ity rather than in the appearance of new bands (*8*). Galactose dehydrogenase specific activity is barely detectable before birth, whereupon a sudden increase results in a maximum in animals 5 days old, which falls slowly to the adult level in animals aged 30 days (*2*). The zymogram patterns suggest that the fall in specific activity after 5 days of age does not result from selective depressions of isoenzymes.

Differences among the various isoenzymes, other than electrophoretic mobility, remain to be determined. No differences in Michaelis-Menten kinetic constants, *p*H optimum, or heat-inactivation behavior were found between liver galactose dehydrogenase isolated from newborn and adult rats (*2*); it is not known whether the enzyme contains a subunit type of structure such as that in LDH. Further purification of the enzyme, and isolation of the individual isoenzymes in high yield, will probably be necessary to resolve these questions. Search for variants of human isoenzymes will be hampered by the limited availability of tissue for study: only liver contains appreciable activity.

PEDRO CUATRECASAS
STANTON SEGAL
Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland

References

1. P. Cuatrecasas and S. Segal, *Science* **153**, 549 (1966).
2. ———, *J. Biol. Chem.*, in press.
3. C. L. Markert and F. Møller, *Proc. Nat. Acad. Sci. U.S.* **45**, 753 (1959); M. M. Dewey and J. L. Conklin, *Proc. Soc. Exp. Biol. Med.* **105**, 492 (1960); E. S. Vessell, *Ann. N.Y. Acad. Sci.* **94**, 877 (1961); N. Ressler and R. Joseph, *J. Lab. Clin. Med.* **60**, 349 (1962).
4. R. P. Metzger, S. S. Wilcox, A. N. Wick, *J. Biol. Chem.* **239**, 1769 (1964).
5. W. A. Skoog and W. S. Beck, *Blood* **11**, 436 (1956).
6. E. S. Vessell and A. G. Bearn, *J. Gen. Physiol.* **45**, 553 (1962).
7. C. L. Markert, *Science* **140**, 1329 (1963); E. S. Vessell, *Proc. Nat. Acad. Sci. U.S.* **54**, 111 (1965).
8. J. Philip and E. S. Vessell, *Proc. Soc. Exp. Biol. Med.* **110**, 582 (1962); D. T. Lindsay, *J. Exp. Zool.* **152**, 75 (1963).

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Polymorphism of Heavy-Chain Genes in Immunoglobulins of Wild Mice

Abstract. *The serums of 123 wild mice from six different geographic locations in the United States contain five of the six known heavy-chain antigenic determinants that have been identified in immunoglobulin of inbred laboratory strains of mice. On the basis of the distribution of determinants in inbred strains, 44 of the mice were judged to be heterozygotes of various combinations and two had combinations of determinants that were unusual and could only have occurred in laboratory inbred mice by recombination.*

We have examined the serums of wild mice for antigenic determinants present in immunoglobulins of inbred laboratory strains. One of the determinants was not found, while other determinants were present in various combinations suggestive of heterozygosity and, in two instances, of recombination in mice.

The wild mice were trapped in different geographic areas of the United States. Antigenic determinants were identified with precipitating isoantiserums; that is, antiserums prepared by immunization of inbred mice with the immunoglobulins from genetically different inbred mice (*1–7*). Some of the isoantiserums provided a means of identification of antigenic determinants on specific heavy-chain immunoglobulin molecules (*4, 6*), while others were used to identify determinants on immunoglobulins not yet assigned to a specific class (*2, 5*). These isoantiserums have made it possible to study the genetic control of heavy-chain antigenic determinants. In the mouse the immunoglobulin genes A, G, and H, controlling the three heavy chains of γA, γG, and γH immunoglobulins, respectively, are closely linked (*3, 4, 8*). Each of four isoantiserums provided identification for specific determinants on proteins that were shown by immunoelectrophoresis to be immunoglobulins (probably γG) (*2*). These determinants, called 2, 3, 4, and 5, were found separately among different groups of homozygous inbred strains designated Asa2, Asa3, Asa4, and Asa5 (Table 1) (*2*; see *9*). Homozygous mice carry one of these determinants, heterozygotes carry two, but no inbred mouse has been found which carries three. In 14 of the 38 inbred strains studied, the 2, 3, 4, and 5 determinants were not found, and this group was designated Asal (al) (*2*; see also *9*). Isoantiserums reacting with a determinant only present in the a1 group are difficult to prepare since most immunoglobulin determinants of the a1 strain are widely distributed among the inbred strains carrying the immunoglobulin determinants 2, 3, 4, and 5. However, we were able to identify antigenic determinants on immunoglobulins in the a1 group with two specific isoantiserums. With one, we identified a determinant G6 on the heavy chain of the γG immunoglobulin, while with the other isoantiserum we identified a determinant H9 on the heavy chain of the γH immunoglobulin (*4, 6*). The specificity of these two isoantiserums could be determined because immunoglobulins carrying specific determinants can be isolated in pure form from BALB/c mice with transplantable plasma cell tumors (*10*). The BALB/c belongs to the a1 group of mice (*2*).

