitated would necessarily reflect hybrid molecules with γL5I^{125}- and γL4I^{131}-chains. Similarly I^{125} radioactivity precipitated by antiserum to A4 which had been added to the 7*S* material derived from the γH5, γL5I^{125}, γL4I^{131} mixture must represent hybrid molecules with two different L-chains.

As control experiments artificial mixtures were made of γ_2-globulin molecules (dissolved in sodium acetate, sodium chloride buffer, *p*H 5.0) reconstituted from γH4, γL4I^{131} and from γH4, γL5I^{125}. When antiserum to A5 was added, the precipitate contained 68 percent of the I^{125} and 5.0 percent of the I^{131}. Similarly, when antiserum to A4 was added to a mixture of γ_2-globulin molecules reconstituted from γH5, γL5I^{125} and from γH5, γL4I^{131}, 48 percent of the I^{131} and 5.5 percent of I^{125} were precipitated. Obviously, minimum cross-precipitation occurred with each antiserum. The results of the experiments on formation of hybrid molecules with γL4- and γL5-chains (Table 1) show significant cross precipitation, an indication that hybrid molecules containing each kind of L-chain were formed. The calculated percentage of hybrid molecules formed corresponds closely with the expected values if the allotypically different L-chains recombined at random with the added H-chains.

Antibodies to the 2,4-dinitrophenyl determinant (anti-DNP) were produced (*7*) in the same rabbits from which the serum prior to immunization had been obtained. Isolated antibodies from one A3A4 animal and one A3A5 animal were used. Non-iodinated antibody H-chains were prepared from

Table 2. Formation of "hybrid" antibody (to 2,4-dinitrophenyl determinant) molecules with L-chain allotypic markers A4 and A5.

Recombination mixtures from which antibody molecules were isolated	Ratio L5/L4 in:			Allotypic antiserum added	Radioactivity precipitated (%)			
					From isolated antibodies		Due to hybrid molecules	
	Total mixture	7S Peak	Isolated antibodies		I^{131} (L4)	I^{125} (L5)	Expected*	Observed
AbH4, AbL4I^{131}, AbL5I^{125} with hapten	0.98	1.10	1.32	anti-A5	53	81	46	53
AbH4, AbL4I^{131}, AbL5I^{125} without hapten	1.0	0.95	1.14	anti-A5	51	90	48	51
AbH5, AbL4I^{131}, AbL5I^{125} with hapten	1.01	.84	0.96	anti-A4	75	38	38	38
AbH5, AbL4I^{131}, AbL5I^{125} without hapten	1.02	.93	1.10	anti-A4	79	38	38	38

* Sample calculation for random recombination is described at foot of Table 1.

both rabbits (designated as AbH4 and AbH5, respectively). The L-chains were prepared from I^{125}-labeled antibodies from the A3A5 rabbit (designated as $AbL5I^{125}$) and from I^{131}-labeled antibodies from the A3A4 animal (designated as $AbL4I^{131}$). An equimolar mixture was prepared from AbH4, $AbL5I^{125}$, and $AbL4I^{131}$, and a similar mixture from AbH5, $AbL5I^{125}$, and $AbL4I^{131}$. The recombination procedure was carried out on samples of each mixture in the presence and absence of the hapten N-2,4-dinitrophenyl ϵ-aminocaproic acid (DNP-aminocaproate) (*5*). Samples of the recombined material were analyzed on sucrose density gradients, and from the amount of I^{125} and I^{131} in the 7*S* peak and from the specific activities of each of the labeled L-chains the molar ratios, AbL5/AbL4, were calculated. The reformed 7*S* molecules contained nearly the same ratio of AbL5/AbL4 that was present in the original mixture (Table 2). Thus recombination occurred at random and was not influenced by hapten.