The determinants identified in 38 homozygous inbred strains by use of the six isoantiserums described (Table 1) are G6, H9 for group a1; 2, H9 for a2; 3, H9 for a3; 4, G6 for a4; and 5, H9 for a5.

Most inbred strains now in use are derived from assorted European and Asian stocks (*11*), and the distribution of immunoglobulin genes in domesticated stock and "wild type" *Mus musculus* should be quite different. We therefore determined the immunoglobulin phenotypes and genotypes of wild *Mus musculus*. Serums from wild *Mus musculus* and *Peromyscus* were collected in 1961–62 (*12*). None of the *Peromyscus* serums tested with a variety of specific determinant antiserums (Table 1) showed a precipitin reaction. The serums were diluted 1:5 and 1:10 when collected, and were then frozen until tested.

Serums collected from 123 wild *Mus Musculus* were each tested in Ouchterlony plates (Table 1). The distribution of determinants among the wild mice and their genotypes based on the determinants present was investigated (Table 2). We see that none of the 123 wild mice showed the 2 determinant found in the a2 group of inbred strains. Among the inbred strains, the incidence of the determinant 2 is fairly high, and of 38 inbred strains nine showed this determinant (*2*). Isoantiserums to determinant 2 are the easiest to prepare, and immunoglobulins of a2 mice appear to be equally highly anti-

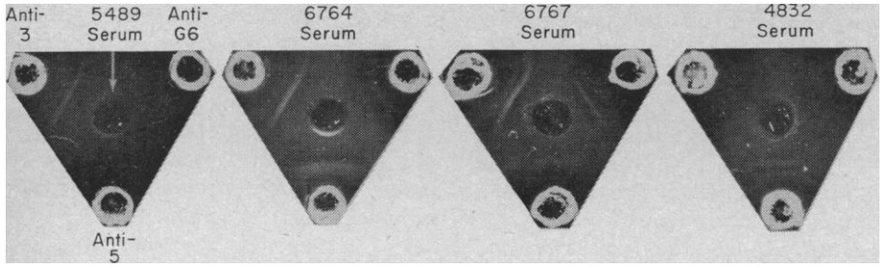

Fig. 1. Precipitin reactions (by Ouchterlony method) of three antiserums with serums of four representative wild mice (5489, 6764, 6767, and 4832). Each serum was tested with mouse antiserums to determinant 3 (BALB/c antiserum to DBA/2), to determinant G6 (CE antiserum to ST), and to determinant 5 [(BALB/c × C57BL/6) F_1 antiserum to NH]. For the designs, center wells contain wild-mouse serums; upper-left wells, antiserum to determinant 3; upper-right wells, antiserum to determinant G6; and bottom wells, antiserum to determinant 5. Determinant 6 is on γG of a1 mice. Determinants 3 and 5, respectively, are on unassigned classes of immunoglobulins in a3 and a5 mice (Table 1).

genic to mice in any of the a1, a3, a4, and a5 groups. This suggests that the immunoglobulins carrying the 2 determinant, so far only found in inbred strains, are the result of an extensive mutation from an ancestral wild type.

We found wild mice that were homozygous and heterozygous for determinants (other than the 2 determinant) present in the inbred strains (Table 2). The mice in group I resemble the inbred strains; that is, they have determinants G6+H9, H9+3, G6+4, and H9+5, which are found in the a1, a3, a4, and a5 strains, respectively (Table 1). In group II, there were 44 mice which resembled the various heterozygotes of known inbred strains. For example, a wild mouse having the determinants G6, H9, and 3 has its counterpart in a heterozygote of a cross of an a1 with an a3 inbred mouse. On this basis in group II, six different heterozygous combinations were found. Group III contains two unusual mice, each of which carries three determinants that are found separately in homozygous inbred strains; that is, the 3 determinant is found in the a3 group, the 5 determinant is found in the a5 group, and the G6 is found in the a1 group. No inbred mouse has been found showing determinants 3, 5, and G6 together. It thus appears very likely that these mice have a new heavy-chain linkage group containing either the 3 or 5 determinant plus the G6. This could be the result of recombination so that the G6 and the 3 or 5 determinants are now linked on the same chromosome.

Precipitin reactions are shown with the serums from four wild mice tested with isoantiserums for specific determinants by the Ouchterlony method (Fig. 1). There are examples of mice having the 3 determinant, the 5 determinant, both the 3 and 5 determinants, and the 3, 5, and G6 determinants in combination.

Group IV (Table 2) contains 20 serums that failed to react with any of the isoantiserums. Possibly this group includes serums that were too dilute or that may not contain any of the known determinants. However, concentrated five times, the serums still showed no reactivity. Individually these serum samples were insufficient to immunize inbred strains of mice, therefore 13 of these concentrated negative serums were pooled and then injected into BALB/c (a1 group), SJL (a2 group), and AL (a4 group) mice. Isoantiserums prepared in BALB/c gave weak precipitin reactions with serums of mice having the 3 and 4 determinants, but they did not react with the serums of mice having the 1, 2, or 5 determinants. Isoantiserums prepared in SJL mice precipitated the se-

Table 1. Isoantiserums, specific for antigenic determinants 6 on γG, 9 on γH, and 2, 3, 4, and 5 on unassigned immunoglobulins in inbred strains, used to test serums of wild *Mus musculus*.

Antiserums		Notation of determinants identified			Allele groups with determinants		Inbred strains having determinants
Strains immunized	Donor immunoglobulin	Lieberman		Herzenberg	Lieberman	Herzenberg	
		Current	Previous				
BALB/c	C57BL/6	2	B	Ig-1.4	Asa2	Ig-1^b	C57BL/HeN; C57BL/6N; C57BL/10ScN; NBL/N; HR/De; LP/J; SJL/J; SM/J; STR/1N
BALB/c	DBA/2	3	C	Ig-1.3	Asa3	Ig-1^c	DBA/1J; DBA/2N; I/An; RF/J; RIII/AN*; STOLI/Lw; SWR/J; YBR/He
BALB/c	AL	4	D	Ig-1.5	Asa4	Ig-1^{d1^e}	A/HeN†; AKR/N; AL/N; BL/LyDe
(BALB/c × C57BL/6)F_1‡	NH	5§	E	Ig-1.11	Asa5	Ig-1^f	CE/J; DE/J; NH/Lw
CE	ST	G6‖	F	Ig-1.6	Asa1a4	Ig-$1^{a1^{d1^e}}$	BALB/cAnN; BDP/J; BRSUNT/N; CBA/J; C3H/HeN; C57BR/cdJ; C57L/N; C58/N; DD/He; MA/J; PL/J; ST/J; STR/N;129/J; and a4 strains
AL	BALB/c γH myeloma protein	H9‖	I	Ig-3.2	Asa1a2a3a5	Ig-$1^{a1^{b1^{c1^f}}}$	a1, a2, a3, and a5 strains and in BL/LyDe.