Subsequently, the active recombined antibodies were isolated from the mixtures as follows. Precipitates were produced at equivalence between the recombined antibodies and the dinitrophenyl groups conjugated to bovine γ_2-globulin. Unrecombined L-chains do not precipitate or coprecipitate under these conditions (*5*). The precipitates were washed and then redissolved in a sufficient amount of the aforementioned DNP-aminocaproate to provide a 200-fold excess of the dinitrophenyl group. About 40 percent of the calculated amount of 7*S* molecules in the mixture were eluted from the precipitate when recombination was performed in the absence of hapten, and 55 percent when the recombination was performed in the presence of hapten. From the amount of I^{125} and I^{131} in the isolated precipitating antibodies, molar ratios of AbL5 to AbL4 were again calculated. The specifically eluted reconstituted antibodies contained nearly the same ratio of AbL5 to AbL4 that was present in the original mixture (Table 2). Hence the active antibody molecules contain the same molar ratio of L5- to L4-chains as that of the total population of recombined molecules.

Antiserums to the allotypic markers were used to precipitate hybrid molecules exactly as already described for the γ_2-globulin obtained prior to immunization. Antiserum to A5 was added to the active antibodies recovered from a mixture of AbH4, $AbL5I^{125}$, and $AbL4I^{131}$, and similarly antiserum to A4 was added to the active antibodies isolated from a mixture of AbH5, $AbL5I^{125}$, $AbL4I^{131}$. The data (Table 2) indicate that hybrid antibody molecules were produced under the conditions of recombination, and the values observed approximate those calculated for random recombination of the AbL4- and AbL5-chains with the added H-chains. The presence of the hapten had no effect. Thus by this assay at least the antibody molecules with two different L-chains were as active as antibodies with identical L-chains.

In additional experiments the γL4-chains of the γ_2-globulin of unimmunized animals were used in the appropriate mixtures in place of the AbL4-chains. In the presence of the hapten, AbH-chains preferentially recombine with AbL-chains from an equimolar mixture of AbL- and nonspecific γL-chains (*5*). Precipitating antibodies were isolated from a mixture of AbH4, $AbL5I^{125}$, and $\gamma L4I^{131}$ and from a mixture of AbH5, $AbL5I^{125}$, and $\gamma L4I^{131}$. Nonspecific γL-chains were present in the eluted antibody molecules (*5*). Thus the AbL5- and the γL4-chains had formed hybrid molecules with both kinds of AbH chains even though the hapten was present during recombination.

Single cells from germinal centers of lymphoid follicles as well as single mature plasma cells produce γ_2-globulin molecules with both A4 and A5 markers in rabbits heterozygous for these allotypes (*8*). On the other hand, the individual γ_2-globulin molecules from such animals contain only one type of L-chain. Structural restrictions due to allotypic differences do not prevent the formation in vitro of hybrid molecules. Recombination studies with "half-molecules" (one H-chain bound to one L-chain) of rabbit γ_2-globulin results in the formation of hybrid molecules containing different H-chain and L-chain allotypic markers (*9*). Similarly, in our study hybrid-functioning antibody molecules and hybrid γ_2-globulin molecules were produced when separated H- and L-chains were recombined.

Human type I and type II L-chains can form hybrid γ_2-globulin molecules (*10*), although the peptide sequence of type I and type II L-chains are vastly different (*11*). On the other hand the molecules formed in vivo do not have two different L-chains (*12*).

These observations suggest that structural restrictions are not responsible for formation in vivo of functional antibody molecules with two allotypically identical L-chains and imply that the cause of this is related to selective polypeptide synthesis.

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Rubella Complement Fixation Test

Abstract. *Complement-fixing antigens specific for rubella were made in tissue cultures of RK-13 rabbit kidney cells and primary cultures of kidney from the African green monkey. The antigens were prepared with the infected cell monolayer diluted to 30 percent with supernatant fluid and frozen and thawed three times. Complement-fixing antibody appeared in patients with rubella soon after the termination of rash and persisted for at least 8 months. In 8 out of 12 individuals who had the disease 10 to 20 years before they were tested, no complement-fixing antibody was found, but neutralizing antibody was present.*

Serologic investigations with rubella have been restricted because of the lack of rapid and inexpensive tests such as complement fixation or hemagglutination. Although several neutralization tests are available, they are technically difficult to perform and require at least 1 week for completion (*1*, *2*). An in-