* RIII is found in Ig-1^g group of Herzenberg. † A/HeN is found in Ig-1^e group of Herzenberg. ‡ This cross made isoantiserums which precipitated only with the serums of the a5 group. § Isoantiserum to just the 5 determinant is difficult to prepare in homozygous inbred strains. ‖ These determinants are on the papain Fc fragments of their respective heavy chains.

rums of a1, a3, a4, and a5 groups of mice but not those of the a2 group. Using a variety of specific molecular types of myeloma immunoglobulins from a1-BALB/c mice with plasma cell tumors, we ascertained which immunoglobulins carried the determinants precipitated by the SJL antiserum to wild mouse. Precipitin reactions were obtained only with BALB/c γG myeloma proteins. Thus in the pool of 13 wild-mouse serums the same determinants as those found in the 103 individual wild-mouse serums were present. Possibly the 13 negative serums individually failed to react because the determinants in the negative serums were too dilute, and the amount of immunoglobulin was too small to be detected by the Ouchterlony method. The antiserums prepared in the AL strain identified an antigenic specificity whose distribution among the inbred strains was completely different from that of any of the known determinants identified thus far. This antigenic specificity was present in the serums of a1 (except BRSUNT, DD, and ST) and a2 (except NBL) mice, and in three of the a3 mice (DBA/1, RIII, and STOLI). There was a possibility that this was a new, widely distributed immunoglobulin determinant. Precipitin tests, however, with a variety of BALB/c myeloma proteins including γM, γA, γF, γG, and γH immunoglobulins, and kappa and lambda light-chain types, and the AL antiserum to wild-mouse immunoglobulins were negative. This antigenic specificity appears to be similar to that of a protein component of complement Hc^1 or MuB1 (*13, 14*), and shows the same distribution pattern among the inbred strains as the MuB1 (*14*). This was confirmed by a genetic test with F_2 progeny of a cross of C57BL/6 × AL mice, which showed that the inheritance of the MuB1 antigenic specificity and the a2 or a4 immunoglobulin allotypic specificity were completely independent.

The antigenic determinants on immunoglobulin molecules identified by isoantiserums are a reflection of the genetic polymorphism of the immunoglobulins in the mouse population. The persistence of the same determinants in wild mice that are found in inbred strains suggests that these mutations in immunoglobulin molecules are of selective advantage. On the basis of our experience with the inbred strains which are homozygous for the immunoglobulin loci, 44 of the 123 wild mice were clearly heterozygous. In the heterozygous mice, two different immunoglobulin heavy-chain loci are present, and evidence has been presented that only one of these loci operates in a given cell at a given time (*7, 15*). Warner and Herzenberg showed that, in plasma cell tumors arising in heterozygous mice, the myeloma protein carries one of the genetically polymorphic antigenic determinants (*7*). Weiler presented evidence that only one of the heavy-chain loci takes part in synthesis of immunoglobulin by normal immunocytes from heterozygous mice (*15*).

ROSE LIEBERMAN
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland
MICHAEL POTTER
National Cancer Institute

Table 2. Geographic distribution of genotypes among wild *Mus musculus* strains based on immunoglobulin antigenic determinants identified in 38 inbred strains of mice. Numbers in parentheses denote different areas in a state.

Determinants	Mice with determinants (total number)	Number of mice: Ga.	Ind.	Fla. (1)	Fla. (2)	Md. (1)	Md. (2)	Group assignment of mice	Presumed genotypes
				Group I: homozygotes					
H9,G6	34	4	11	4	2	4	9	a1	$\frac{*-,G^6,H^9}{-,G^6H^9}$
3,H9	8	0	0	2	3	1	2	a3	$\frac{^3,G^6-,H^9}{^3,G^6-,H^9}$
4,G6	3	1	0	1	0	0	1	a4	$\frac{^4,G^6,H^9-}{^4,G^6,H^9-}$
5,H9	12	2	7	2	0	0	1	a5	$\frac{^5,G^6-,H^9}{^5,G^6-,H^9}$
				Group II: heterozygotes					
3,H9,G6	7	1	0	2	1	2	1	a3/a1	$\frac{^3,G^6-,H^9}{\dagger-,G^6,H^9}$
4,H9,G6	4	0	2	1	0	0	1	a4/a1	$\frac{^4,G^6,H^9-}{\ddagger-,G^6,H^9}$
5,H9,G6	22	11	1	3	0	5	2	a5/a1	$\frac{^5,G^6-,H^9}{\S-,G^6,H^9}$
3,4,H9,G6	2	0	0	1	0	0	1	a3/a4	$\frac{^3,G^6-,H^9}{^4,G^6,H^9-}$
3,5,H9	8	0	0	1	0	7	0	a3/a5	$\frac{^3,G^6-,H^9}{^5,G^6-,H^9}$
4,5,H9,G6	1	0	0	1	0	0	0	a4/a5	$\frac{^4,G^6,H^9-}{^5,G^6-,H^9}$
				Group III: recombinants or new alleles					
3,5,H9,G6	2	0	0	0	0	2	0	a3/a5a1	$\frac{^3,G^6-,H^9}{^5,G^6,H^9}$
								or, a3a5/a1	$\frac{^3,^5,G^6-,H^9}{G^6,H^9}$
								or, a3a1/a5	$\frac{^3,G^6,H^9}{^5,G^6-,H^9}$
								or, a1a3a5/–	$\frac{^3,^5,G^6,H^9}{---}$
				Group IV: Unknown					
None	20	6	4	3	2	4	1		

* Determinants 2, 3, 4, and 5 not found. † Determinants 2, 4, and 5 not found, 2, 3, and 5 found. ‡ Determinants § Determinants 2, 3, and 4 not found.

References and Notes

1. A. Kelus and J. K. Moor-Jankowski, *Nature* **191**, 405 (1961).
2. R. Lieberman and S. Dray, *J. Immunol.* **93**, 584 (1964).
3. L. A. Herzenberg, *Cold Spring Harbor Symp. Quant. Biol.* **29**, 455 (1964).
4. S. Dray, M. Potter, R. Lieberman, *J. Immunol.* **95**, 823 (1965).
5. L. A. Herzenberg, N. L. Warner, L. A. Herzenberg, *J. Exp. Med.* **121**, 415 (1965).
6. M. Potter, R. Lieberman, S. Dray, *J. Mol. Biol.* **16**, 334 (1966).
7. N. L. Warner, L. A. Herzenberg, G. Goldstein, *J. Exp. Med.* **123**, 707 (1966).
8. R. Lieberman, S. Dray, M. Potter, *Science* **148**, 640 (1965); R. Lieberman and M. Potter, *J. Mol. Biol.* **18**, 516 (1966).
9. For brevity the 5 groups of strains designated as having the allotypic specificities Asa1, Asa2, Asa3, Asa4, and Asa5 will be referred to as a1, a2, a3, a4, and a5 groups, respectively.
10. M. Potter and C. L. Robertston, *J. Nat. Cancer Inst.* **25**, 847 (1960); M. Potter and C. R. Boyce, *Nature* **193**, 1086 (1962); M. Potter and J. L. Fahey, *J. Nat. Cancer Inst.* **24**, 1153 (1960); J. L. Fahey, J. Wunderlich, R. Mishell, *J. Exp. Med.* **120**, 223, 243 (1964); M. Potter and E. L. Kuff, *J. Nat. Cancer Inst.* **26**, 1109 (1961); ———, *J. Mol. Biol.* **9**, 537 (1964); M. Potter, W. D. Dreyer, E. L. Kuff, K. R. McIntire, *ibid.* **8**, 814 (1964); M. Potter, E. Appella, S. Geisser, *ibid.* **14**, 361 (1965).
11. C. E. Keeler, *Laboratory Mouse* (Harvard Univ. Press, Cambridge, 1931).
12. Drs. Robert Huebner and Janet Hartley of NIAID supplied the wild-mouse serums.
13. R. P. Erickson, D. K. Tachibana, L. A. Herzenberg, L. T. Rosenberg, *J. Immunol.* **92**, 611 (1964).
14. B. Cinader, S. Dubiski, A. C. Wardlaw, *J. Exp. Med.* **120**, 897 (1964).
15. E. Weiler, *Proc. Nat. Acad. Sci. U.S.* **54**, 1765 (1965).
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